Unraveling the Mechanism of Immunity and Inflammation Related to Molecular Signatures Crosstalk Among Obesity, T2D, and AD: Insights From Bioinformatics Approaches

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Kumar Vishal[1,2,3*], Piplu Bhuiyan[4*], Junxia Qi[1,2,3], Yang Chen[1,2,3], Jubiao Zhang[1,2,3], Fen Yang[3] and Juxue Li[1,2,3,5,6]

[1]State Key Laboratory of Reproductive Medicine, Nanjing Medical University, Nanjing, China. [2]Key Laboratory of Human Functional Genomics of Jiangsu Province, Nanjing Medical University, Nanjing, China. [3]Department of Biochemistry & Molecular Biology, School of Basic Medical Sciences, Nanjing Medical University, Nanjing, China. [4]Department of Anesthesiology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China. [5]The Affiliated Eye Hospital, Nanjing Medical University, Nanjing, China. [6]The Second Affiliated Hospital of Nanjing Medical University, Nanjing, China.

ABSTRACT: Individuals with type 2 diabetes (T2D) and obesity have a higher risk of developing Alzheimer disease (AD), and increasing evidence indicates a link between impaired immune signaling pathways and the development of AD. However, the shared cellular mechanisms and molecular signatures among these 3 diseases remain unknown. The purpose of this study was to uncover similar molecular markers and pathways involved in obesity, T2D, and AD using bioinformatics and a network biology approach. First, we investigated the 3 RNA sequencing (RNA-seq) gene expression data sets and determined 224 commonly shared differentially expressed genes (DEGs) from obesity, T2D, and AD diseases. Gene ontology and pathway enrichment analyses revealed that mutual DEGs were mainly enriched with immune and inflammatory signaling pathways. In addition, we constructed a protein-protein interactions network for finding hub genes, which have not previously been identified as playing a critical role in these 3 diseases. Furthermore, the transcriptional factors and protein kinases regulating commonly shared DEGs among obesity, T2D, and AD were also identified. Finally, we suggested potential drug candidates as possible therapeutic interventions for 3 diseases. The results of this bioinformatics CC analysis provided a new understanding of the potential links between obesity, T2D, and AD pathologies.

KEYWORDS: Alzheimer disease, type 2 diabetes, obesity, bioinformatics

Introduction

Alzheimer disease (AD) is the most common form of neurodegenerative dementia globally.[1] The global prevalence of AD is rapidly increasing, particularly among people aged 65 years and over. A lack of detection ability characterizes the early stages of AD disease. Neuronal loss, impaired synaptic plasticity, dystrophic neuritis, abnormal protein phosphorylation, ubiquitination, and accumulation of the toxic Aβ peptide as senile plaques and tau aggregation hyperphosphorylated as neurofibrillary tangles (NFTs), are all pathology hallmark of AD pathogenesis.[2,3] Alzheimer disease is increasingly being considered correlated with metabolic dysfunction,[4] altered glucose metabolism in the brain,[5] increased insulin abnormalities, and insulin-like growth factor (IGF) resistance.[6] Insulin resistance can disrupt the blood-brain barrier (BBB), causing it to become less permeable. Cerebrovascular dysfunction results from this disruption, resulting in synaptic plasticity and cognitive impairments.[7] Insulin resistance exacerbates Aβ and tau pathologies in type 2 diabetes (T2D) patients, elucidating the pathophysiological aspects of synaptic dysfunction, inflammation, and autophagic impairments that are common to both disorders and have an indirect influence on Aβ and tau functions in neurons.[8]

Type 2 diabetes has become more prevalent in different regions of the world.[9] The growing body of research demonstrated that T2D has been linked to cognitive impairment and dementia in elderly people.[10] The number of elderly T2D patients with cognitive impairment has been rising worldwide. Cognitive impairment, vascular dementia, and AD development are considerably exacerbated in patients with metabolic disorders.[11] Type 2 diabetes is connected to severe deficits in numerous aspects of cognitive function, thus resulting from obesity and metabolic disorder.[12]

Obesity is a multifactorial, chronic disease defined by excessive body fat deposition. The relationship between being overweight or obese and brain health is perhaps less well

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*These authors contributed equally to this work.

understood. A higher body mass index (BMI) and mid-life obesity have increased the risk of dementia.[13] Several studies reported that obesity and AD tend to induce similar brain dysfunctions.[14,15] However, specific findings are debatable, and further research into the underlying mechanisms of obesity-associated with pathogenic processes that cause AD is needed. Obesity and T2D patients are more likely to develop AD-related cognitive deterioration.[16] Obesity, AD, and T2D have common characteristics, such as brain atrophy, decreased cerebral glucose, and central nervous system (CNS) insulin resistance.[17] Given the evidence above, it is not unexpected that individuals with T2D and obesity are more likely to develop AD. However, the molecular pathways and linking biomarkers remain unclear.

Studies on transgenic mice models of AD and T2D may throw some lights on inflammatory signaling pathways as a possible mechanistic connection between AD and T2D, as inflammatory processes play a crucial involvement in the pathogenesis of both disorders.[18,19] Takeda et al crossed AD transgenic mice (APP23) with diabetes mice (ob/ob) and investigated the metabolism and pathology of the brains. They observed that up-regulation of receptors for advanced glycation end-products and inflammatory changes in the cerebral vasculature had been shown in those double mutant mice even before the onset of cerebral amyloid angiopathy, implying that T2DM-induced cerebrovascular inflammation is the source of enhanced AD pathogenesis.[20] Obesity causes the inflammatory signaling pathways c-Jun N-terminal kinase (JNK) and nuclear factor-kappa B (NF-κB). Once triggered, these pathways cause adipocytes to produce several pro-inflammatory cytokines, leading to insulin resistance and the influx of pro-inflammatory macrophages.[21] Type 2 diabetes and obesity are considered risk factors for AD. However, it is unclear whether the 2 diseases are connected pathologically.

According to gene transcriptome investigations in the mouse brain, astrocytes are extremely immune reactive and up-regulate distinct sets of genes that can either promote or impede recovery depending on the immunologic trigger in AD.[22] When the reactive microglia compared with non-reactive microglia, the reactive microglial population expressed increasing levels of CD11c, CD14, CD86, CD44, programmed death-ligand 1, and major histocompatibility complex-II (MHC-II), and lower levels of the microglial homeostatic checkpoint markers CX3CR1, MerTK (C-MER proto-oncogene tyrosine kinase), and Siglec-H.[23] This study suggested that major histocompatibility complex (MHC) linked with immunologic dysfunction has been implicated in susceptibility to more progressive AD pathogenesis. Microarray analysis revealed that AD subjects with mild/moderate dementia hippocampus showed elevated gene expression of the inflammatory molecule MHC-II compared with non-demented high-pathology controls.[24] Major histocompatibility complex-II protein levels were similarly elevated and were shown to be negatively associated with cognitive

ability. In addition, compared with controls, the mild/moderate AD dementia sufferers had a lower number of T cells in the hippocampus and cortex. Major histocompatibility complex-II genes and molecules have been linked to several diseases, particularly autoimmune type 1 diabetes (T1D). Interferon gamma (IFN-γ) promotes the expression of MHC-II, which is required for antigen presentation.[25] In addition, Foss-Freitas et al[26] found decreased IFN-γ levels in the T2D group compared with the normal control group, suggesting that IFN-γ enhances granulocyte activation and phagocytic capability, lowering infection susceptibility. A microarray study of primary adipocytes reported that numerous genes involved in MHC-II antigen processing and presentation associated with inflammatory pathways were increased in obese women.[27]

The main purpose of this research is to explore the crosstalk of similar sharing therapeutic targets and molecular signaling pathways of obesity, T2D, and AD using system biology approaches. To better understand the pathophysiological mechanisms that underlie AD linked with T2D and obesity, we identified potential biomarkers, signaling molecules, and pathways for early diagnosis. Understanding the mechanisms underlying signaling pathways in the development of obesity, T2D, and AD may aid in identifying novel targeted therapeutics for pharmacologic treatments.

