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Putative disease gene identification and drug repurposing for DEGENERATIVE DISEASE OF NERVOUS SYSTEM

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GROUP 7

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ABSTRACT

This work explores various computational approaches to study the role of protein-protein interactions (PPI) in disease gene association, specifically focusing on "Degenerative diseases of nervous system". These disorders are broadly characterized by the progressive dysfunction of neuronal systems, leading to severe clinical outcomes. The primary goal is to identify relevant genes within the disease-specific interactome and evaluate computational tools for predicting putative disease genes. To achieve this, several algorithms - DIAMOnD, DiaBLE, and heat diffusion - were examined, comparing their accuracy and robustness through cross-validation and performance metrics such as Precision, Recall and F1 score. Followed by further validation through enrichment analysis for the predicted putative disease genes, DiaBLE was identified as the best-performing algorithm. Additionally, drug repurposing efforts were undertaken to propose therapeutic strategies targeting the identified genes. Finally, an additional study was conducted using the PROCONSUL algorithm, which was further evaluated through Enrichment Analysis. All steps of the analysis were carried out using Python because of its flexibility for network visualization and computational workflows.

INTRODUCTION

“Degenerative nervous system diseases” is an umbrella term that includes a wide spectrum of conditions responsible for the slow and progressive loss of neural function, affecting over 50 million people worldwide. Although these disorders vary in their clinical manifestations, they often share common features such as neurodegeneration and impaired signaling pathways, which can result in motor dysfunction, cognitive decline and other neurological symptoms. Despite ongoing research, much remains unknown about the molecular basis of these conditions and effective therapeutic options are limited [1]. This study aims to identify potential genes driving these conditions and validate their biological relevance through protein-protein interaction (PPI) networks by constructing a disease-specific interactome. Furthermore, the application of computational methods offers a unique opportunity to integrate large-scale datasets and uncover patterns that would otherwise be challenging to identify. The ultimate goal is to provide a computational analysis to enhance our understanding of degenerative nervous system diseases and propose potential therapeutic targets.

MATERIALS AND METHODS

1. PPI and GDA data gathering and interactome reconstruction

In this study, the Human Protein-Protein Interaction (PPI) Network was constructed using data for the latest version of the BioGRID database (4.2.240). Protein interactions were filtered to retain only “physical” interactions among human proteins. Furthermore, self-loops were removed from the network, while redundancies were irrelevant as the analysis was performed on an undirected graph implemented with the NetworkX package. The resulting network consisted of 19972 nodes and 861240 edges. The largest connected component (LCC) corresponded to the entire graph, as the human proteome is highly interconnected. While some proteins perform highly specific roles with minimal or no interactions, they remain indirectly connected through shared biological pathways or regulatory functions.

Disease-associated genes linked to neurodegenerative diseases were obtained from DisGeNET, a comprehensive repository of human gene-disease associations. Gene symbols were validated using the HGNC database BioMart tool and database IDs were verified via Ensembl BioMart. During the analysis, a discrepancy was identified for the SELENOP gene. While SELENOP is the approved gene symbol for a disease-associated gene, its interaction data in the BIOGRID PPI dataset was listed under the alias SELP, as confirmed via the HGNC database. To maintain consistency, SELP was replaced with SELENOP in the PPI dataset.

Several centrality metrics were computed for the disease LCC, including node degree, betweenness centrality, eigenvector centrality, closeness centrality, and the betweenness-to-degree ratio. These were used to identify the most influential nodes in the network.

2. Putative disease genes identification algorithms

In this study, three algorithms were employed to identify putative disease genes. The distribution of disease-associated genes within protein-protein interaction networks is non-random; these genes aggregate in specific regions, forming distinct disease modules. Identifying these modules is a critical step toward understanding the underlying biological mechanisms of a disease and guiding the search for therapeutic targets.

