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Research Article

One path, two solutions: Network-based analysis identifies targetable pathways for the treatment of comorbid type II diabetes and neuropsychiatric disorders

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ABSTRACT

Comorbid diseases complicate patient outcomes and escalate healthcare costs, necessitating the need for a deeper mechanistic understanding. Neuropsychiatric disorders (NPDs) such as Neurotic Disorder, Major Depression, Bipolar Disorder, Anxiety Disorder, and Schizophrenia significantly exacerbate Type 2 Diabetes Mellitus (DM2), often leading to suboptimal treatment outcomes. The neurobiological mechanisms underlying this comorbidity remain poorly understood. To address this gap, we developed a novel pathway-based network computational framework to identify critical shared disease mechanisms between DM2 and these five prevalent comorbid NPDs. Our approach involves reconstructing an integrated DM2 ∩ NPDs KEGG pathway-pathway network and employs two complementary analytical methods, including the "minimum path to comorbidity" method to identify the shortest path fostering comorbid development. This analysis uncovered shared pathways like the PI3K-Akt signaling pathway and highlighted key nodes such as calcium signaling, MAPK, estrogen signaling, and apoptosis pathways. Dysregulation of these pathways likely contributes to the development of DM2-NPDs comorbidity. These findings have significant clinical implications, as they identify promising therapeutic targets that could lead to more effective treatments addressing both DM2 and NPDs simultaneously. Our model not only elucidates the intricate molecular interactions driving this comorbidity but also identifies promising therapeutic targets, paving the way for innovative treatment strategies. Additionally, the framework developed in this study can be adapted to study other complex comorbid conditions, advancing personalized medicine for comorbidities and improving patient care.

1. Introduction

Comorbidity, defined as the presence of two or more diseases in the same individual, is associated with worse patient outcomes, more complicated treatments, and increased healthcare costs [1,2]. Understanding the etiology of comorbid diseases is essential for effective treatment and the prevention of their emergence.

Type 2 Diabetes mellitus (DM2) is a chronic metabolic disorder affecting approximately 6.28 % of the world's population, corresponding to 462 million people [3], with projections indicating a rise to 700 million by 2045 [4]. Characterized by insulin resistance and hyperglycemia, DM2 accounts for 90 % of all diabetes cases [5]. It occurs when pancreatic islet β cells fail to produce sufficient insulin to maintain normal glucose metabolism [6,7]. The origin of DM2 is multifactorial, involving the complex interaction of genetic, environmental and lifestyle factors [8]. While DM2 most often develops in people older than 45, its incidence is increasing among younger populations due to obesity, increased food intake, and lack of physical activity [9,10]. The risk of DM2 increases with age, with approximately one-third of the individuals suffering from DM2 being above 50 years old [3].

DM2 patients frequently experience emotional and behavioral symptoms, such as anxiety and depression, which exacerbate disease severity [11]. There is a bidirectional relationship between DM2 and depression, with each condition increasing the risk of the other [12].

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Moreover, individuals with bipolar disorder (BD) [13] and schizophrenia [14] have a three- to fivefold times higher risk of developing comorbid DM2. Current evidence suggests a notably high prevalence of comorbidity between DM2 and neuropsychiatric disorders (NPDs), with around 40 % of patients with DM2 also suffering from a non-psychotic psychiatric disorder [15]. Common NPDs in DM2 include anxiety disorders (20–25 %), major depressive disorder (MDD) (10–30 %), schizophrenia (10 %) and BD (10 %) [15–19]. Comorbid NPDs in DM2 patients are linked with impaired quality of life [20], reduced treatment adherence [21], decreased glycemic control control [22], and poorer treatment prognosis [23,24].

Despite recognition of DM2-NPDs comorbidity, the underlying mechanisms remain unclear. No pharmacological interventions specifically target this comorbidity, and some treatments for DM2 can exacerbate NPDs and vice versa [25–28]. Understanding these mechanisms is crucial. Additionally, there is a lack of transcriptomic data from human patients and animal models with comorbid DM2 and NPDs in public repositories like ArrayExpress [29] and Gene Expression Omnibus (GEO) [30], making it difficult to study these mechanisms. This gap necessitates alternative approaches, such as computational modeling and pathway-based analyses. Our study addresses this issue by employing network-based approaches to investigate the comorbidity between DM2 and NPDs, including MDD, Neurotic Disorder (ND), Anxiety Disorder (AD), BD, and Schizophrenia. Unlike previous studies that focused on isolated conditions, our research provides novel insights into shared molecular pathways and potential therapeutic targets for this specific set of comorbidities.

Network-based approaches, including protein-protein interaction (PPI) networks, have been instrumental in unravelling shared pathological mechanisms between comorbid diseases [31–33]. Studies by Barabasi on network medicine and Brunak on integrated molecular networks have advanced our understanding of molecular interactions in human diseases [34,35]. Recent research has combined disease networks with predictive modeling to better understand chronic diseases and comorbidities [36], while another study used network analysis on electronic medical records of 496,408 DM2 patients to identify core diseases and trends [37]. However, few studies have focused on the comorbidity between DM2 and NPDs, such as MDD, ND, AD, BD, and Schizophrenia.

Previous research primarily explored DM2 comorbidities with

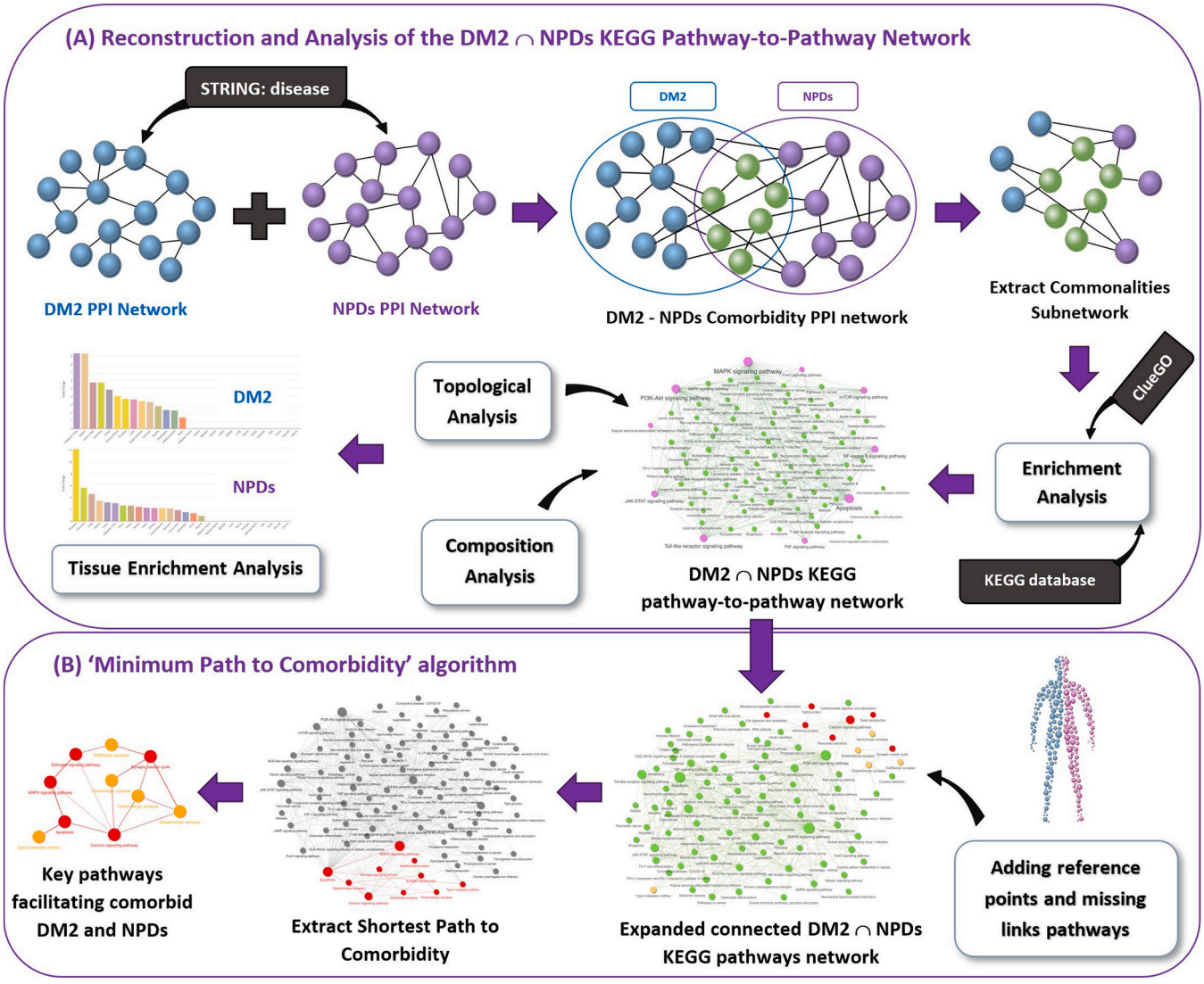

Fig. 1. Schematic illustration of the methodology used in this paper. The aim was to isolate key pathways contributing to the development of comorbid DM2 and NPDs. (A) We represent the various data sources and the methodology used to reconstruct and analyze the DM2 ∩ NPDs KEGG pathway-pathway network. Topological analysis was employed to identify the top 10 high centrality pathways, and composition analysis was performed to determine the subclass to which the common pathways belong. (B) We also devised the 'minimum path to comorbidity' method, which allows us to isolate the shortest path that facilitates the development of comorbid DM2 and NPDs.

individual disorders, such as Alzheimer's Disease [33,38], or studied NPDs in isolation, such as with Schizophrenia [39], using transcriptomic and network-based approaches. Network-based methods have also been used to study NPDs, unravelling pathogenic mechanisms [40] and identifying potentially drug treatments [41]. Pathway-based analyses of large-omics data have further advanced our understanding of complex diseases [42]. Bioinformatics and machine learning have also been applied to find molecular biomarkers for DM2 with neurological diseases [43], and study the effects of central nervous system disorders on glioblastoma progression [44]. However, there is a need for more comprehensive approaches that examine a wider range of NPDs in combination with DM2.