Methods and Materials

Data acquisition

RNA sequencing (RNA-seq) data (GSE53697, GSE92724, and GSE106289) were downloaded from the GEO database. The RNA expression levels of 3 data sets were compared between healthy control and obesity, T2D, and AD in human, respectively. The GSE53697 data were obtained from 9 cases of AD patients and 8 controls by the GPL16791 Illumina HiSeq 2500. The GSE92724 data were obtained from 4 cases of T2D and 6 controls by the GPL20301 Illumina HiSeq 4000. The GSE126169 data were obtained from 10 cases of obesity and 10 healthy volunteers by the GPL10999 Illumina Genome Analyzer IIx. Subsequently, we analyzed the 3 data sets and collected overlapping genes among AD, T2D, and obesity for further analysis.

Identification of differentially expressed genes

GREIN[28] used a generalized negative binomial linear model in edgeR. To determine DEGs between control and AD, T2D, and obese patients, we used the GREIN web application for 3 RNA-seq data sets. Algorithms employed with the Log2 transformation and the Benjamini-Hochberg approach[29] to limit the rate of false discovery of numerous correction methods to find deregulated DEGs using the LIMMA package in R. The statistically significant DEGs were then filtered from 3 RNA-seq data sets using the mathematically used cut-off criteria:

DEGi = Up-regulated if the *P*-value < .5 and log2FC ≥1.

Down-regulated if *P*-value < .5 and log2FC ≤−1.

We identified the overlap DEGs across the 3 data sets (GSE53697, GSE92724, and GSE106289) from both up-regulated and down-regulated using the above mathematically cut-off formula. In addition, commonly shared DEGs among obesity, T2D, and AD were recognized from 3 RNA-seq data sets using the Bioinformatics & Evolutionary Genomics Venn diagram web application tool.

Functional enrichment analysis of commonly shared differentially expressed genes

The biological characteristics of common DEGs between obesity, T2D, and AD were determined using gene ontology (GO) and pathway enrichment analyses. To evaluate the biological insights of the identified DEGs, GO terms were focused on their 3 main components: biological process, cellular component, and molecular function (MF). Gene ontologies were annotated with a *P*-value < .01 from statistically significant results of shared DEGs using EnrichR[30] (a web-based complete gene enrichment analysis tool). To investigate pathway enrichment analysis of commonly shared DEGs among obesity, T2D, and AD, we employed 3 computational approaches databases: Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome 2016, and BioCarta 2019. Choosing the top listed pathways depends on the cut-off criteria of a *P*-value of .01.

Integration of protein–protein interaction network and hub genes identification

Protein-protein interaction (PPI) network analysis approaches are a valuable strategy for accelerating our understanding of molecular crosstalk and biological processes (BPs) driving the complexity of diseases. Using the STRING database, the PPI network was retrieved based on the physical interaction of DEGs with a high confidence score of >0.7. Then, we optimized the PPI network using Cytoscape for better visualization. Furthermore, the top PPI network clusters were identified using the Cytoscape MCODE plugin.[31] Using the CytoHubba plugin of Cytoscape, we employed different topological parameters, including maximal clique centrality (MCC), maximum neighborhood component (MNC), degree nodes, closeness, and betweenness, to identify hub genes from the PPI network.

Recognition of transcription factors from mutual differentially expressed genes

Transcription regulation is complicated and multidimensional, including the direct binding of transcription factor (TF) to a target gene's regulatory element and a complex interplay between TFs and TF binding proteins. X2K[32] is a web-based bioinformatics algorithm (regulatory networks platform) that uses the hypergeometric *P*-value from the ChIP-seq experiments (ChEA) database to find the top transcriptional factors. Based on commonly shared DEG signatures, the X2K online tool generates inferred TF networks associated with PPI, resulting in downstream regulatory pathways. The Genes2Networks[33] (G2N) algorithm was used to find transcriptional factors by looking for proteins that physically connect with them. G2N is a robust command-line and web-based tool that analyzes DEGs in genomic and proteomic data based on empirically proven PPI networks or protein complexes.

Identification of protein kinases associated with protein and transcriptional network

One of the most significant aspects of activation is the requirement for strict controls that allow appropriate enhancement and reduction of function. Protein kinase has been identified using the X2K kinase enrichment analysis module. Kinase enrichment analysis (KEA) is command-line software that can match mammalian protein DEG lists to the protein kinases anticipated to phosphorylate them. The HPRD, PhosphoSite, phospho.ELM, NetworKIN, and Kinexus databases were used to create an extended subnetwork encompassing protein kinases, PPIs, and TFs with phosphorylation.

Uncovered the potential biological signaling pathways

The FunRich[34] interactive bioinformatics tool (http://www.funrich.org) was used against the human genomic and proteomic data sets, which was merged with the FunRich background, to identify the potential biological signaling pathways associated with commonly shared DEGs.

Potential therapeutic candidates

Using the drug signature database (DSigDB),[35] we identified therapeutic targets based on DEGs generally shared among obesity, T2D, and AD. Drug signature database is a global database that helps identify targeted communications. It is a unique gene set and valuable resource for analyzing new genes and connecting therapeutic compounds to their target genes. There are now 22 527 gene sets, 17 389 different compounds, and 19 531 genes in DSigDB. Drug signature database gene sets ultimately meld with GSEA software, allowing transcriptional activation to be linked to drugs for pharmacologic applications and research purposes. The pharmacologic signature database is linked to differentially expressed genes. These drugs might have inhibitory properties against obesity, T2D, and AD.

Results

Identification of mutual differentially expressed genes among Alzheimer disease, type 2 diabetes, and obesity

Using GREIN interactive web platform, we acquired using 3 RNA-seq data sets (GSE53697, GSE92724, and GSE106289). Based on significant *P*-values ($P<.05$) and log2FC ≥ 1 or ≤ -1, we screened a total of 2398, 3522, and 6782 from the data sets of GSE53697, GSE92724, and GSE106289, respectively. Up-regulated genes were identified at 1028, 1704, and 3025 among obesity, T2D, and AD, respectively, whereas numbers of down-regulated genes were observed at 1370, 1818, and 3757, respectively. Figure 1A to C represents the hierarchical heatmap gene expression patterns among obesity, T2D, and AD diseases. Figure 1D to F illustrates all the DEGs with a log2FC vs the $-$log10 (*P*-value) among those 3 diseases and control individuals in an MA plot. Furthermore, we used the Venn diagram tool to identify commonly shared overlapping DEGs among 3 diseases. We found 132 up-regulated and 92 down-regulated common genes among 3 data sets, a total number of 224 mutual DEGs (Figure 1G). The functional annotation of genes, pathways enrichment analysis, identification of predicated TFs and signaling molecules, potential molecular and biological pathways, and drug candidates employing those commonly deregulated DEGs were then analyzed using bioinformatics and system biology approaches.

Gene ontology term and pathway enrichment analysis of mutually differentially expressed genes

We employed gene annotation and pathways enrichment analysis to see how the underlying BPs link complicated diseases to each other. We used EnrichR, a bioinformatics resource, to perform gene set enrichment analysis to discover pathways. We evaluated 4 pathway databases to conduct tests using commonly shared DEGs frequent in obesity, T2D, and AD. The study of GO terms was used to explore the activities of BPs, MFs, and cell components (CCs) of common overlapping DEGs. In BP (Figure 2A), the mutual DEGs were enriched with an IFN-γ-mediated signaling pathway, cellular response to IFN-γ, antigen processing and presentation of endogenous peptide antigen, antigen processing and presentation of endogenous peptide antigen via MHC class I via ER pathway, antigen processing and presentation of endogenous peptide antigen via MHC class I via ER pathway, transporter associated with antigen processing (TAP)-independent, regulation of T-cell-mediated cytotoxicity, regulation of immune response, antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-independent, antigen processing and presentation of peptide antigen via MHC class I, and antigen processing and presentation of exogenous peptide antigen. For the category of MF (Figure 2B), commonly shared DEGs were significantly linked with MHC class II receptor activity, transporter associated with antigen processing 1 (TAP1) binding, MHC class II protein complex binding, steroid hydroxylase activity, oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, MHC class Ib protein binding, MHC class I protein binding, icosatetraenoic acid-binding, arachidonic acid-binding, and C3HC4-type RING finger domain binding. In the aspect of CC (Figure 2C), commonly shared genes expression was mainly involved with MHC protein complex, lumenal side of endoplasmic reticulum (ER) membrane, integral component of lumenal side of ER membrane, MHC class II protein complex, ER to Golgi transport vesicle membrane, coated vesicle membrane, transport vesicle membrane, endocytic vesicle membrane, coat protein complex type II (COPII)-coated ER to Golgi transport vesicle, and integral component of ER membrane. These findings suggest that immunogenetics dysregulation processes might be involved in the pathomechanisms of obesity, T2D, and AD.