The DIAMOnD algorithm [2] was used to extend the set of known disease genes by identifying additional genes with significant connectivity to the seed set. This method leverages network structure to reliably expand the pool of potential disease-associated genes, providing valuable context for understanding their role in the interactome. The DiaBLE algorithm [3], a variation of DIAMOnD, refines the approach by using a more localized background model. Instead of considering the entire interactome, DiaBLE dynamically adjusts the gene universe to focus on the smallest local neighborhood around the seed set during each iteration. Additionally, Heat-Diffusion algorithm was implemented [4] and ran with three different diffusion times (t=0.002, 0.005, 0.01). This algorithm employs network propagation to identify related genes. This method measures connectivity through iterative information flow across the network, estimating the relevance of nodes based on their proximity to the seed genes. All algorithms were run using default parameters (*Appendix Figures 6, 7, 8* for the resulting putative disease modules, when predicting 100 genes).

3. Algorithm selection

The criteria for algorithm selection were principally 5-fold cross-validation and Enrichment analysis. They were both applied on all the algorithms. The first one is used to evaluate the algorithms' performance, and it was conducted dividing the set of known disease genes into five subsets, using one subset as a probe set while the remaining subsets served as the training set. Enrichment analysis, instead, is a method used to determine groups of genes or proteins over-represented within a larger set of genes or proteins, potentially linked to specific biological functions or disease phenotypes [5]. One of the techniques used in this project was Gene Ontology (GO), which systematically associates genes with functional biological terms, and provides enriched GO categories within the given gene list. Another technique was Pathway analysis, that focuses on identifying cellular pathways based on a set of proteins. In this context, Pathways represent processes within cells, and help in finding significant diseases associated with the observed proteins. To measure the significance, Enrichment analysis uses a statistical approach, i.e. the p-value, that quantifies the probability of observing the obtained enriched terms for a specific category, by chance. This analysis was performed on the 100 putative disease genes predicted by the algorithms, through EnrichR.

4. Drug repurposing

Drug repurposing is a technique aimed at identifying alternative applications for existing or experimental drugs beyond their originally intended medical use [6]. Using the DiaBLE algorithm, the top 20 candidate disease genes were identified and then linked to potential drugs using data for the Drug-Gene Interaction Database (DGIdb). Starting with the drugs associated with the highest number of these 20 genes, a ranking was generated. The top three drugs in this ranking were then checked on https://clinicaltrials.gov to determine if they are currently being tested in clinical trials for degenerative diseases of the nervous system. While associating drugs with genes, it was noticed that the same drug-gene interaction could be reported multiple times due to different interaction mechanisms, data from different sources or studies or different updates in database's versions. To address this, repeated drug-gene interactions were subsequently included in the ranking only if the type of interaction differed between entries.

5. Optional Task - PROCONSUL algorithm

The PROCONSUL algorithm [7] extends the DIAMOnD one, adding probabilistic features. Similarly to DIAMOnD, it begins with a set of seed genes and evaluates every neighbor based on the statistical connectivity significance of this specific node. To find the putative disease genes, nodes with the lowest p-values are iteratively added to the seed set that will form the list of putative genes. Moreover, PROCONSUL assigns an average ranking score to every gene based on its position in the list across the iterations. As for the other algorithms, it was run using default parameters. Finally, an analysis was performed on the top 20 putative disease genes obtained.