Here, we developed and applied a pathway-based network computational framework (see Fig. 1) to uncover key pathological mechanisms contributing to the comorbidity of DM2 and NPDs. To the best of our knowledge, no prior studies have used network-based approaches specifically to study the emergence of this comorbidity. This study makes several significant contributions: First, we take a comprehensive approach by concurrently analyzing the shared pathophysiological mechanisms between DM2 and five prevalent NPDs (ND, MDD, AD, Schizophrenia and BD), offering new insights into the molecular interactions that drive these comorbid conditions. We reconstructed and analyzed five DM2-NPDs comorbidity PPI networks to identify common disease pathways shared between DM2 and all five NPDs. The shared pathways between DM2 and these NPDs were then used to reconstruct the DM2 ∩ NPDs KEGG pathway-pathway network. Next, we employed two complementary strategies to analyze this network. The first strategy identified the top 10 high-centrality pathways that could potentially exert system comorbid effects. The second strategy introduced the 'minimum path to comorbidity' method, which uses graph theory techniques to isolate the shortest path that might facilitate the development of comorbid DM2 and NPDs. This approach highlighted key disease pathways that functionally interact and are in close proximity with the reference pathways representing DM2 and NPDs, suggesting that targeting these pathways pharmacologically could have a significant therapeutic impact. These contributions collectively enhance the understanding of DM2-NPD comorbidities and open new avenues for therapeutic development, offering potential treatment strategies based on the identified pathways.

The paper is organized as follows: The Methods section provides a detailed explanation of the network-based approaches and data sources used in the study. The Results section presents key findings, including shared pathways and potential therapeutic targets. The Discussion interprets these findings within the context of current literature and outlines possible implications for treatment. Lastly, the Conclusion summarizes the study's contributions and suggests directions for future research.

Overall, this study advances our understanding of DM2-NPD comorbidity by developing a novel computational framework, identifying crucial shared molecular pathways, and suggesting potential therapeutic targets that could be explored for treating these interconnected conditions.

2. Methods

2.1. Reconstruction of the integrated DM2-NPDs comorbidity PPI networks

To identify common pathological mechanisms between DM2 and each of the five NPDs, we reconstructed five DM2-NPDs comorbidity PPI networks (Table 1). Following established studies [40, 45–48], we utilized the *STRING disease* app, integrated within Cytoscape app [49], to collect the top 200 disease-associated proteins, ranked by the highest disease association score, for each of the six conditions: DM2 (DOID:9352), MDD (DOID:1470), ND (DOID:4964), AD (DOID:2030), BD (DOID:3312), and Schizophrenia (DOID:5419). The *STRING disease* app sources its data from the DISEASES database [50,51], which collects gene-disease associations from various types of evidence, including automatic text mining, a rigorously maintained and frequently updated resource that integrates that integrates gene-disease associations from multiple evidence types, including automatic text mining, manually curated databases like UniProt Knowledgebase (UniProtKB), genome-wide association studies (GWAS), and cancer mutation data. The DISEASES database is updated weekly, ensuring that the data we used are both current and robust. The gene-disease associations are then unified and assigned a confidence score, with 5 stars indicating high confidence and 1 star indicating low confidence in the association being a true positive. By focusing on the top 200 high ranking disease-associated proteins, we prioritize those proteins strongly implicated in the diseases of interest, facilitating a more precise identification of biological processes closely associated with disease progression [52]. This method ensures that our analysis is based on high-confidence data, allowing us to accurately explore the common pathological mechanisms underlying comorbid DM2 and NPDs.

Table 1
Characteristics of the DM2-NPDs comorbidity PPI networks and commonalities subnetworks.

DM2-NPDs comorbidity PPI networks	Nodes	Edges	Common disease-associated proteins (DM2 ∩ NPDs)	Commonalities subnetwork human proteins
DM2 and AD	368	1610	32	197
DM2 and MDD	381	1558	19	177
DM2 and Schizophrenia	379	1610	21	181
DM2 and BD	381	1588	19	170
DM2 and Neurotic Disorder	360	1531	40	206

To reconstruct the five DM2-NPDs comorbidity PPI networks, we merged the DM2 PPI network with each of the five NPDs PPI networks using the "merged" function in Cytoscape app [53] (see Fig. 1A). The confidence cut-off score for the PPI was set at 0.8. This score is determined based on the nature and quality of the supporting evidence for the PPIs, ranging from 0 (indicating low confidence) to 1.0 (indicating high confidence). Therefore, the higher the score, the greater the likelihood that the PPIs are true positives [54]. It's worth noting that a recommended cut-off for high confidence is above 0.7 [55]. As a result, a stronger cut-off of 0.8 was selected.

2.2. Commonalities subnetwork extraction and enrichment analysis

We, then extracted the commonalities subnetwork from each DM2-NPDs comorbidity PPI network. These subnetworks include common disease-associated proteins between DM2 and each NPD, along with their first neighbors. The number of common disease-associated proteins between DM2 and each NPDs found from each comorbidity network, along with the number of human proteins contained in each extracted commonalities subnetwork, is listed in Table 1. Using the isolated human proteins from each subnetwork, we then performed enrichment analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [56]. Pathway enrichment analysis allows us to gain mechanistic insight associated with a list of proteins [57]. Enrichment analysis was conducted in the ClueGO app [58] in Cytoscape, utilizing the KEGG database. Only statistically significant enriched terms with an adjusted p-value ≤ 0.05 (corrected with Bonferroni step-down) were retained.

2.3. Reconstruction and analysis of the DM2 ∩ NPDs KEGG pathway-pathway network

2.3.1. Integrated DM2 ∩ NPDs KEGG pathway-pathway network

To identify common pathological pathways between DM2 and all the

NPDs included in our analysis, we reconstructed an integrated DM2 ∩ NPDs KEGG pathway-pathway network. In this network, nodes represent pathways, and edges denote functional relationships between these pathways. To reconstruct the integrated network, we first compared the isolated significantly enriched KEGG pathways found between DM2 and each of the five NPDs using enrichment analysis. This allowed us to identify the KEGG pathways involved in DM2 that are also found in all five NPDs. Overall, we identified 87 KEGG pathways shared between DM2 and all five NPDs.

To ensure that the identified 87 common pathways do not contain any false positive results of pathways containing only DM2- or NPD-associated genes, we checked that all the pathways contained at least one disease-associated gene for each condition. Using this method, we did not identify any false positive results, as all of the 87 common pathways were found to contain at least two genes associated with each condition.

To reconstruct the integrated DM2 ∩ NPDs KEGG pathway-pathway network and create functional interactions between the 87 common pathways, we utilized the KEGGREST package [59] in R to parse the KEGG database [56]. We parsed each of the 353 KEGG pathways (Homo sapiens) entries to extract information of the functional relationships of each pathway with other pathways. We then combined all the interactions obtained, which resulted into 1914 functional pathway-pathway interactions. Functional relationships between pathways, represent the interconnectedness and communication that occurs between different pathways to accomplish complex physiological processes, such as where components from one pathway influence the activity or regulation of components in another pathway. We then isolated the functional relationships between the 87 common pathways and reconstruct a direct DM2 ∩ NPDs KEGG pathway-pathway network, composed of 87 nodes and 328 edges. Therefore, these 87 pathways represent shared biological mechanisms that concurrently play a role in both diseases, with the edge interactions denoting functional relationships between the pathways.

2.3.2. Pinpointing essential comorbidity disease communicator nodes

After reconstructing the DM2 ∩ NPDs KEGG pathway-pathway network, we used the igraph package [60] in R to conduct network topological analysis and identify high centrality pathways. Specifically, we used the 'hub_score' function within igraph to assess node centrality, focusing on those nodes that act as hubs within the network. The 'hub_score' function measures how well-connected a node is to other highly connected nodes, assigning higher scores to nodes that are linked to multiple hubs or nodes with high degrees, indicating their pivotal role in the network. Nodes with high hub scores are crucial because they have extensive connections and interact with other central nodes, making them key facilitators of information flow and interaction within the network. By applying the 'hub_score' function, we identified nodes that are central to the network's structure and functionality. These high centrality nodes, or hubs, are crucial for understanding the comorbidity between DM2 and NPDs. They play significant roles in the systemic interactions driving the emergence of comorbid conditions by functionally connecting with multiple pathways. Consequently these hubs act as key "comorbidity disease communicator nodes" in the network [45]. The term "comorbidity disease communicator nodes" refers to nodes that are crucial for bridging different pathways related to DM2 and NPDs, thereby facilitating and amplifying interactions that contribute to the comorbidity between these diseases. These nodes are essential in both promoting the emergence and deepening the understanding of comorbid DM2 and NPDs. To pinpoint these critical nodes, we focused on the top 10 nodes with the highest hub scores, which serve as indicators of high centrality. These nodes are integral to the network's functionality and offer valuable insights into the mechanisms underlying the comorbidity between DM2 and NPDs, as well as potential therapeutic targets.