Furthermore, we also performed pathways enrichment analysis from KEGG, Reactome, and BioPlanets of overlapping mutual DEGs to find critical signaling pathways that may relate to obesity, T2D, and AD pathogenesis. The top signaling pathways were chosen for pathway analysis based on the significance of an adjusted *P*-value of less than .1 as criteria. Using the KEGG pathway database, critical biological pathways may be involved in the activator protein-1 (AP-1) TF network, endogenous Toll-like receptor (TLR) signaling pathway, interleukin 12 (IL12)-mediated signaling pathway, epidermal growth factor receptor (EGFR)-dependent endothelin signaling pathway, CXCR4-mediated signaling pathway, ErbB receptor signaling network, regulation of p38-alpha and p38-beta, syndecan-4-mediated signaling, IL12 signaling mediated by STAT4, IL1-mediated signaling pathway, amb2 integrin signaling pathway, FOXM1 TF network, Syndecan-1-mediated signaling pathway, and T-cell receptor (TCR) signaling pathway (Figure 2D). Reactome 2016 pathway showed that commonly DEGs were significantly enriched with an IFN-γ signaling pathway, interferon signaling pathway, immune system, cytokine signaling in the immune system, MHC class II antigen presentation, adaptive immune system, interferon-alpha/beta signaling pathway, programmed cell death protein 1 (PD-1) signaling pathway, endosomal/vacuolar pathway, ER-phagosome pathway, TCR signaling pathway, receptor-interacting protein (RIP)-mediated NF-κB activation pathway, tumor necrosis factor receptor-associated factor 6 (TRAF6)-mediated NF-κB activation pathway, transforming growth factor β activated kinase-1 (TAK1) activates NF-κB by phosphorylation, and initial triggering of complement (Figure 2E). Results from the BioCarta 2019 pathways database revealed the most important signaling pathways associated with antigen processing and presentation, activation of COOH-terminal Src kinase (Csk) by 3',5'-cyclic adenosine monophosphate (cAMP)-dependent protein kinase inhibits
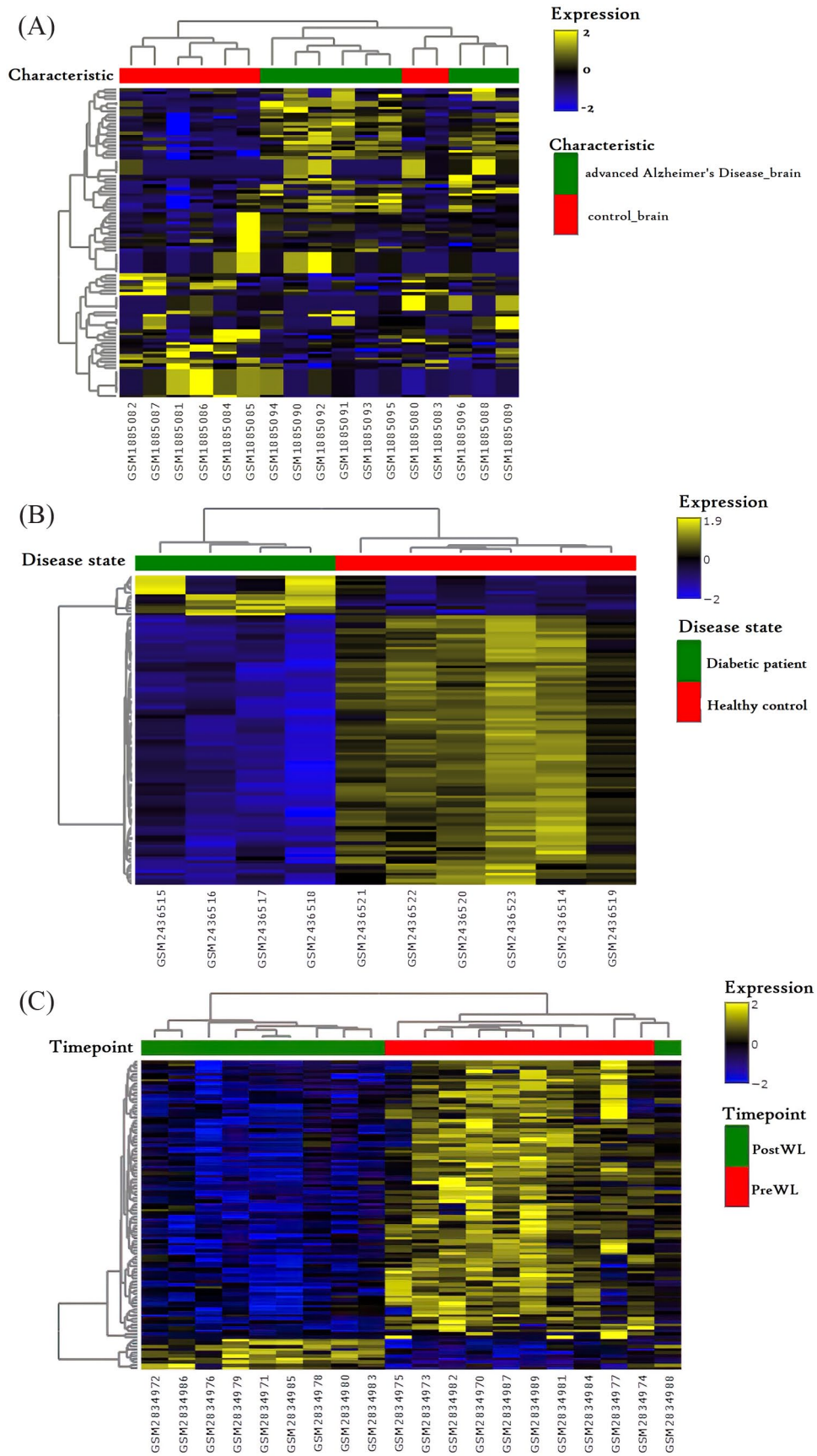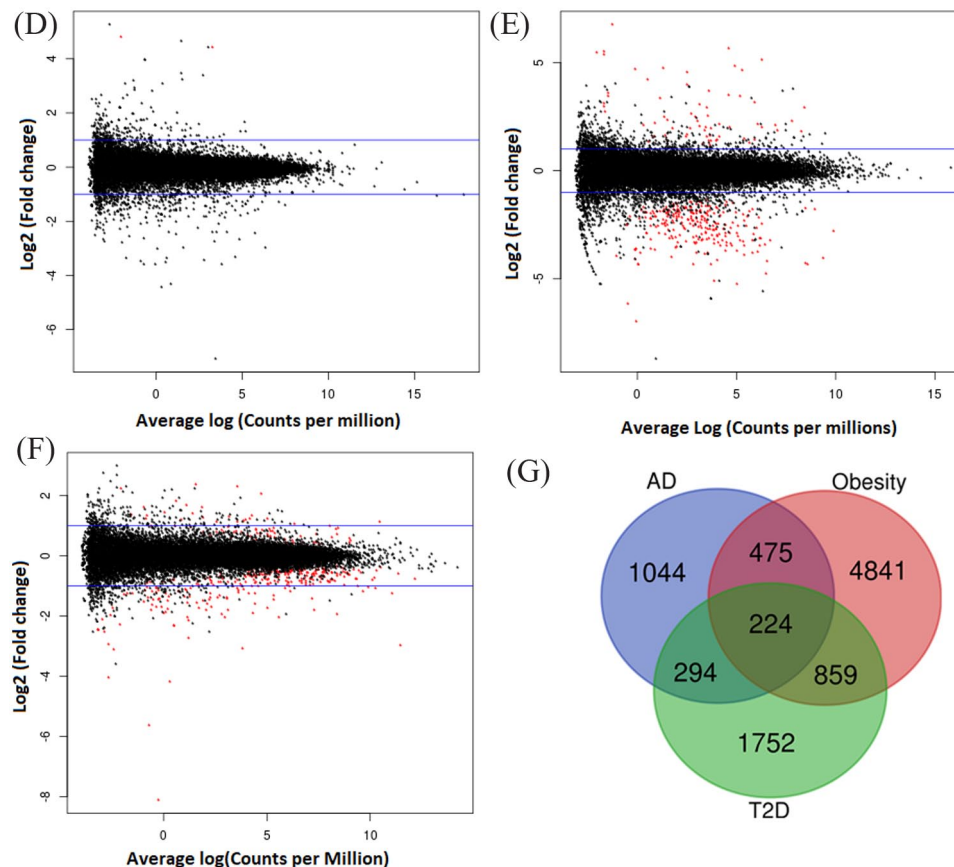
Figure 1. *(Continued)*