RESULTS AND DISCUSSION

1. Interactome reconstruction

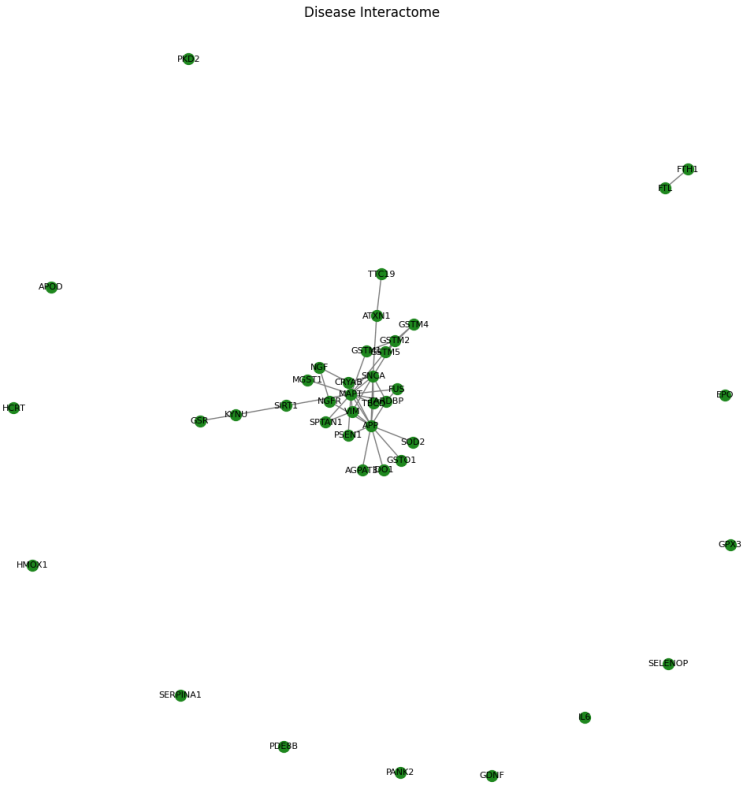
All 40 disease-associated genes from the DisGeNET database are present in the human interactome (*Table 1*), while the disease LCC consisted of 26 genes and 41 edges. Disease genes in the LCC are ranked primarily by degree and, in cases of ties, by betweenness (*Appendix Table 2*). The centrality metrics revealed MAPT and APP as the most connected nodes, with the highest degree (12), followed by SNCA (8) highlighting their roles as central hubs in the disease network. Conversely, many nodes have low degrees, reflecting the sparsity

typical of biological networks [8]. These findings are consistent with existing literature. MAPT, APP, and SNCA are well-known key players in neurodegenerative diseases such as Alzheimer's and Parkinson's. Their roles involve tau aggregation, beta-amyloid deposition, and alpha-synuclein accumulation [9].

Table 1: Summary of GDAs and basic network data

disease name	*UMLS disease ID*	*MeSH disease class*	*number of associated genes*	*number of genes present in the interactome*	*LCC size of the disease interactome*
Degenerative disease of nervous system	*C0524851*	*D019636*	*40*	*40*	*26*