2.4. Composition analysis and tissue-specificity enrichment analysis of DM2-NPDs interactions

Additionally, we conducted composition analysis on the DM2 ∩ NPDs KEGG pathway-Comorbidity, defined as the presence of two or more diseases in the same individual, is associated with worse patient outcomes, more complicated treatments, and increased healthcare costs [1,2]. Understanding the etiology of comorbid diseases is essential for effective treatment and the prevention of their emergence. network to determine the subclasses to which the 87 common disease pathways belong. To achieve this, we used the KEGGREST package [59] in R to extract the subclass classification of each of the 87 pathways based on the KEGG database. This analysis offers valuable insights into the categorization of pathways and their functional relevance in the context of comorbid DM2 and NPDs.

Moreover, in order to identify potential overlapping tissues where the dysregulation of the 87 identified pathways could occur between DM2 and NPDs, we performed tissue-specific gene enrichment analysis. This analysis involved using the disease-associated genes identified to participate in these pathways from each condition. We performed the analysis using the TissueEnrich web application [61], and chose the GTEx database [62]. The GTEx database provides the most comprehensive information on normal tissue expression across 56 distinct tissues. It is important to note that in the TissueEnrich application, samples from sub-tissues are combined into broader categories. For example, different regions of the brain are grouped under the term "brain", resulting in 29 human tissue categories. To identify tissue-specific genes, we applied the "tissue enriched" criterion, which defines genes as tissue-specific if their expression levels are at least five times higher in a particular tissue compared to all other tissues. In addition, we employed the fold-change test to determine the statistical significance of the tissue-specific genes.

2.5. The 'minimum path to comorbidity'

To isolate the shortest path that may facilitate the development of comorbid DM2 and NPDs, we developed a method termed the 'minimum path to comorbidity' (see Fig. 1B). This approach leverages graph theory methods, specifically the shortest path algorithm, to identify key routes that contribute to the emergence of comorbid DM2 and NPDs. The 'minimum path to comorbidity' is designed to identify the most direct and influential pathways that may play a role in the development of comorbid DM2 and NPDs. To illustrate this concept, think of it as finding the shortest and most efficient route on a map connecting two cities, where each city represents a disease (DM2 and NPDs), and the roads between them represent biological pathways. The 'minimum path' is the most direct route connecting the key pathways of both diseases, emphasizing the crucial molecular interactions that are most likely to contribute to the comorbidity. By focusing on this minimum path, we can pinpoint critical pathways that could represent therapeutic targets to address both DM2 and NPDs simultaneously, thereby reducing the risk or severity of comorbid DM2 and NPDs.

2.5.1. Highlighting and adding missing reference points on the DM2 ∩ NPDs KEGG pathway-pathway network

To determine the "minimum path to comorbidity" between DM2 and NPDs, we first selected five KEGG pathways as reference points: (i) the Type II diabetes mellitus (hsa04930) pathway, representing DM2, and (ii) four KEGG pathways—Dopaminergic synapse (hsa04728), Glutamatergic synapse (hsa04724), Serotonergic synapse (hsa04726), and GABAergic synapse (hsa04727)—representing NPDs. The selection of these pathways was guided by several considerations. Firstly, specific disease pathways for the five NPDs under investigation (MDD, BD, ND, AD, and Schizophrenia) are not available in the KEGG database. This limitation required a strategic approach to accurately represent the molecular mechanisms underlying NPDs.

We selected these four pathways to collectively represent the NPDs reference point "pathway," because they are widely recognized in the literature as playing critical roles in the pathophysiology of all the NPDs studied [63–66]. These pathways were not selected arbitrarily; they are fundamental to neuropsychiatric function and are directly targeted by the pharmacological treatments currently employed for these disorders. For example, selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine and sertraline, are commonly prescribed for the treatment of MDD, BD, ND and AD, specifically targeting the serotonin system. Similarly, the antidepressant ketamine, used for treatment-resistant depression, modulates glutamate neurotransmission, highlighting the importance of the Glutamatergic synapse pathway [67,68]. Benzodiazepines like lorazepam, which enhance GABAergic activity in the brain, are widely used to treat MDD, ND and AD [69]. Furthermore, atypical antipsychotic drugs such as risperidone affect both the dopaminergic and serotonergic systems and are used to treat BD, MDD and Schizophrenia [70]. These pathways were selected as reference points not only because of their central role in neurotransmission but also because of their direct involvement in the therapeutic mechanisms of action for these NPDs. This selection process allows us to overcome the absence of specific KEGG pathways for NPDs by focusing on pathways that are biologically and therapeutically relevant, ensuring that our network analysis is rooted in robust biological evidence.

The pathways serve as anchors in our "minimum path to comorbidity" analysis, facilitating the identification of the shortest and most biologically significant routes that connect the reference points for DM2 and NPDs. This approach enables us to explore potential mechanisms of comorbidity and identify key molecular interactions that could serve as therapeutic targets, making our study both data-driven and biologically meaningful.

Our approach works by first highlighting the selected reference points on the DM2 ∩ NPDs KEGG pathway-pathway network in yellow. However, only two reference points, the Dopaminergic synapse and Type II diabetes mellitus pathways, were initially present on the network. The absence of the other reference points highlights a limitation of enrichment analysis. Despite the critical roles of Glutamatergic, Serotonergic, and GABAergic synapse pathways in the pathogenesis of these NPDs, they were not identified as statistically significant during the enrichment analysis. To overcome this limitation, our methodology adds the three missing reference points (Glutamatergic synapse, Serotonergic synapse, and GABAergic synapse) to the network. To add the missing reference points, the algorithm calculates all the shortest paths between the reference points and all the nodes on the DM2 ∩ NPDs KEGG pathway-pathway network, using the functional relationships of all 353 KEGG pathways collected from KEGG database. It then identifies the shortest path with the smallest length for each missing reference point and extracts the relevant edge interactions between the reference points and the pathways on the network.

The shortest path between two nodes in the network is calculated as follows:

$$d(v_i, v_j) = \min\left(\sum_{e \in E} length(e)\right)$$

where v_i and v_j represent nodes (pathways in the network), E is the set of all edges (pathways functional interactions) in the network, and $length(e)$ represents the weight or distance of each edge e. The min function identifies the path with the smallest total length, representing the shortest route between v_i and v_j.

In the case where no direct interactions exist between the missing reference points and the nodes on the network, the algorithm introduces additional missing nodes and edge interactions that are required to connect the missing reference points with the rest of the network to ensure connectivity. In addition, if multiple shortest paths with the smallest length exist, the algorithm adds the interactions for all of these paths to the network.

2.5.2. Adding missing pathways on the expanded DM2 ∩ NPDs KEGG pathway-pathway network

Moreover, the algorithm aims to create a fully connected network, where all nodes are connected with each other, so it identifies all nodes with degree values of 0 and 1 and determines all pairs that exist between these nodes and the remaining nodes on the expanded network.

The degree of a node in the network is calculated as:

$$\deg(v) = \sum_{e \in E} \mathbb{I}(v \in \quad e)$$

where $\deg(v)$ represents the degree of node v, and $\mathbb{I}(v \in e)$ is an indicator function that equals 1 if the node v is part of edge e (i.e., if there is a direct interaction between v and another node), and 0 otherwise. This summation counts the number of edges incident to node v.

It then calculates all the shortest paths between the pairs using the functional relationships collected from KEGG database. For each node of interest, the algorithm isolates the shortest path with the smallest length. Finally, the algorithm extracts and adds the relevant edges and missing nodes to create a fully connected expanded DM2 ∩ NPDs pathway-pathway network. The reliability of our graph expansion approach in introducing relevant comorbidity disease pathways in a Disease ∩ Disease KEGG pathway-pathway network is further analyzed in Supplementary File 1.

2.5.3. Isolating the minimum path to DM2 and NPDs comorbidity

To identify the key pathways that facilitate the development of comorbid DM2 and NPDs, we utilized the connected expanded DM2 ∩ NPDs KEGG pathway-pathway network to isolate the minimum path to DM2 and NPDs comorbidity. This process involved calculating the shortest paths with the smallest length for each of the four pairs that exist between the Type II diabetes mellitus pathway (DM2 reference point) and each of the four pathways (Dopaminergic, Glutamatergic, Serotonergic and GABAergic synapses) that represent the NPDs reference points.

The shortest path for each pair was computed as:

$$\text{min\_path}(P_{DM2}, P_{NPD}) \quad = \min(d(P_{DM2}, P_{NPD})$$

where P_{DM2} represents the Type II diabetes mellitus pathway, and P_{NPD} represents one of the four NPD pathways (Dopaminergic, Glutamatergic, Serotonergic, or GABAergic synapse). $d(P_{DM2}, P_{NPD})$ is the shortest path (distance) between the DM2 pathway and the NPD pathway.

We then highlighted the corresponding edges on the fully connected expanded DM2 ∩ NPDs pathway-pathway network. This enabled us to isolate the minimum path between DM2 reference point and the four NPDs reference points.

2.6. Validation of the importance of the central and "shortest path to comorbidity" pathways in DM2 and NPDs comorbidity using prefrontal cortex data

To validate the importance of the central and "shortest path to comorbidity" pathways in the context of DM2 and NPDs comorbidity, we conducted an analysis using microarray gene expression data from the prefrontal cortex (PFC). We accessed this data through the GEO database, a repository with transcriptomic data [30]. We sought PFC microarray studies associated with NPDs, DM2, and comorbid DM2 and NPDs. Unfortunately, there were no available datasets for PFC samples from humans with DM2 or datasets from either animal models or humans with comorbid DM2 and NPDs. However, we identified dataset GSE34451, which includes three samples from the PFC of male Goto-Kakizaki rats (a model of Type II diabetes) and three samples from male Wistar rats (control) [71]. Additionally, we analyzed dataset GSE12654, which contains human PFC samples from patients with various NPDs [72].