Figure 1. Hierarchical heatmap clustering of gene expression patterns from 3 RNA-seq data sets (A) GSE53697, (B) GSE92724, and (C) GSE106289. Up-regulated gene expressions are colored by yellow, down-regulated gene expressions are illustrated by blue color, and insignificant gene expressions are represented by black color in these hierarchical heatmaps. Determination of DEGs from 3 RNA-seq data sets. (D to F) MA plots represented a significant up-regulated and down-regulated of DEGs. (G) Identification of mutual DGEs among obesity, T2D, and AD.
AD indicates Alzheimer disease; DEGs, differentially expressed genes; RNA-seq, RNA sequencing; T2D, type 2 diabetes.

signaling through the T-cell receptor, Lck and Fyn tyrosine kinases in the initiation of TCR activation, interleukin-4 (IL-4) signaling pathway, the co-stimulatory signal during T-cell activation, role of MEF2D in T-cell apoptosis, T-cell receptor signaling pathway, inhibition of matrix metalloproteinases, regulation of mitogen-activated protein kinase (MAP) pathways, alternative complement pathway, G-protein signaling, interleukin-4 (IL-10) anti-inflammatory signaling pathway, gamma-aminobutyric acid receptor signaling pathway, chaperones modulate interferon signaling pathway, and NF-κB signaling pathway (Figure 2F).

Protein–protein interaction network analysis and hub genes identification

We built putative PPI networks using our enriched common DEGs gene sets using the web-based visualization resources, including STRING, BioGrid, OmniPath, InWeb_IM, IntAct, and MatrixDB via Metascape. We employed the molecular complex detection (MCODE) algorithm to identify significant gene clusters from mutual DEGs among obesity, T2D and AD (filter criteria: degree cut-off = 2; node score cut-off = 0.2; k-core = 2; max depth = 100). We identified 7 gene

clusters that have played a critical in the disease's progression of obesity, T2D, and AD (Figure 3). The top hub genes were identified from 5 CytoHubba algorithms, including maximal clique centrality (MCC), MNC, degree, closeness, and betweenness algorithm of Cytoscape, as shown in Figure 4A to E. In addition, the 5 algorithms of CytoHubba results were merged to determine hub genes that were commonly found in Figure 4F.

Transcription factors analysis of commonly differentially expressed genes and identification of protein kinase with upstream regulatory network

Transcription factors are the proteins that control gene expression by binding to specific DNA sequences. Differentially expressed genes are regulated by TFs, which are essential for a range of biological and cellular processes and have been connected to the development of neurological disorders and other chronic diseases. Using the ChEA database, the X2K bioinformatics tool identified transcriptional factors that influence common DEGs expression of obesity, T2D, and AD. The top 20 TF candidates were chosen using transcription factor enrichment analysis (TFEA) based on the

hypergeometric *P*-value. The name of the top 20 TFs was TP63, SUZ12, CREB1, EGR1, EZH2, PPARD, ZEB1, E2F1, RFX5, ESR1, SPI1, KLF4, TCF3, FOXA2, YY1, NANOG, and FOXM (Figure 5A). To evaluate the links between PPIs and TFs, the G2N algorithm uncovered proteins that physically interact with them (Figure 5B). The regulatory network of connected TFs and their functionally and physically interacting proteins was represented based on the degree of nodes. Pink nodes indicated TFs, whereas gray nodes expressed proteins.

Protein kinases, comprised of sensors and effectors from signal transduction cascades, govern mRNA translation, cell proliferation and survival, and nuclear genomic response to cellular stresses. Dysregulation of protein kinase activity has been related to various diseases, from inflammatory to neurodevelopmental. We used the mutual DEGs to investigate potential neurotherapeutic protein kinase targets using the Kinase Enrichment Analysis module of X2K. We found the top 20 potential protein kinases that play a crucial role in intracellular signaling pathways related to obesity, T2D, and AD. Kinase enrichment analysis revealed that the top 20 protein kinases were CDK1, MAPK14, CSNK2A1, AKT1, GSK3B, CDK4, HIPK2, ABL1, MAPK3, MAPK1, CDK2, ATM, GSK3BETA, PKBALPHA, JNK1, ERK2, TGFBR2, MAPK8, and CDC2 to regulate the expression of common DEGs (Figure 5C). Then, the human protein reference database (HPRD), PhosphoSite, phospho.ELM, NetworKIN, and Kinexus were used to construct the regulatory network of kinase-substrate phosphorylation interactions based on ranked enriched kinases (Figure 5D).
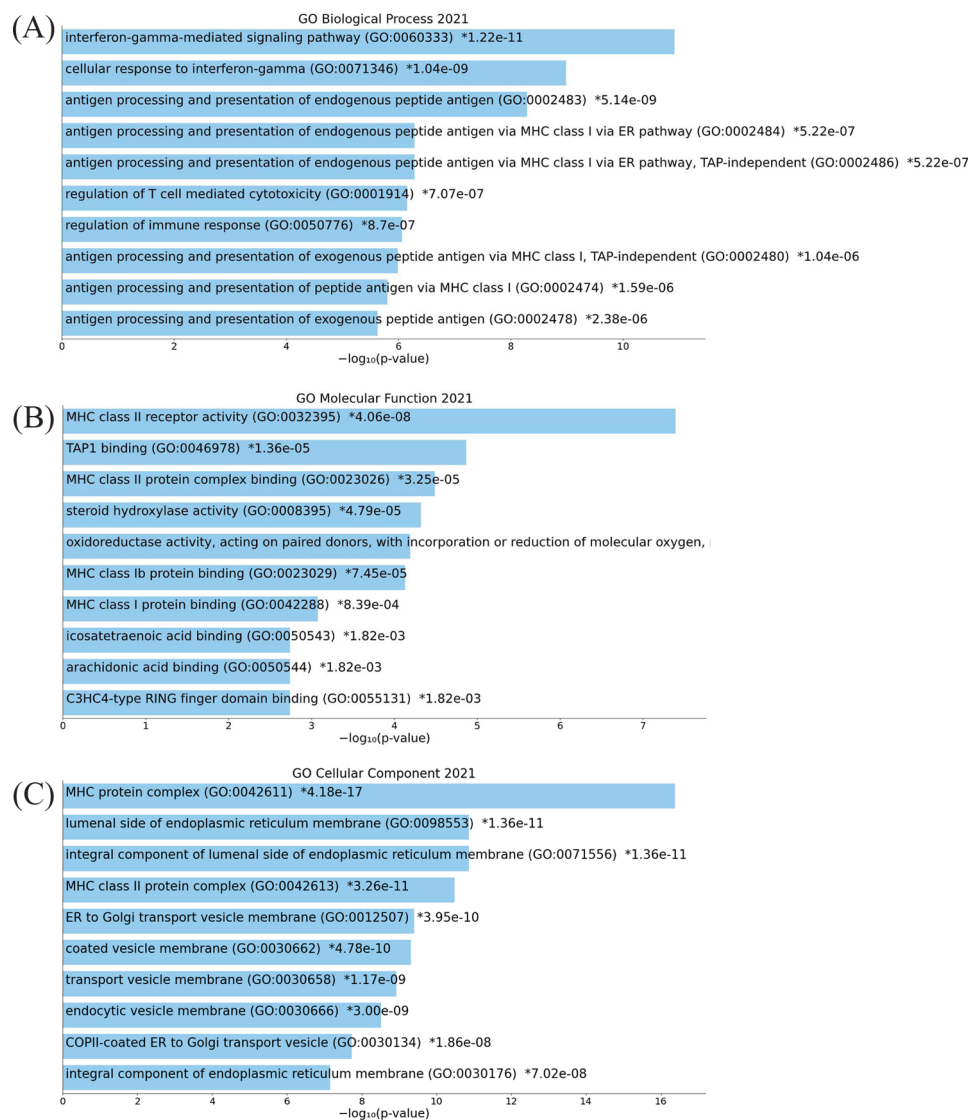