Figure 1: Disease interactome



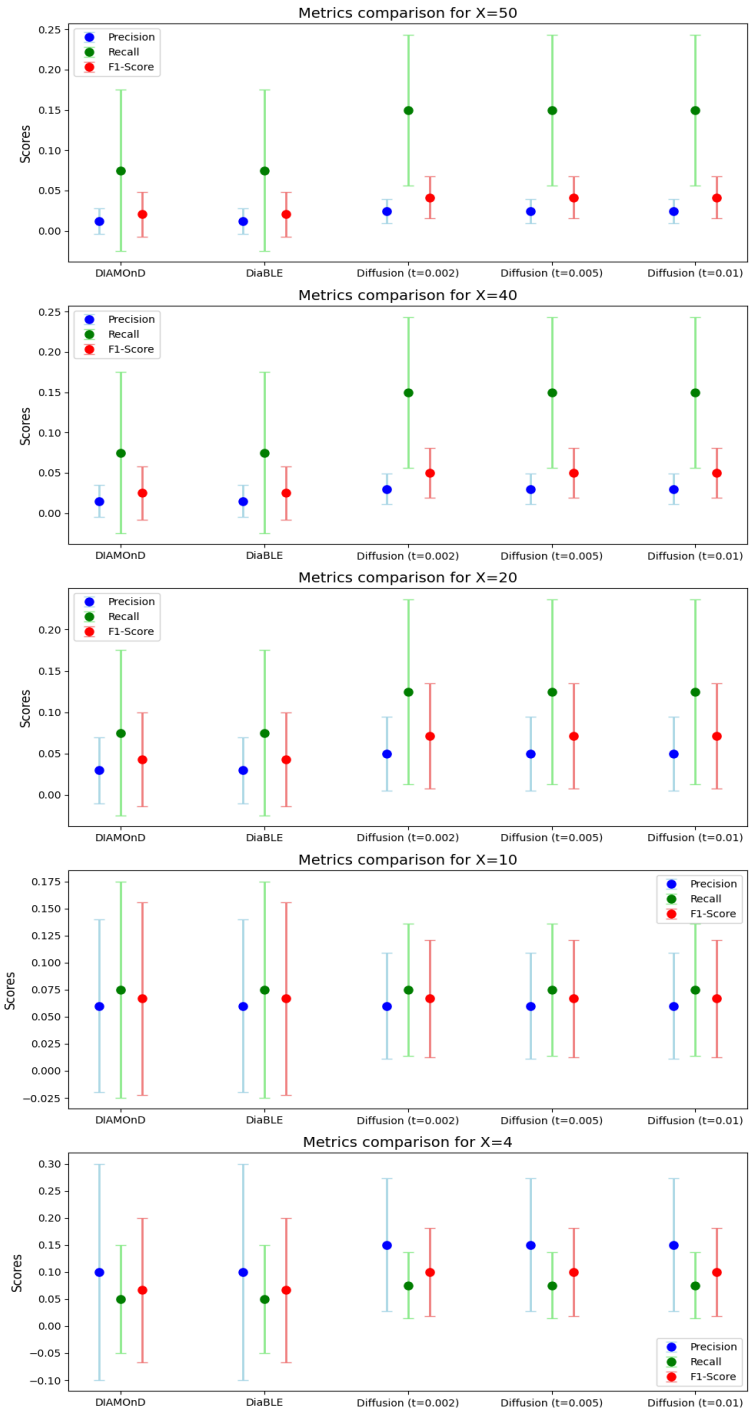
2. Performance Results

The three different algorithms were compared through the method of 5-fold cross validation. The metrics calculated were Precision, Recall and F1 Score and the performances were analyzed for different levels X of top genes. In the graph below (*Fig. 1*), there are also the standard deviations, i.e. the uncertainty, of each metric for each algorithm, represented by error bars. As it is possible to see, in all the algorithms, there are better Precision and F1 Score for lower X, while Recall performs better for higher X. This makes sense, due to the inverse relationship between Precision and Recall metric, known in literature [10].

The best algorithm from this analysis and based on this data, seems to be the Heat-Diffusion algorithm, since the performances are higher than DIAMoND and DiaBLE. While the results from 5-fold cross-validation provide an initial evaluation of algorithm performance, the small dataset size (40 genes per fold, with 32 training and 8 test genes) raises concerns about the reliability of these metrics. To address these limitations, enrichment analysis was performed on the putative disease genes generated by all algorithms.

Despite the slightly higher performance metrics of the Heat-Diffusion one, this led us to choose the DiaBLE algorithm's predictions due to its best enrichment analysis results, as detailed in Section 3.

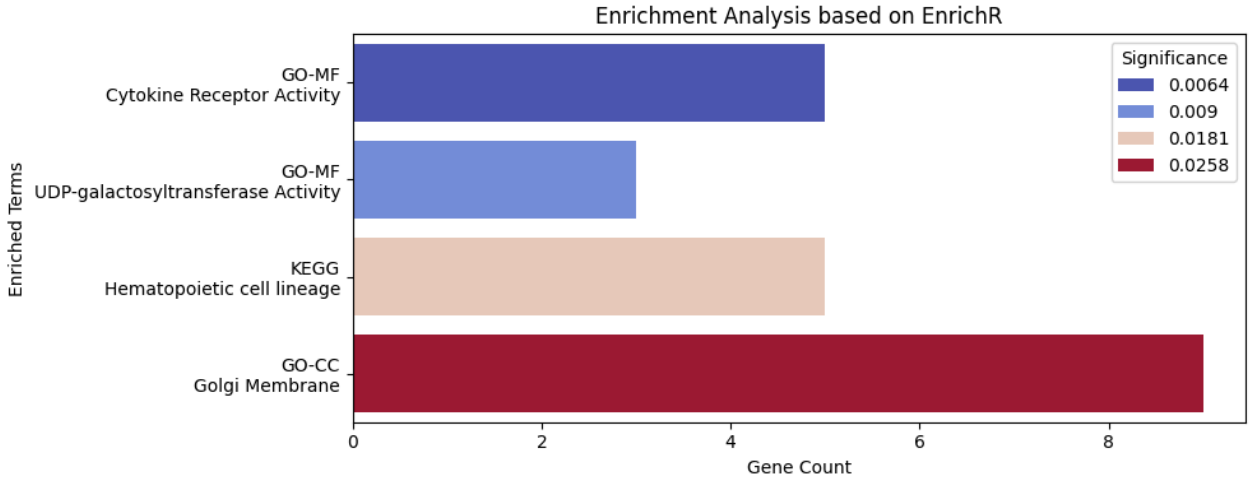
Figure 2: Performance algorithms comparison for different levels of top genes