The Limma R package [73], which allows for the identification of differentially expressed genes (DEGs) from microarray experiments, was used to analyze each dataset and identify DEGs between each condition and the control samples. Both datasets were normalized and $\log_2$ transformed. DEGs with p-value < 0.05 were considered as statistically significant. Subsequently, pathway enrichment analysis was performed using the ClueGO app [58] in Cytoscape, leveraging the KEGG database, to identify statistically significant enriched pathways with an adjusted p-value ≤ 0.05 (corrected with Benjamini-Hochberg) that are related to the DEGs.

3. Results

3.1. Comparison between the five NPDs

Comparison between the top 200 disease-associated proteins of the five NPDs (see Methods) using a Venn diagram [74] revealed that they share 43 common disease-associated proteins (Fig. 2). Table 2 indicates the major biological processes to which the disease-associated proteins of the NPDs belong.

Table 2
Major biological processes in which the common disease-associated proteins of NPDs participate.

Biological Process	NPDs common disease-associated proteins
Serotonergic neurotransmission	5-hydroxytryptamine receptor 1 A (HTR1A), 5-hydroxytryptamine receptor 2 A (HTR2A), Tryptophan 5-hydroxylase 1 (TPH1), Sodium-dependent serotonin transporter (SLC6A4), 5-hydroxytryptamine receptor 1B (HTR1B), 5-hydroxytryptamine receptor 2 C (HTR1C)
Dopaminergic neurotransmission	Monoamine oxidase A(MAOA), D2 dopamine receptor (DRD2), Dopamine D4 receptor (DRD4)
Glutaminergic neurotransmission	Glutamate receptor ionotropic, kainate 2 (GRIK2), Glutamate receptor ionotropic NMDA 2 A (GRIN2A), Glutamate receptor ionotropic, NMDA 2B (GRIN2B), Glutamate decarboxylase 2 (GAD2)
Inflammation	Corticotropin releasing hormone (CRH), Interleukin 10 (IL10), Tumor Necrosis Factor (TNF), Corticotropin releasing hormone receptor 1 (CRHR1), Interleukin−1 beta (IL1B), Glucocorticoid receptor (NR3C1) and Interleukin−6 (IL6)
Neurodegeneration	Apolipoprotein E (APOE), Microtubule-associated protein (MAPT)
Diabetes	Insulin (INS)

3.2. Analysis results of the DM2 ∩ NPDs KEGG pathway-pathway network

We reconstructed and analyzed the DM2 ∩ NPDs KEGG pathway-pathway network (see Fig. 3), which consists of 328 functional relationships (edges) between the 87 common pathways (nodes) identified through our analysis to be shared between DM2 and all NPDs.

3.2.1. Composition of the DM2 ∩ NPDs KEGG pathway-pathway network

The composition analysis of the DM2 ∩ NPDs KEGG pathway-pathway network indicated that the 87 common pathways belong to 21 subclasses (Table 3), according to the KEGG database classification system. The results revealed that 23 pathways belong to the subclass of infectious diseases (viral, bacterial and parasitic), while 10 pathways belong to the endocrine system subclass. In addition, 11 pathways were classified under the signal transduction subclass, and 12 pathways were associated with various cancer subclasses.

3.2.2. Comorbidity (DM2 ∩ NPDs) disease communicator nodes

Topological analysis of the network led to the identification of the top 10 hubs nodes (Fig. 3, Table 4), which are high-centrality nodes that communicated with several of the other common disease pathways in the network. Therefore, these pathways, acting as potential comorbidity disease communicator nodes, are hypothesized to play a crucial role in facilitating the comorbidity between DM2 and NPDs. Their centrality in the network suggests they are essential due to their high connectivity and potential systemic influence.

The rationale behind identifying 'communicator nodes' within our network stems from the need to prioritize and understand the most influential pathways among the numerous shared ones. While all 87

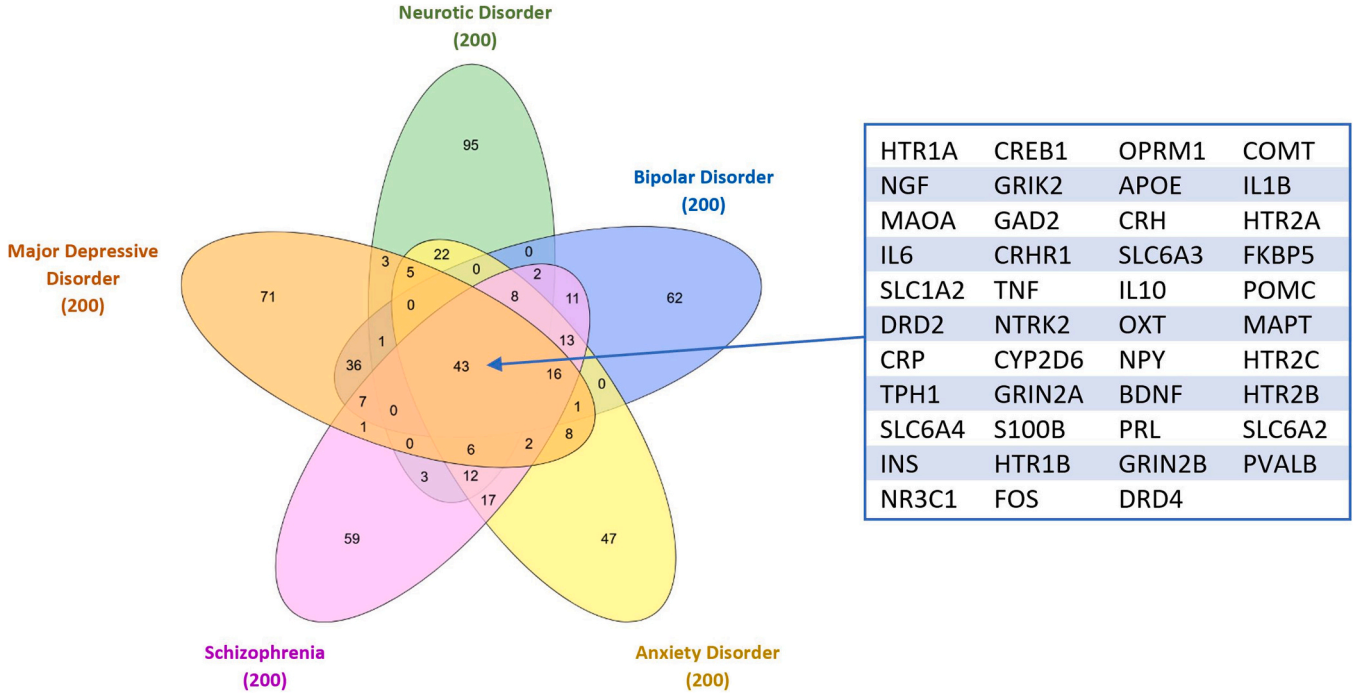

Fig. 2. Comparison of the top 200 disease-associated proteins among the five NPDs (Schizophrenia, MDD, ND, AD and BD), indicating the presence of 43 common disease proteins.