(A) GO Biological Process 2021

(B) GO Molecular Function 2021

(C) GO Cellular Component 2021
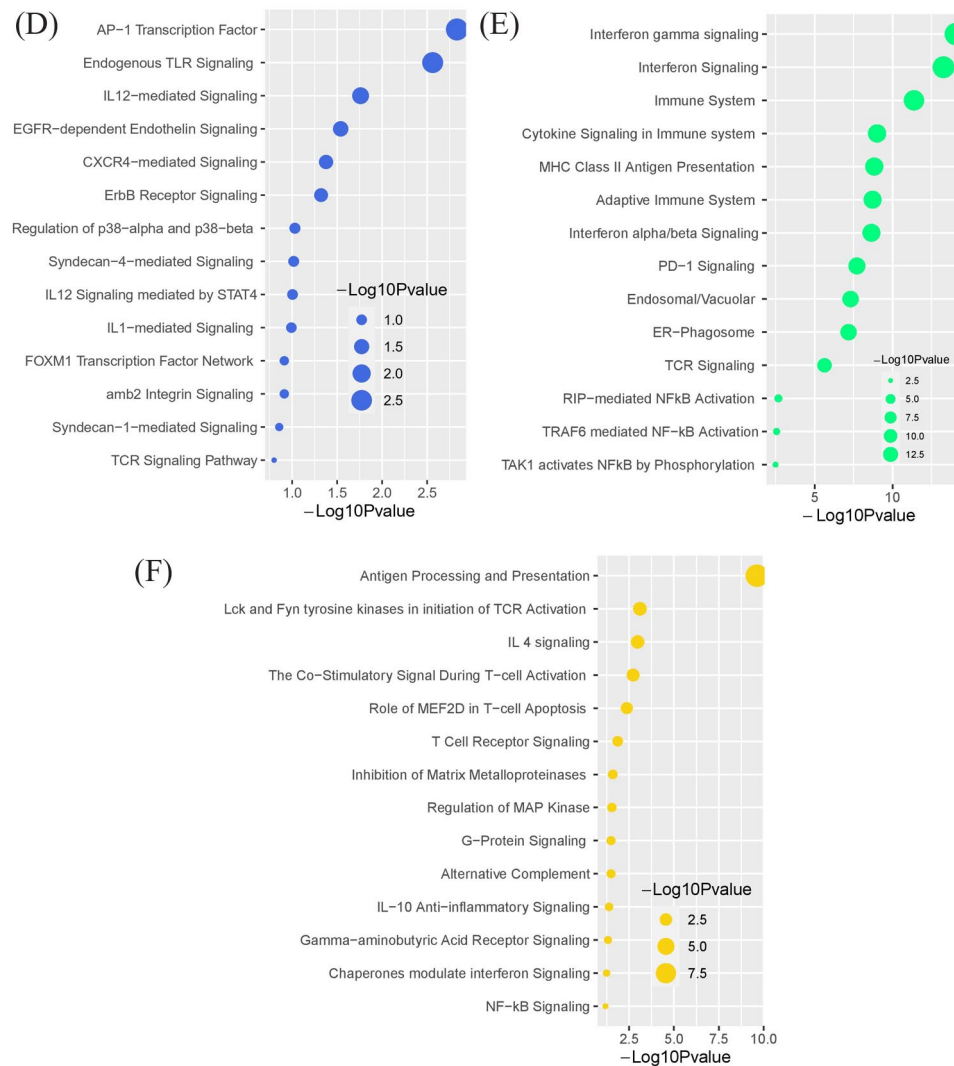
Figure 2. *(Continued)*

Figure 2. Gene ontology enrichment analysis of mutual DEGs from AD, T2D, and obesity. (A) GO biological processes, (B) GO molecular function, and (C) GO cellular component. Pathway enrichment analysis of commonly shared DEGs crosstalk links with obesity, T2D, and AD. (D) KEGG pathway, (E) reactome pathway, and (F) BioCarta pathway.

AD indicates Alzheimer disease; DEGs, differentially expressed genes; GO gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; RNA-seq, RNA sequencing; T2D, type 2 diabetes.

Prediction of potential biological signaling pathways and potential therapeutic drugs

We investigated the potential biological signaling pathways linked with obesity, T2D, and AD using the FunRich bioinformatics tool (Figure 6A). Our results revealed that the critical biological pathways of obesity, T2D, and AD are involved in the immune system (17.9%), complement cascade (4.5), antigen presenting-cross presentation (9%), glypican pathway (19.4%), mechanistic target of rapamycin (mTOR) signaling pathway (17.9%), IFN-γ pathway (17.9%), vascular endothelial growth factor–vascular endothelial growth factor receptor (VEGF-VEGFR) signaling network (17.9%), mesenchymal-to-epithelial transition (14.9%), metabolism (14.9%), IFN-γ signaling (9%), ER-phagosome pathway (9%), interferon signaling (9%), endosomal-vacuolar pathway (6%), integrin-linked signaling pathway (10.4%), ALK1 pathway (6%), metabolism of non-coding RNA (1.5%), ATR (ataxia telangiectasia and Rad3-related) signaling pathway (7.5%), direct p53 effectors (6%), and IL12-mediated signaling pathway (6%).

It is critical to correlate protein-drug findings to understand better the structural properties of small molecules in receptor sensitivity. Using the EnrichR web server from the DSigDB database, the top 10 potential therapeutic compounds and significant DEGs interacting with them were identified based on *P*-value (*P* < .01). Predicated drug molecules were D-penicillamine CTD 00006475, beryllium sulfate CTD 00001005, isotretinoin PC3 UP, phencyclidine CTD 00005881, cyclophosphamide CTD 00005734, 5253409 MCF7 UP, primaquine CTD 00006613, nicardipine CTD 00006388, flupirtine CTD 00001630, and tamibarotene CTD 00002527 (Figure 6B).
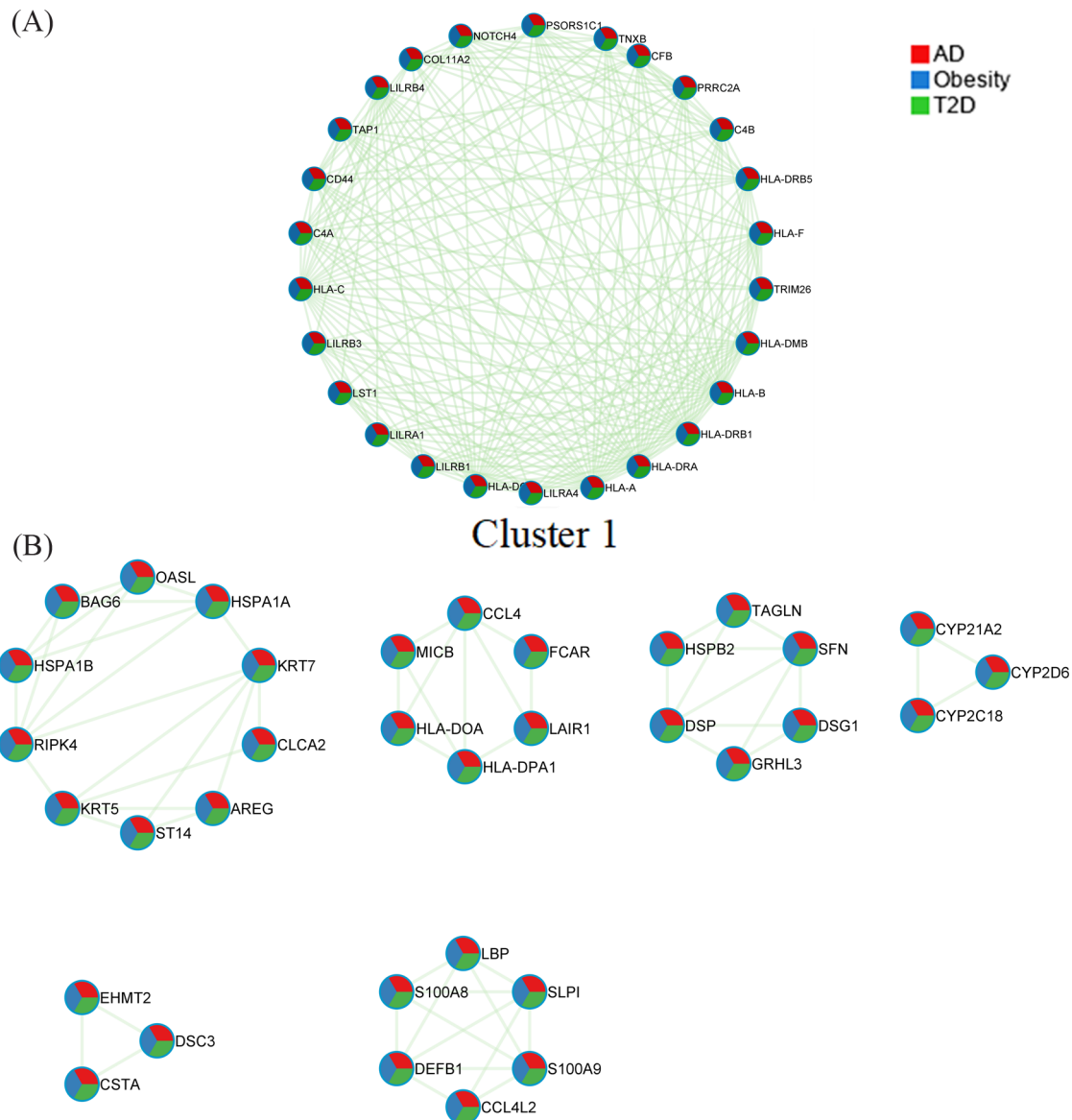
(A)