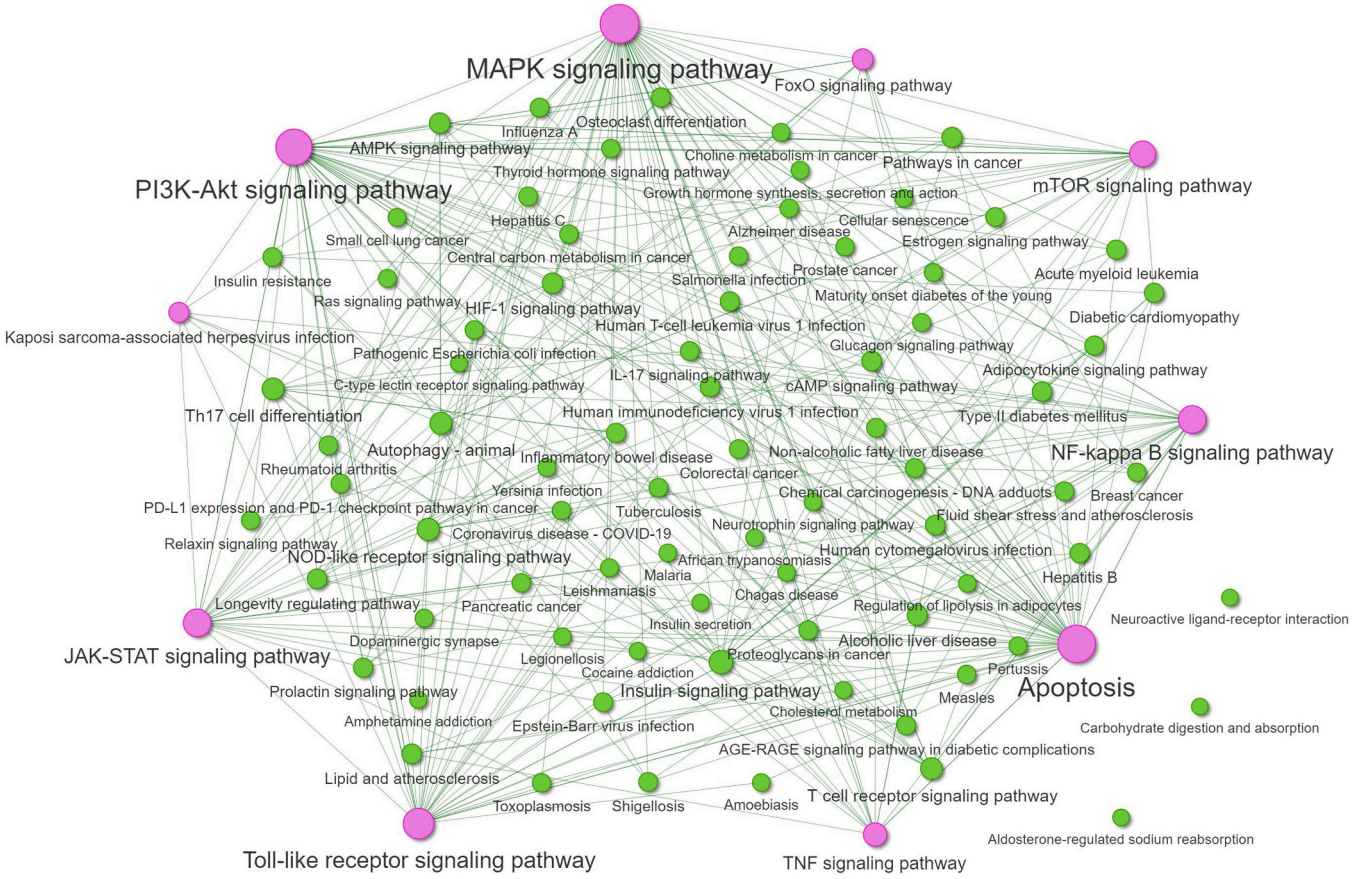

Fig. 3. Visualization of the DM2 ∩ NPDs KEGG pathway-pathway network, indicating the functional relationships between the 87 common pathways between DM2 and NPDs. The top 10 hubs are highlighted in purple color. The size of the nodes is proportional to their degree. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Top 10 subclasses found in the DM2 ∩ NPDs KEGG pathway-pathway network and the number of pathways in each subclass.

Rank	Subclass	Frequency (pathways)
1.	Signal transduction	11
2.	Endocrine system	10
3.	Infectious disease: viral	10
4.	Infectious disease: bacterial	7
5.	Cancer: overview	6
6.	Cancer: specific types	6
7.	Endocrine and metabolic disease	6
8.	Immune system	6
9.	Infectious disease: parasitic	6
10.	Cardiovascular disease	3

Table 4
Top 10 high centrality nodes that facilitate the emergence of DM2 and NPD comorbidity and their classification.

Rank	KEGG pathway name	Classification
1.	Apoptosis (hsa04210)	Cell growth and death
2.	PI3K-Akt signaling pathway (hsa04151)	Signal transduction
3.	MAPK signaling pathway (hsa04010)	Signal transduction
4.	Toll-like receptor signaling pathway	Immune system
5.	NF-kappa B signaling pathway (hsa04064)	Signal transduction
6.	JAK-STAT signaling pathway (hsa04630)	Signal transduction
7.	TNF signaling pathway (hsa04668)	Signal transduction
8.	mTOR signaling pathway (hsa04150)	Signal transduction
9.	Kaposi sarcoma-associated herpesvirus infection (hsa05167)	Infectious disease: viral
10.	FoxO signaling pathway (hsa04068)	Signal transduction

pathways identified contain genes associated with both DM2 and NPDs, network analysis allows us to pinpoint pathways that are central and highly connected, suggesting they might have a more significant systemic impact. This approach helps in prioritizing pathways for further study and potential therapeutic targeting. Moreover, network analysis provides a structured way to hypothesize about the functional relationships and interactions between pathways, which might not be apparent from gene overlap alone. The identification of high centrality pathways as potential 'communicator nodes' offers a focused direction for subsequent experimental validation and therapeutic exploration.

According to KEGG database classification system, seven of the communicator nodes belong to the subclass of 'Signal transduction' (Table 4). In contrast, Apoptosis belongs to the subclass of 'Cell growth and death', the Kaposi sarcoma-associated herpesvirus infection pathway belongs to the subclass of 'Infectious disease: viral', and the Toll-like receptor signaling pathway belongs to the 'immune system' subclass.

3.2.3. *Tissue specificity analysis results of DM2-NPDs interactions*

Comorbid conditions often involve multiple tissues and organs. Therefore, considering the interplay of molecular changes in different tissues and their contributions to comorbidity is essential. To address this, we conducted tissue-specific enrichment analysis of the disease-associated genes from DM2 and NPDs that were found to participate in the 87 common disease pathways. This analysis aimed to identify potential overlapping tissues where the dysregulation of these pathways could occur between these two conditions. The tissue-specific

enrichment analysis revealed 14 statistically significant tissues related to the disease-associated genes in DM2 (Fig. 4A) and 17 statistically significant tissues in NPDs (Fig. 4B). This approach highlighted 11 overlapping tissues (spleen, pancreas, cervix uteri, fallopian tube, colon, pituitary, small intestine, esophagus, stomach, muscle, brain) between DM2 and NPDs where the dysregulation of the 87 common disease pathways could occur.

3.3. Isolating the shortest path to DM2 and NPDs comorbidity

3.3.1. Adding reference points and missing pathways on the DM2 ∩ NPDs KEGG pathway-pathway network

Our objective was to identify the most critical path that might facilitate the development of comorbid DM2 and NPDs. To achieve this, we utilized the DM2 ∩ NPDs KEGG pathway-pathway network, which comprised the 87 common disease pathways identified between DM2 and NPDs. We selected five pathways to act as reference points for the two conditions (see Methods) and highlighted them on the DM2 ∩ NPDs KEGG pathway-pathway network. We also employed the shortest path approach (see Methods) to add any missing reference points and ensure connectivity within the network. In addition, to create a fully connected network, we identified nodes with degree values of 0 and 1, and employed the shortest path approach to add additional missing pathways to the network (see Methods) to generate a fully connected expanded DM2 ∩ NPDs KEGG pathway-pathway network (Fig. 5), consisting of 97 nodes and 391 edge interactions. The remaining unconnected pathway lacked any direct functional interactions based on the data collected from the KEGG database (Fig. 5). Additionally, our approach introduced seven additional pathways (highlighted in red) to establish a fully connected network. Our model highlights the significance of the added Calcium signaling pathway (hsa04020) in facilitating the development of comorbid DM2 and NPDs, as it exhibits functional relationships with several common disease pathways (DM2 ∩ NPDs) identified between DM2 and NPDs (Fig. 5).

3.3.2. Highlighting the shortest path to DM2 and NPDs comorbidity

To isolate the shortest path that contributes to the development of comorbid DM2 and NPDs, we initially calculated and isolated all the shortest paths with the smallest length between the DM2 reference point (Type II diabetes mellitus) and each of the four pathways representing NPDs (Dopaminergic synapse, Glutamatergic synapse, Serotonergic synapse and GABAergic synapse). Subsequently, we extracted the relevant nodes and edges from these shortest paths and highlighted them on the expanded DM2 ∩ NPDs KEGG pathways network (Fig. 5), which allowed to isolate the shortest path that might lead to the emergence of DM2 and NPDs comorbidity.

The shortest path leading to NPDs and DM2 comorbidity encompasses three high centrality pathways: Calcium signaling pathway,