Cluster 1

(B)

Figure 3. (A) Construction protein-protein interaction (PPI) network of the mutual overlapping DEGs and cluster analysis. (B) Identification of 7 gene clusters using MCODE algorithm from mutual DEGs presented with obesity, T2D, and AD.
AD indicates Alzheimer disease; DEGs, differentially expressed genes; T2D, type 2 diabetes.

Discussion

Obesity and T2D are recognized to influence neurological disease but how they do their crosstalk during disease progression remains unclear, although specific vascular-based processes are frequently considered. We used bioinformatics methods and analytical approaches to examine functional disease overlaps in genes and pathways and identify potential therapeutic biomarkers involved in these comorbidity interactions among obesity, T2D, and AD. Using a network-based bioinformatics pipeline, we investigated RNA-seq data sets from publicly available repositories. We found mutual DEGs common to obesity, T2D, and AD and constructed diseasome networks to get insight into how these comorbidities interact using these DEGs. These DEGs facilitated the identification of connected dysregulated molecular pathways and related GO terms. We

analyzed regulatory patterns, molecular key pathways, PPI interactions, TF identification, upstream protein kinases, and potential biological pathways to look at differential genes expression of these 3 diseases and find these pathways and molecular signatures that could serve as possible treatment targets or biomarkers for obesity, T2D, and AD.

In this study, mutual DEGs from obesity, T2D, and AD were enriched in GO terms like IFN-γ-mediated signaling pathway, antigen processing, and presentation of endogenous peptide antigen via MHC class I via ER pathway, regulation of T cell-mediated cytotoxicity, regulation of immune response, antigen processing and presentation of exogenous peptide antigen via MHC class I, MHC class II receptor activity and TAP1 binding consistent with this result; it has been reported that the IFN-γ-mediated signaling pathway evokes several potentially