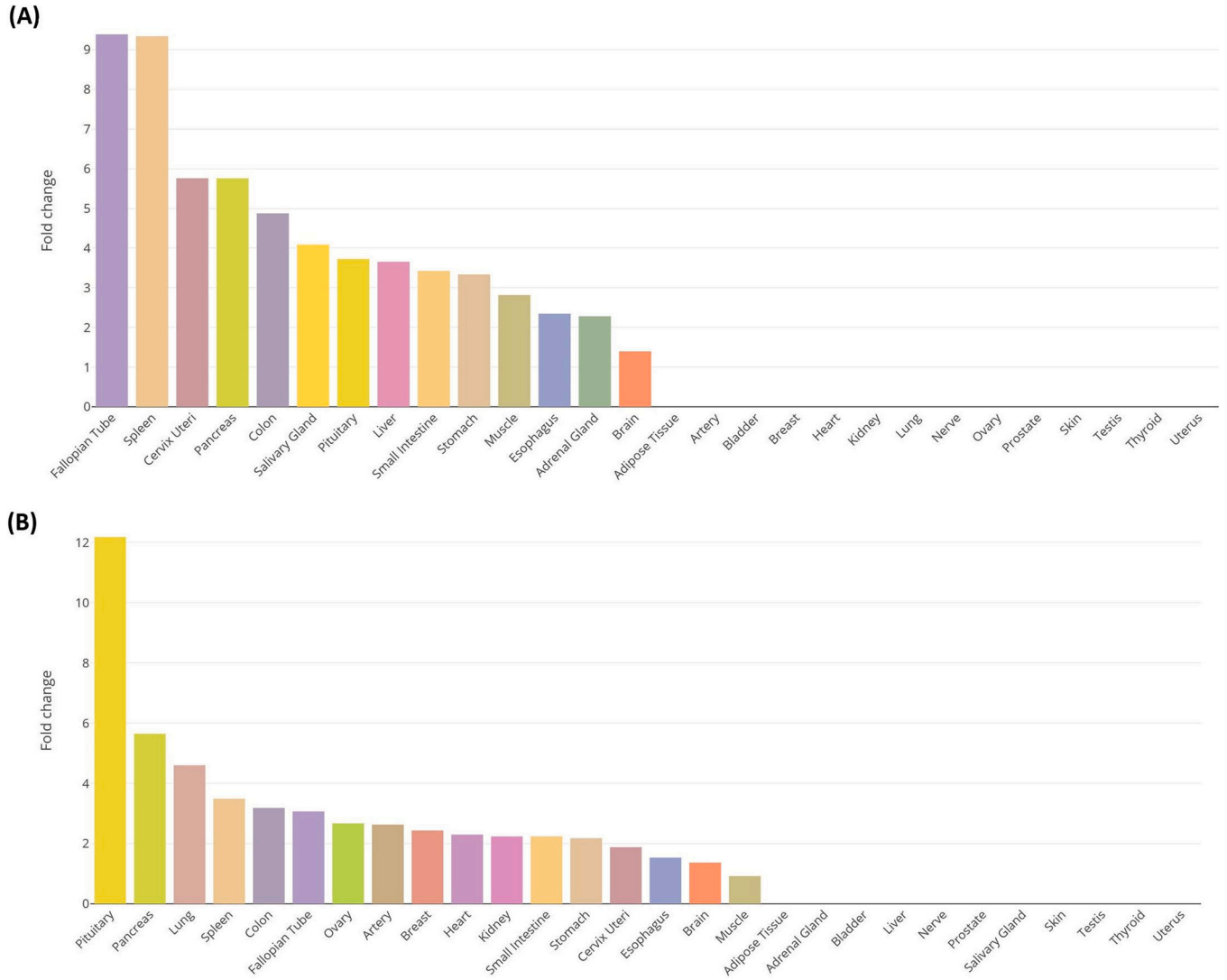

Fig. 4. Tissue enrichment analysis results of disease-associated genes from (A) DM2 and (B) NPDs, which participate in the 87 common disease pathways.

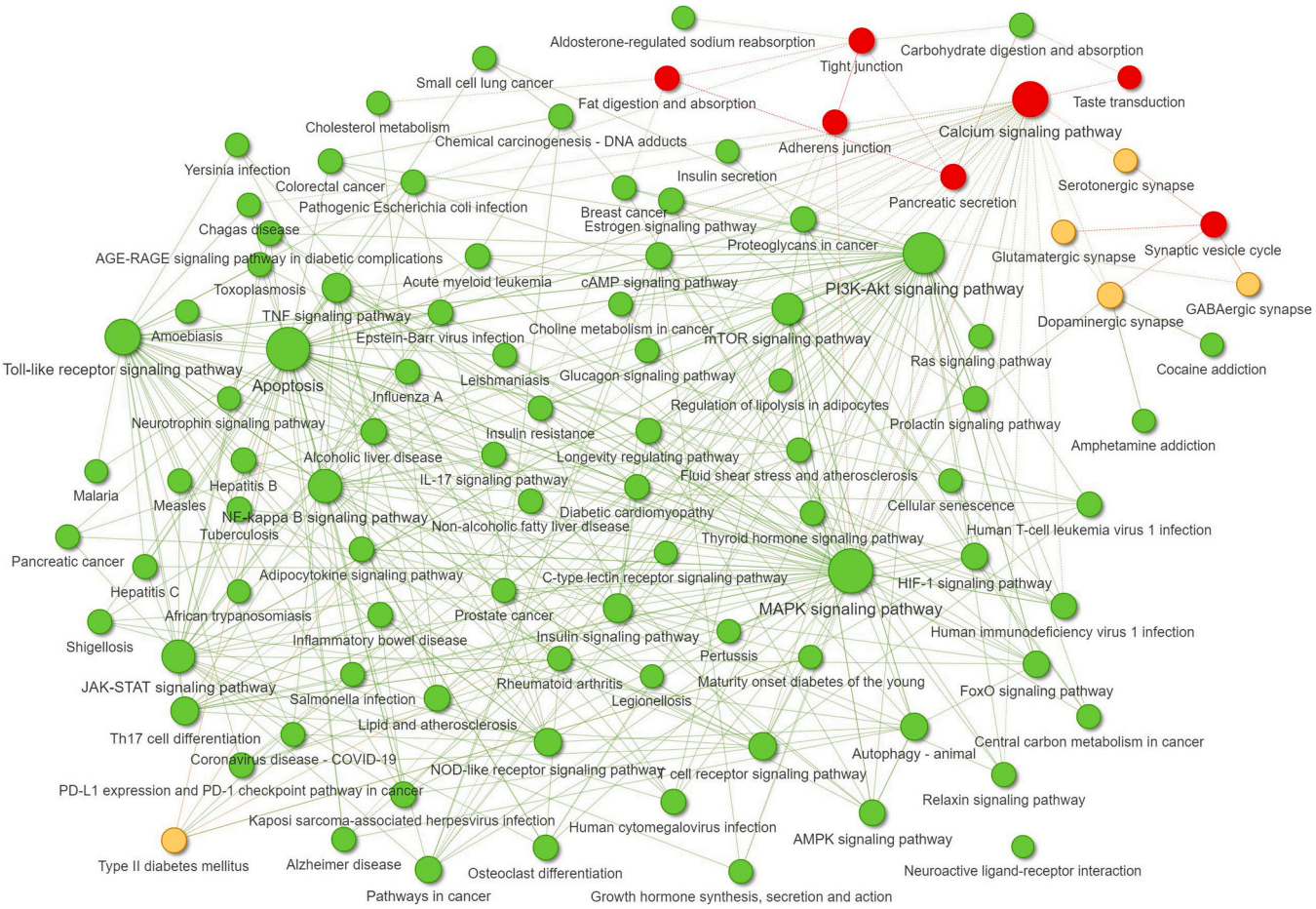

Fig. 5. Visualization of the expanded DM2 ∩ NPDs KEGG pathway-pathway network, where the reference points are highlighted in yellow, the additional pathways added are shown in red, and the remaining common disease pathways between DM2 and NPDs are depicted in green. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

MAPK signaling pathway and Apoptosis (Fig. 6). These pathways not only exhibit functional interactions with one another but also have the potential to exert systemic comorbid pathogenic effects within the expanded DM2 ∩ NPDs KEGG pathway-pathway network (Fig. 5). Therefore, dysregulation of these pathways can facilitate the development of comorbid DM2 and NPDs. Notably, the MAPK signaling and Apoptosis pathways are high centrality nodes in the initial DM2 ∩ NPDs KEGG pathway-pathway network. Moreover, the Calcium signaling pathway was an additional missing node added on the network to establish connections between the unconnected NPDs reference points and the rest of the network. It also interacts with several of the common disease pathways. In addition, the shortest path to comorbidity includes the Estrogen signaling pathway, which exhibits functional interactions with the GABAergic synapse (NPDs reference point), the Calcium signaling pathway (added missing pathway), and the MAPK signaling pathway (comorbidity disease communicator nodes).

3.4. Validation of the importance of the central and "shortest path to comorbidity" pathways in DM2 and NPDs comorbidity using PFC data

As described in the Methods section, we validated the importance of the central and "shortest path to comorbidity" pathways using microarray gene expression data from the PFC obtained from the GEO database.

Pathway enrichment analysis of the DEGs from each dataset revealed 8, 21, 13, and 28 statistically significant KEGG pathways associated with depression, schizophrenia, BD, and DM2, respectively (Supplementary File 2–3). The pathway enrichment analysis yielded several significant findings. First, the PI3K-Akt signaling pathway, identified as one of the top 10 high centrality nodes (hubs) in our computational model contributing to DM2 and NPDs comorbidity, was statistically significant in all three NPDs datasets (depression, schizophrenia, BD). Additionally, it showed statistical significance in the DEGs from the PFC of DM2 rats. Second, the Kaposi sarcoma-associated herpesvirus infection pathway, which was recognized by our analysis as one of the top 10 high centrality disease communicator nodes, displayed statistical significance in both DM2 and schizophrenia. Finally, the MAPK signaling pathway, highlighted in our analysis as participating in the shortest path to DM2 and NPDs comorbidity and as one of the top 10 high centrality nodes, demonstrated statistical significance in both NPDs (schizophrenia, BD) and DM2.

Previous studies have successfully used transcriptomics to infer shared and distinct molecular mechanisms underlying comorbid diseases, providing valuable insights into the pathophysiological links and potential therapeutic targets for complex comorbidities [75]. The findings from the PFC transcriptomic datasets (GSE34451, GSE12654) indicate a strong convergence between the pathways identified through our network-based computational framework and those deemed statistically significant in the DEGs from the PFC. This convergence validates the reliability and robustness of our computational model by demonstrating that it can independently capture relevant pathways.

However, it is important to acknowledge the limitations of our validation approach. While the use of animal models, such as the Goto-Kakizaki rats in dataset GSE34451, provides valuable insights into the molecular mechanisms of DM2, these models may not fully replicate the complexity of human disease. Differences in physiology, brain structure,

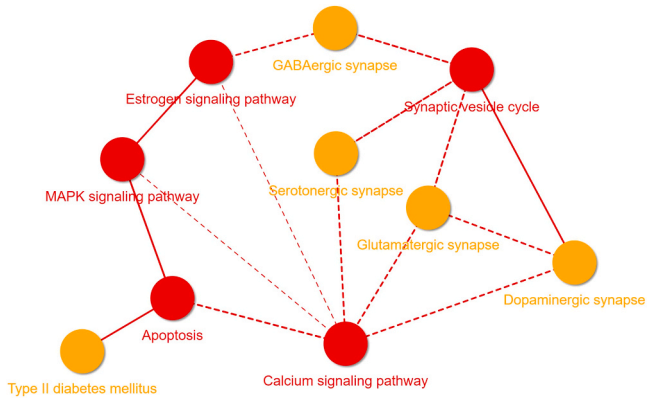

Fig. 6. Shortest path to DM2 and NPDs comorbidity. Illustration of the subnetwork representing the shortest path to DM2 and NPDs comorbidity. It highlights the five reference points representing DM2 and NPDs in yellow color, as well as the minimum number of pathways (shown in red) required to connect the Type II diabetes mellitus pathway (representing DM2) with each of the four reference points: Dopaminergic synapse, Glutamatergic synapse, Serotonergic synapse and GABAergic synapse (representing NPDs). Dotted edges represent additional interactions that were required to be added to create the fully connected expanded DM2 ∩ NPDs KEGG pathway-pathway network. On the other hand, undotted edges depict the interactions present in the initial DM2 ∩ NPDs KEGG pathway-pathway network, which included only the 87 common disease pathways found to be shared between DM2 and NPDs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and disease progression between rats and humans may result in gene expression variations that do not perfectly reflect human conditions. Therefore, findings from animal models must be interpreted cautiously when extrapolating to human disease.

Additionally, due to the lack of comparable human PFC samples for DM2, we were required to compare the rat results with human datasets from PFC samples of individuals with NPDs. This introduces another layer of complexity, as the gene expression profiles in the PFC of diabetic rats may not align precisely with those in humans, particularly in the context of NPDs. This limitation underscores the challenge of validating comorbid disease mechanisms when direct human data for both conditions in the same tissue are unavailable. As a result, the conclusions drawn from this comparison, while informative, must be considered indicative rather than definitive, emphasizing the need for future studies that directly examine comorbid DM2 and NPDs in human tissues. Nevertheless, the alignment of our computational findings with PFC-specific data reinforces the significance of these pathways in the comorbidity of DM2 and NPDs, confirming our model as a robust tool for uncovering critical pathological mechanisms.

Despite these limitations, the convergence of our computational findings with PFC data underscores the effectiveness and reliability of our model in capturing critical interactions. Although the model is not tissue-specific, it offers a comprehensive understanding of the comorbid mechanisms of DM2 and NPDs. This validation process, though constrained by the available datasets, provides strong evidence supporting our computational findings and highlights the robustness of our pathway network-based approach in identifying key pathological mechanisms underlying DM2-NPD comorbidity.

4. Discussion

Our network-based computational framework makes significant contributions by providing a novel method for identifying key pathways and determining the shortest path to comorbidity between DM2 and NPDs. By focusing on high centrality (hub) pathways and uncovering the most direct and biologically relevant connections between these diseases, our model reveals critical molecular interactions that may serve as potential therapeutic targets. This approach surpasses traditional transcriptomic analyses, especially considering the lack of available transcriptomic data from human patients or animal models with comorbid DM2 and NPDs. Consequently, our model provides novel insights into shared biological mechanisms that might be overlooked by conventional methods. The framework can be applied to identify therapeutic targets that address both metabolic and neuropsychiatric conditions simultaneously, making it a versatile tool for exploring complex disease interactions and guiding personalized treatment strategies. By leveraging a graph-based approach, we pinpointed the top 10 high centrality disease pathways and uncovered the shortest path between DM2 and NPDs. These findings are critical, as they highlight specific molecular interactions that may serve as therapeutic targets for addressing the comorbidities of these diseases. While the framework does not aim to capture every possible mechanism contributing to comorbidity, it emphasizes key pathways that are critical to its emergence through shared disease pathways. The ability of the framework to identify these pathways in DM2-NPDs comorbidity was validated using transcriptomic data from the PFC.

We first reconstructed five DM2-NPDs comorbidity PPI networks and extracted their commonalities subnetworks. We then performed enrichment analysis on the human proteins contained in each subnetwork. This allowed to identify 87 common disease pathways shared between DM2 and all five NPDs (ND, MDD, BD, AD and Schizophrenia), which could represent possible crossroad pathological mechanisms that facilitate the emergence of comorbid DM2 and NPDs. Furthermore, we reconstructed the DM2 ∩ NPDs KEGG pathway-pathway network using the identified 87 common disease pathways and their 328 functional relationships.

Topological analysis of the DM2 ∩ NPDs KEGG pathway-pathway network allowed us to identify the top 10 high centrality (hubs) pathways, which can act as comorbidity disease communicator nodes and play a crucial role in promoting systemic comorbid pathogenic effects. Nodes that act as hubs in a network have a high degree of connectivity, meaning they are connected to a large number of other nodes in the network, this centrality renders them crucial for the network's overall structure and functioning. Consequently, perturbations affecting these hub nodes can have significant implications for the stability, functioning, and overall behavior of the network.

Our network analysis identified the top 10 high centrality pathways that could possibly lead to DM2 and NPDs comorbidity, suggesting their essential role due to their high connectivity and potential systemic influence. These pathways, including PI3K-Akt signaling, mTOR signaling, and Toll-like receptor signaling pathway, which have been previously implicated in the pathophysiology of both DM2 and NPDs. For instance, the PI3K-Akt signaling pathway is crucial for insulin signaling and glucose homeostasis and is known to be dysregulated in DM2 [76]. Dysregulation of this pathway in the brain has been associated with synaptic dysfunction and neuroinflammation [77], which are critical factors in the development of NPDs such as schizophrenia and depression. The mTOR signaling pathway, another high centrality node, plays a vital role in cell growth, proliferation, and survival. Alterations in mTOR signaling have been associated with both metabolic disorders like DM2 [76] and NPD conditions such as MDD [78,79], BD [80] and Schizophrenia [81]. Evidence from preclinical models suggests that impaired mTOR signaling contributes to insulin resistance and β-cell dysfunction in DM2 and affects synaptic plasticity and neurogenesis in NPDs [76, 78–81]. Chronic inflammation is a common feature in DM2 and has been increasingly recognized in the pathology of NPDs, including the possible role of Toll-like receptor signaling in contributing to this inflammation [82–84]. The identification of immune-related pathways suggests that inflammatory processes may be a shared mechanism contributing to the comorbidity.

We have also analyzed the composition of the 87 common disease pathways, which showed that the majority belong to the subclasses of Signal transduction, Endocrine system, Cancer, and Infectious diseases.

More specifically, 23 pathways belong to the infectious disease subclasses, including viral, bacterial, and parasitic infections. Several pathogenic organisms have been considered as environmental risk factors for the development of various diseases, including DM2 [85–90] and NPDs [91,92]. Based on the 'associated risk factors' etiological model, comorbidity can arise when risk factors, such as viral infections, for Disease "A" correlate with the risk factors of Disease "B" [1, 93, 94]. For example, the COVID-19 infectious disease is associated with the development of both DM2 and several NPDs, including depression, schizophrenia, and AD [40, 87, 88, 92, 95]. Viruses have the ability to modulate and dysregulate disease-associated pathways via virus-host PPIs and lead to the emergence of diseases [40, 45, 46], thereby leading to the development and exacerbation of comorbid conditions. Understanding these mechanisms highlights the importance of infectious disease pathways in contributing to the comorbidity of DM2 and NPDs, emphasizing the need for integrated therapeutic strategies that address both metabolic and neuropsychiatric aspects.

In addition, we performed tissue-specific gene enrichment which led to the identification of eleven overlapping tissues (spleen, pancreas, cervix uteri, fallopian tube, colon, pituitary, small intestine, esophagus, stomach, muscle, brain) between DM2 and NPDs. These tissues could represent possible sites where the dysregulation of the 87 common pathways could occur. The pancreas and the brain are particularly notable, as their reciprocal interaction plays an important role in maintaining glucose homeostasis both in the brain and peripheral tissues [96]. Pancreatic islets communicate with the brain, and vice versa; brain circuits regulate the endocrine functions of the pancreas. The brain is a key player in the regulation of energy metabolism and glucose homeostasis, as it integrates various peripheral metabolic inputs, including signals from the pancreas [97]. Glucose metabolism is critical for brain functioning, and disruption of glucose metabolism is a primary pathophysiological characteristic of NPDs [98]. Hence, the brain is particularly vulnerable to the metabolic effects of DM2. Moreover, evidence suggests the involvement of brain pathways in the regulation of pancreatic islet physiology; however the exact brain regions that communicate with the pancreas have not yet been fully defined [99, 100]. Therefore, communication between common disease pathways from different tissues can also facilitate the development of comorbidities.

It is notable that the fallopian tube appeared as the most enriched tissue in our analysis of DM2 (Fig. 4A). This unexpected result underscores the need for further investigation to understand its relevance. One possible explanation is the association between DM2 and polycystic ovarian syndrome (PCOS) in women. Women of reproductive age who have PCOS and are obese have an eight times greater chance of developing DM2 [101]. This is because most PCOS women exhibit metabolic syndrome symptoms such as insulin resistance [102]. Additionally, women with PCOS have a higher prevalence of NPDs, including anxiety, depression, and BD [103]. This link suggests that molecular changes in the fallopian tube related to PCOS might also be relevant in the context of DM2 and NPDs comorbidity.

Finally, we devised the 'minimum path to comorbidity' algorithm that allowed us to identify the shortest path that might facilitate the development of comorbid DM2 and NPDs. Existing tools utilizing different methods allow for the recreation, visualization, and analysis of pathway-pathway networks, such as ComPath [104], ClueGO [58], PANEV [105], PathExNET [106] and PathwayConnector [107]. For instance, ComPath [104] is specifically designed for the systematic comparison and visualization of biological pathways across different databases. It allows researchers to explore the similarities and differences between pathways from various sources like KEGG, Reactome, and WikiPathways. Thus, ComPath mainly focuses on comparing existing pathways across databases and identifying pathway modules and clusters. PathwayConnector introduces complementary pathways to create a fully connected pathway-pathway network [107], and PathExNET is a tool specifically designed to create pathway-pathway expression networks by incorporating over- and under-expression data from differential gene expression analyses.