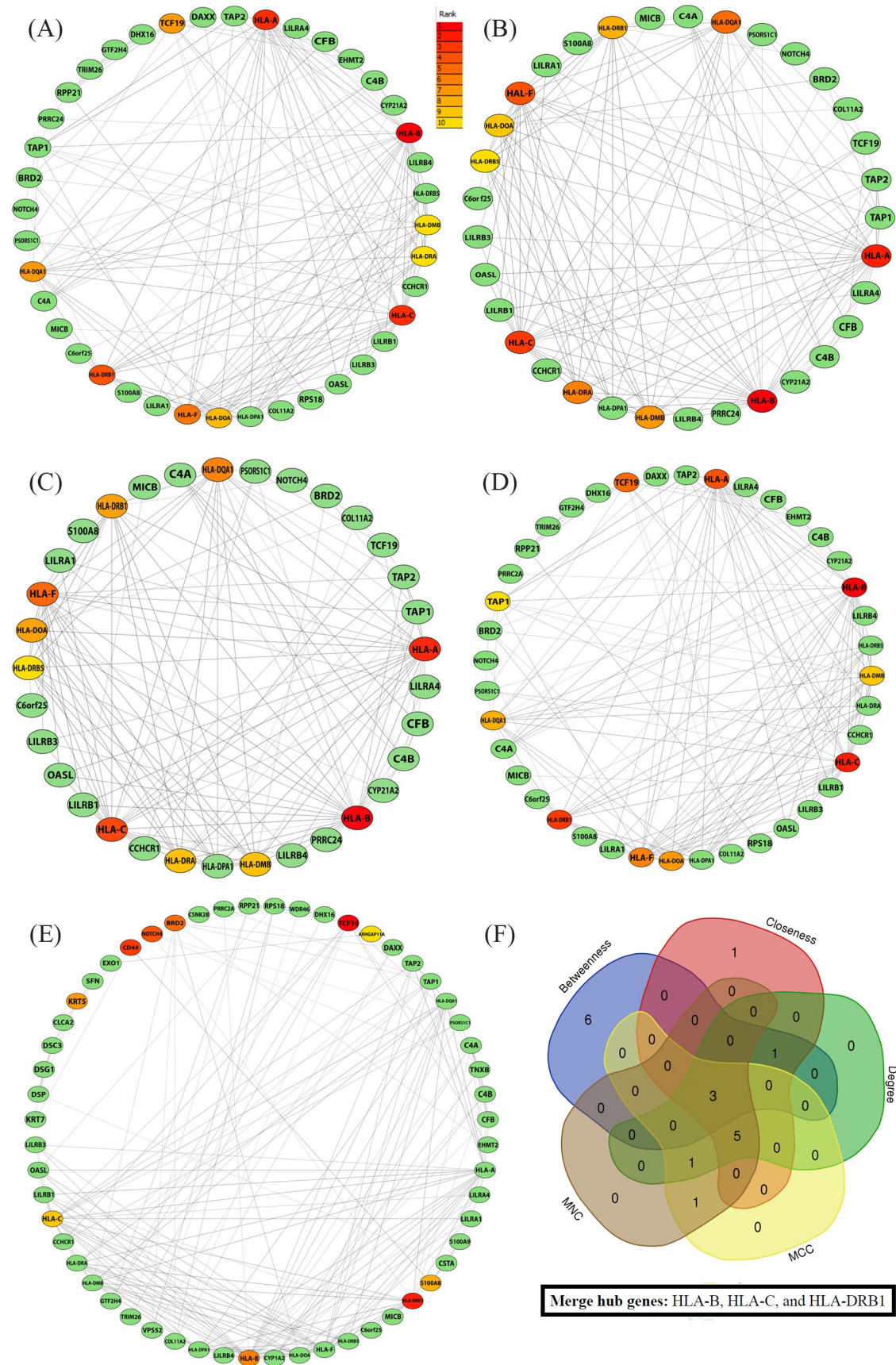**Figure 4.** Hub genes identification using the CytoHubba plugin algorithm. We employed different topological parameters for identifying hub genes, including (A) degree nodes, (B) MCC, (C) MNC, (D) closeness, and (E) betweenness. (F) Venn diagram represents commonly shared similar hub genes from MCC, MNC, degree nodes, closeness, and betweenness parameters.
MCC indicates maximal clique centrality; MNC, maximum neighborhood component.
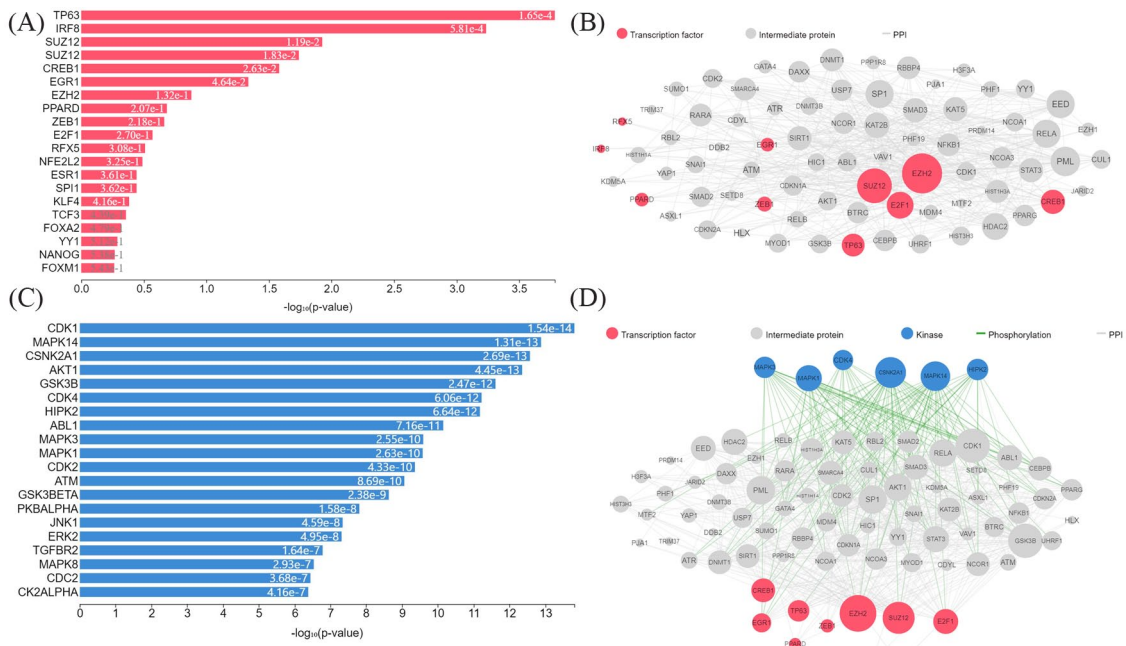
Figure 5. Transcriptional factors analysis and protein-protein network with transcription factors of mutual DEGs. (A) Identification of top 20 transcription factors between obesity, T2D, and AD based on hypergeometric *P*-value, (B) The G2N algorithm is used to observe the interaction of transcription factors with a known protein-protein network. Determination of kinase enrichment analysis of shared overlapping DEGs with transcription factors and protein-protein interaction network with phosphorylation. (C) Recognition of top 20 transcription factors from shared overlapping DEGs among AD, obesity, and T2D. (D) Upstream pathway involvement transcription factors to kinases through known protein-protein interactions of mutual overlapping DEGs. AD indicates Alzheimer disease; DEGs, differentially expressed genes; T2D, type 2 diabetes.
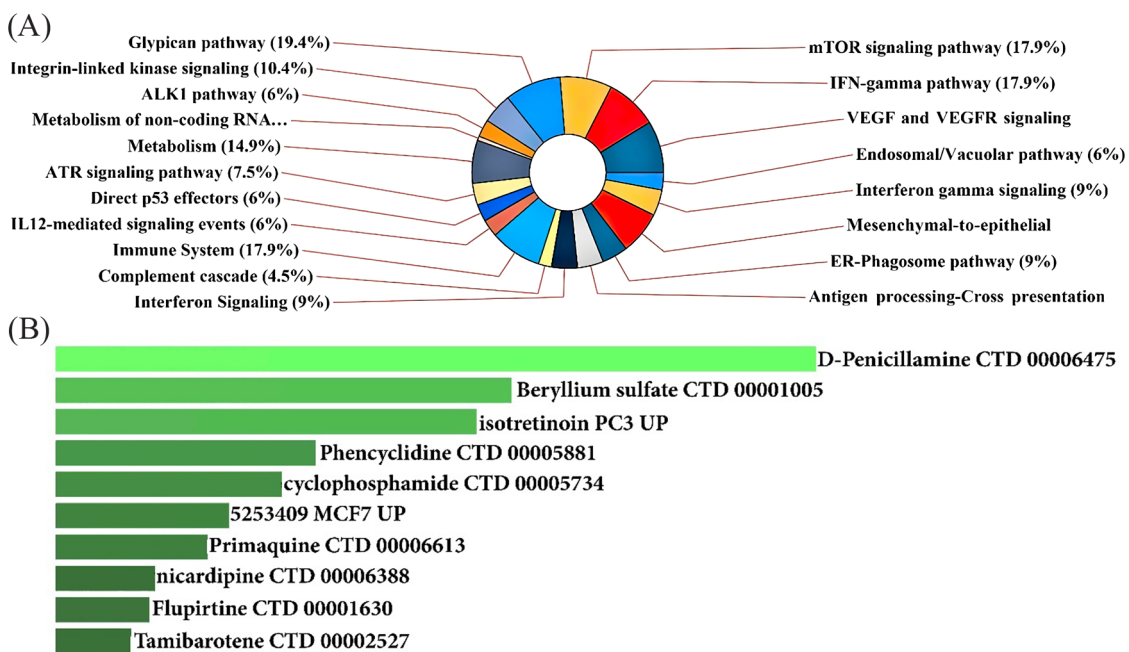
Figure 6. (A) Identification of potential biological pathways associated with pathological hallmark among AD, obesity, and T2D pathogenesis. (B) The potential drug candidates were recognized using the drug signature database (DSigDB).
AD indicates Alzheimer disease; T2D, type 2 diabetes.

contradicting effects in the context of a brain undergoing AD-related degeneration by apparently driving both disease-promoting disease-ameliorating functions.[36] The study reported that the expression levels of INF-γ are significantly reduced due

to T2D immune response.[37] However, it remains unclear how the INF-γ signaling pathway regulates immunity of T2D. Multiple putative pathways—including effects on adipogenesis, cytokine expression, and macrophage phenotype—have

been proposed for INF-γ in regulating inflammation and glucose homeostasis in obesity.[38] Based on our findings, we postulated that the INF-γ signaling pathway plays a critical in the disease progression of obesity, T2D, and AD. Gate et al[39] reported that T cells from AD patients were more clonally increased in cerebrospinal fluid analyses than T cells from healthy individuals, indicating an antigen-specific immune response in AD. It has reported that class II molecules are expressed on selected cells by pancreatic cells from diabetes mellitus donors, along with other critical genes in those pathways and inflammation-related genes.[40] Class II molecule expression in pancreatic cells suggests that they may interact directly with islet-infiltrating CD4+ T cells and have an immunopathogenic role. Obesity is characterized by chronic adipose tissue inflammation, which leads to obesity-induced insulin resistance. T cells pro-inflammatory activity is enhanced in human and mouse fat tissue.[41] These T cells secrete IFN-γ to activate macrophages. However, which key signal causes T-cell activation in adipose tissue is unclear. Deng et al[42] found that the expression of genes involved in MHC class II antigen presentation and processing was enhanced in adipocytes derived from biopsies of 44 obese women. Our findings suggested that improved MHC class II antigen presentation in adipocytes is linked with obesity, which leads to increased pro-inflammatory T-cell activity in adipose tissue. The GO results of our investigation suggested that the IFN-γ signaling pathway may be correlated with MHC class II antigen presentation and processing in the development of complex disease mechanisms of obesity, T2D, and AD.

Kyoto Encyclopedia of Genes and Genomes pathway analysis of commonly shared DEGs revealed that they were highly significantly involved in the AP-1 TF network, TLR signaling pathway, and IL12-mediated signaling pathway. Our results agreed with a previous study, which reported that miR-144 is a negative regulator of ADAM10, and AP-1 is involved in regulating miR-144 expression. Activator protein-1 may be activated by Aβ and is linked to AD pathogenesis.[43] Similarly, evidence reported that toll-like receptor 4 (TLR4)/AP-1 siRNA transfection attenuated systemic and hepatic inflammation, obesity, and insulin resistance caused by a high-fat diet.[44] The intricate crosstalk between the TLR, complement, and inflammasome signaling pathways has been demonstrated to the function of immunologic response in the brain, leading to neuroinflammation and Aβ accumulation in AD.[45] Toll-like receptor signaling pathway promotes unregulated adipogenesis and metaflammation, making it a potential therapeutic target for T2D and obesity.[46] It is worth noting that the IL-12 pathway is linked to inflammatory cascades, which are considered to play a crucial role in AD.[47] The regulation of IL-12 family cytokines in white adipose tissue (WAT) is influenced by the developmental stage of obesity as well as the inflammatory process associated with obesity.[48] Disruption of IL-12 stimulates angiogenesis, which protects tissues from prolonged

ischemia in T2D.[49] Both Reactome and BioCarta 2019 analysis revealed that mutual DEGs were enriched with inflammatory signaling pathways associated with MHC class II antigen presentation. Our pathway enrichment analyses suggested that the complicated interaction between obesity, T2D, and AD has shared pathomechanism immune-privileged dysfunction related to the inflammatory signaling pathway.