However, PathExNET [106], PathwayConnector [107] and ComPath [104], lack the flexibility to introduce specific pathways as disease reference points, which is crucial for disease-focused research. This limitation becomes particularly challenging when disease-specific pathways are not available in databases like KEGG. In contrast, our approach overcomes this by allowing the introduction and selection of specific pathways to represent diseases, even in the absence of predefined pathways. For instance, in the case of NPDs, were no direct KEGG pathways were available, we selected four relevant pathways to represent NPD reference points in our network using a knowledge-based approach.

Most importantly, unlike PathExNET [106], PathwayConnector [107] and ComPath [104], our novel approach allows for the identification of all the shortest paths on a KEGG pathway-pathway network between selected pathways that act as disease reference points. This feature enables us to pin point the minimum path that might facilitate the development of comorbid diseases, a functionality not available in these existing tools. The 'minimum path to comorbidity' algorithm is designed to identify the most direct and functionally relevant connections between disease pathways that contribute to the comorbidity of DM2 and NPDs. While it leverages the shortest path principle, it integrates biological relevance by focusing on pathways with established roles in both conditions. This targeted approach ensures that the identified paths are not only the shortest but also biologically significant. Therefore, the 'minimum path to comorbidity' algorithm is not merely a shortest path algorithm but a biologically informed method that integrates network centrality, functional relevance, and enrichment validation. This approach provides meaningful insights into the pathophysiological mechanisms underlying the comorbidity of DM2 and NPDs and identifies potential therapeutic targets.

The 'minimum path to comorbidity' algorithm allowed us to highlight the Calcium signaling pathway, MAPK signaling pathway, Apoptosis pathway, and the Estrogen signaling pathway as pathways within the shortest path that leads to DM2 and NPDs comorbidity. The identification of these pathways suggests their critical role in the interplay between DM2 and NPDs. The fact that the MAPK signaling pathway and Apoptosis pathway are also ranked among the top 10 high centrality nodes within the DM2 ∩ NPDs KEGG pathway-pathway network further supports their central role in the emergence of comorbid DM2 and NPDs. These findings suggest that pharmacological interventions targeting these pathways may offer a promising approach to simultaneously address both diseases, due to their close proximity to the reference points of DM2 and NPDs and their functional interactions. Despite the existence of other possible paths, our hypothesis is that targeting these pathways would have a higher drug impact effect than targeting more distant disease pathways or longer paths involving additional pathways. This hypothesis is supported by network-based approaches that have modeled the effects of drugs and have shown that proximity between drug targets and the disease pathways provides new insights into the therapeutic effects of pharmacotherapies [108, 109].

Calcium signaling plays an important role in regulating insulin secretion from pancreatic islet β-cells [110] and its dysregulation results in deficient insulin secretion, increasing the risk for the development of DM2 [111]. In turn, insufficient pancreatic insulin release triggers calcium dysregulation in neuronal cells, leading to impaired synaptic plasticity in the brain [112] and neuronal cell death [113,114], which contributes to the development of brain diseases, including NPDs and neurodegenerative diseases (NDs) such as Alzheimer's disease and Parkinson's disease. Disturbances in neuronal calcium signaling has been reported in various NPDs, including schizophrenia and BD [115]. Notably, it was shown that remission of acute psychosis symptoms in patients with schizophrenia correlates with increased calcium levels in the cerebrospinal fluid [116]. This underscores the critical role calcium

signaling plays not only in insulin secretion but also in neuronal function, and its dysregulation can lead to both metabolic and cognitive impairments.

Additionally, calcium signaling is linked to the MAPK (Mitogen-Activated Protein Kinase) signaling pathway, which further amplifies its impact on cellular health. Calcium can promote neuronal apoptosis via the activation of the MAPK signaling pathway [117,118]. The MAPK pathway is a critical signal transduction pathway involved in regulating various cellular processes, including growth, differentiation, and survival [119]. In the brain, MAPK activates transcription factors that are crucial for learning and memory, highlighting its role in cognitive function [120,121]. However, abnormal activity of the MAPK signaling pathway has been implicated in the development of NPDs, contributing to disorders such as depression, schizophrenia, and BD [120–122]. This dysregulation leads to altered neuronal plasticity and increased susceptibility to stress-induced neuronal damage, both of which are key features in the pathophysiology of these disorders. Furthermore, the MAPK signaling pathway is also involved in insulin signaling, and its improper activation is linked in diabetic complications, including insulin resistance and vascular damage [123]. This dual role in both metabolic and neurological processes highlights the MAPK signaling pathway as a critical junction point in the intersection of DM2 and NPDs.

Moreover, estrogen signaling has neuroprotective effects and regulates glucose metabolism, with low estrogen levels being linked to increased risk of both DM2 and NPDs. Estrogen, which is a sex hormone, exerts neuroprotective effects in the brain, through activation of the MAPK signaling pathway [124–126]. Consequently, low estrogen levels in the brain result in reduced activation of the MAPK signaling pathway, diminishing the neuroprotective actions of estrogen, leading to brain diseases like NPDs and NDs [124]. In addition, estrogen is an important regulator of glucose homeostasis, and low levels of estrogen in postmenopausal women are associated with an increased risk of developing DM2, insulin resistance, and decreased calcium secretion, which can be reversed with estradiol treatment [127,128]. Premenopausal women have reduced DM2 risk and exhibit enhanced sensitivity to insulin compared to age matched controls [129], which might explain why DM2 is more common in the elderly female population. Estradiol treatment in menopausal women increases insulin and calcium secretion [128]. Experimental evidence suggests that estrogen potentiates calcium signaling in pancreatic insulin-releasing β-cells and abolishes calcium oscillations generated by low glucose levels in glucagon releasing α-cells [130]. In contrast to women, increased estradiol levels in men are associated with increased risk of developing DM2 [131]. The identification of these pathways as central to the comorbidity of DM2 and NPDs suggests that they could represent potential pharmacological targets for treatment. Additionally, estradiol's impact on serotonin, glutamate, and dopamine systems [132] underscores the potential of targeting hormonal pathways to manage both DM2 and NPDs. Given the role of the estrogen signaling pathway in both DM2 and NPDs, future research should investigate how hormonal fluctuations influence these comorbidities to guide the development of sex-specific personalized treatments. Tissue-specific transcriptomic analysis could offer valuable insights into sex-specific molecular patterns, aiding in more precise therapeutic targeting [133].

Furthermore, the PI3K-Akt pathway is integral to insulin signaling and glucose uptake in peripheral tissues [134]. Dysregulation of this pathway leads to insulin resistance, a hallmark of DM2 [135]. This insulin resistance, in turn, further impairs the PI3K/AKT pathway, creating a vicious cycle [135]. Moreover, the PI3K-Akt pathway also plays a critical role in neuronal survival, growth, and plasticity with impairments in this pathway have been associated with NPDs, including MDD [136]. This suggests that interventions targeting the PI3K-Akt pathway could address both metabolic and cognitive symptoms.

The findings from our network-based computational framework have significant translational potential in clinical settings. By identifying key pathways such as the PI3K-Akt signaling, MAPK signaling, and Calcium signaling pathways as central to the comorbidity between DM2 and NPDs, our study provides a robust foundation for the development of targeted therapies. These pathways are not only critical to the pathophysiology of DM2 and NPDs but also serve as viable therapeutic targets. Modulating these pathways through pharmacological interventions could potentially address both DM2 and NPDs simultaneously, thereby improving treatment outcomes. Additionally, our approach offers a comprehensive understanding of the molecular interactions driving comorbidity, which could guide the design of personalized treatment strategies in the future. This has the potential to significantly impact clinical practice by enabling more effective, targeted treatments for patients suffering from these complex and often challenging comorbid conditions.

Our methodology is grounded in sound biological knowledge, drawing on a wealth of existing research on DM2 and NPDs. It has also been validated utilizing publicly available transcriptomic experimental data. Overall, our study represents a significant advance in the field of comorbid disease research, with the potential to impact clinical practice by facilitating the development of more effective treatments for these complex conditions. Additionally, our methodology can serve as a paradigm for identifying key pathological mechanisms underlying other comorbid diseases, making it a valuable resource for researchers and clinicians alike.

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Code availability

All of the programs we use in this study for analysis are free and open-access software.

Information on the version of the open-source software and its official website:

1. Cytoscape (v3.8.2) https://cytoscape.org/
2. stringAPP (v2.0.1) https://apps.cytoscape.org/apps/stringapp
3. ClueGO (v2.5.10) https://apps.cytoscape.org/apps/cluego
4. KEGGREST (v1.42.0) https://bioconductor.org/packages/release/bioc/html/KEGGREST.html
5. TissueEnrich web application (v1.22.0) https://tissueenrich.gdcb.iastate.edu/
6. Igraph R package (v1.5.1) https://igraph.org/

The software referenced in this study is open-access and can be obtained from their respective repositories. All other codes that support other findings of this study are available from the corresponding authors upon request.

CRediT authorship contribution statement

Panos Zanos: Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization. **Anna Onisiforou:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supporting information

Supplementary File 1_ Reliability of the graph expansion approach. Supplementary File 2_ DEGs. Supplementary File 3_ Enriched terms. Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csbj.2024.10.011.

Data Availability

The data used in this article are derived from publicly available sources. The microarray GSE34451 and GSE12654 used for this study can be found in the Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/).

Additional data used in this study were:

KEGG database (https://www.genome.jp/kegg/pathway.html);.

STRING disease app (https://apps.cytoscape.org/apps/stringapp);.

DISEASES database (https://diseases.jensenlab.org/Search).

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