Through network-based analysis, we assessed overlapping DEGs from 3 RNA-seq data sets. Protein-protein interaction networks have been critical in understanding the shared etiology of obesity, T2D, and AD. The PPI network revealed top hub genes such as HLA-B (major histocompatibility complex, class I, B), HLA-C (major histocompatibility complex, class I, C), and HLA-DRB1 identification from merged 5 algorithms of CytoHubba results that shared the most interconnecting relationships with other DEGs in the network and then were associated with several pathways such as inflammation mediated by the immune response, T-cell transendothelial migration, infection, brain development, and plasticity.[50,51] Previously, it has been reported that *HLA-B* gene expression was observed in early and late-onset AD.[52] A meta-analysis of T2D genome-wide association studies in African Americans found a connection between polymorphism in the HLA-B (class I gene) with T2D.[53] A Mendelian randomization study observed HLA-B variation linked with obesity.[54] Individual immunoglobulin GM (γ marker) alleles, alone or in combination with a known HLA risk allele, were related to AD development.[55] Evidence reported that the development of autoimmune diabetes in adulthood and obesity interacts with HLA high-risk genotypes as well as genes linked to T2D.[56] Meta-analysis data revealed that a single-nucleotide polymorphism (SNP) rs9271192 within HLA-DRB1 has been recognized as a risk factor for the development of AD in genome-wide association studies (GWAS).[57] HLA-DRB1 protects against T2D by increasing self-tolerance and protecting against autoimmune-mediated insulin secretion suppression.[58] In the cohort study reported HLA-DRB1 significant interaction was observed in obesity, thus increasing the risk of developing multiple sclerosis.[59]

Transcription factors are essential regulators of their different target genes because their levels can be used to uncover possible common biomarkers for obesity, T2D, and AD. We identified important top 20 TFs as mutual DEG regulators in our work using TF-protein interaction networks connected to the pathophysiology of obesity, T2D, and AD. The interconnection of 20 TFs with 224 mutual DEGs has been identified as key in understanding pathogenesis, clinical functions, and potential therapeutic targets. This system biology research identified potential top 5 TFs, including TP63, SUZ12, CREB1, EGR1, and EZH2. Cluster analysis of GWAS results revealed novel SNPs proximal to the *TP63, EPHA4,* and *NXPH1* genes and supporting SNPs in APOE and TOMM40 as highly related to multiple brain regions in AD.[60] Sitai Liang

et al[61] discovered that TP63 is potentially involved as a novel candidate gene implicated in insulin receptor substrate 1 (IRS-1) regulation, which might be employed as a new therapeutic target to prevent diabetic kidney problems. Given a large number of genes downstream of p63 that might have a wide range of biological impacts, our understanding of p63 metabolic functions is rather limited. Lineage-dependent chromatin modification (SUZ12) observed dedifferentiation and neuronal repression in PSEN1 mutant hiPSC-derived neurons in AD.[62] The PRC2 (polycomb repressive complex 2) complex—which includes the subunits SUZ12, EZH2, RBBP4 (RbAp48), RBBP7 (RbAp46), and EED—is involved in histone methyltransferase activity associated with T2D and obesity transcriptional reprogramming.[63] The β-amyloid (Aβ) peptide, which plays a critical role in the pathogenesis of AD, alters hippocampal-dependent synaptic plasticity and memory and induces synapse loss via the CREB signaling pathway.[64] Ling Qi et al[65] reported that adipocyte CREB acts as an early detection indication in the development of T2D. Early growth response-1 (EGR1), a TF, may have a role in maintaining cholinergic function in the brain throughout the preclinical phases of AD.[66] In both mice and humans, EGR-1 expression in WAT was strongly linked to dietary-induced obesity and insulin resistance.[67]

Targeting-specific protein kinases may potentially help to prevent or delay the development of disease progression. Our KEA findings indicated the top 20 protein kinases from mutual 224 DEGs with a similar pathophysiological mechanism involved in obesity, T2D, and AD. Identification of protein kinases such as CDK1, MAPK14, CSNK2A1, AKT1, and GSK3B was enriched with common DEGs to regulate their expression. The agreement with previous scientific evidence demonstrated that kinases had been shown to have a role in AD progression, particularly the cell cycle regulatory kinase cyclin-dependent kinase 1 (cdk1).[68] Cyclin-dependent kinase 1 is identified in susceptible neurons in AD brains. It has been reported that the activation of mitochondrial respiratory complex I as a critical mediator of obesity-related metabolic remodeling in β-cells, and CDK1 as a complex I regulator that improves β-cell glucose sensing.[69] MAPK14 has primarily been considered an anti-inflammatory pathway to target innate immune responses in the AD brain,[70] notably microglial activation, and to attenuate inflammation-induced synaptic toxicity as the option for AD treatment target.[71] The considerably abnormal mitogen-activated protein kinase (MAPK) signaling and differential expression of the *MAPK14* gene in both insulin-sensitive organs revealed that the p38-MAPK-dependent pathway plays a shared role in T2D pathogenesis.[72] It has been demonstrated that CSNK2A1 is responsible for the direct phosphorylation of Ser7 and Ser9 of Presenilin-2 (PS-2), a protein involved in APP processing, and therefore is part of the γ-secretase complex.[73] The expression levels of CSNK2A1 were up-regulated in T2D and obesity.[74] The PI3K-Akt1

signaling pathway is a crucial modulator of insulin effects and is involved in T2D pathogenesis and AD.[75] The activation of AKT1 is impaired in insulin-resistant states such as obesity and T2D.[76] GSK3B promotes tau hyperphosphorylation, increased Aβ formation from APP (through β and γ secretase-mediated cleavage), and learning and memory deficits, as well as perhaps enhancing microglial-mediated inflammatory responses in the region of Aβ plaques.[77] GSK3 may have a role in obesity-induced inflammation. Also, GSK3 has a unique role in the involvement of macrophage polarization and its therapeutic potential for obesity-induced inflammation and diabetes mellitus.[78]

We have suggested potential drug molecular substances like D-penicillamine, beryllium sulfate, isotretinoin, phencyclidine, cyclophosphamide, primaquine, nicardipine, flupirtine, and tamibarotene as a possible treatment for AD linked with T2D of obesity. Our predicted also supported by existing clinical trials and pharmacologic studies. A clinical trial revealed that D-penicillamine reduces oxidative stress in AD patients.[79] D-penicillamine has been linked to various immunologic abnormalities, including the production of insulin autoantibodies.[80] Isotretinoin therapy is an option for treating T2D.[81] However, the mechanism of action of isotretinoin remains unclear for the treatment of T2D pathogenesis. Tamibarotene is considered a promising drug candidate for treating AD because of its transcriptional regulation of several target genes implicated in the genesis and pathophysiology of AD.[82] Nicardipine, which was well tolerated, appears to be a promising therapy choice for T2D individuals with moderate essential hypertension.[83] Our anticipated candidate therapeutic molecules are a possible effective pharmacologic target for obesity, T2D, and AD. At the same time, further large-scale research and head-to-head trials are needed to fully understand their mechanism of action and clinical importance.

This study used a bioinformatics technique to investigate gene expression transcriptome patterns to find potential biomarkers that might shed light on crucial pathobiological pathways regulating obesity, T2D, and AD. The common responsive gene among obesity, T2D, and AD was uncovered using overlap, core connection, and gene filtering. We subsequently identified signaling pathways and GO processes, ultimately constructing a PPI network for common DEGs. Furthermore, we used transcriptional analysis to determine TFs and TF-protein interactions network and suggest prospective therapeutic drugs. As a result, our approach will assist in improving the decision-making process in the field of personalized health care.

Author Contributions

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