3. Enrichment analysis

As mentioned in previous Section 2, the first investigation through the Enrichment analysis method was applied on putative disease genes identified by Heat-Diffusion algorithm. It provided only one significant enriched term (*Figure 2*) with a level of significance of 5% for each biological knowledge resource. As shown in *Table 3* (*Appendix*), to reach significantly enriched terms from each database used in the analysis, it is necessary to set at least a level of significance of 25% (quite high). This may suggest that these genes are not highly correlated as they likely do not share multiple functional pathways or biological processes. Moreover there were no overlaps between these terms and the ones obtained from the Enrichment analysis via the original disease genes. Due to these unremarkable results, the analysis proceeded to the other algorithms.

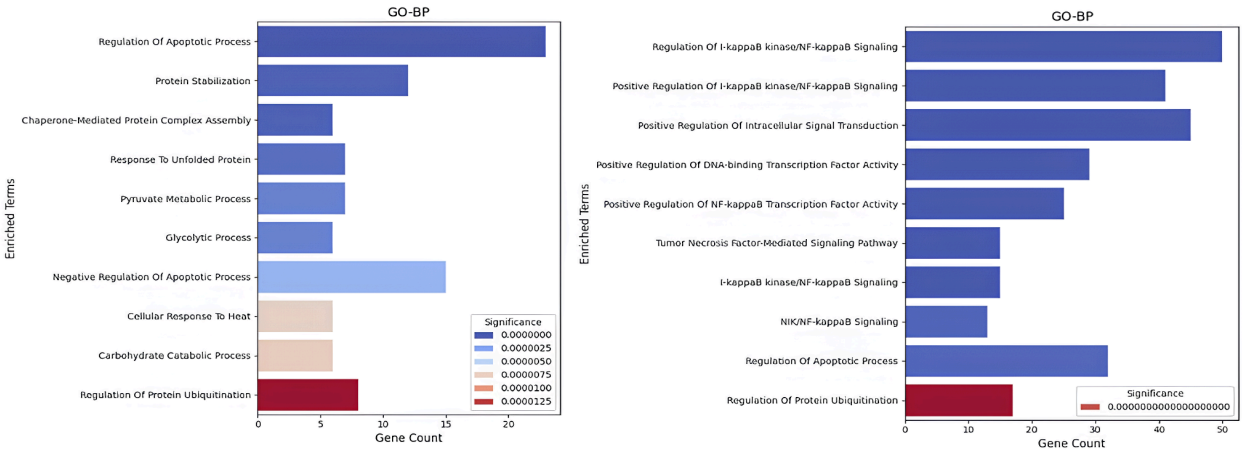
Figure 3: Significant terms for Heat-Diffusion algorithm



Having analyzed the enrichment analysis results for both DIAMOnD and DiaBLE putative disease-genes, it is evident that both are linked to neurodegenerative diseases. However, focusing on the bioinformatics resources used to classify and annotate gene functions such as Gene Ontology Biological Process terms (*Figure 3*), DiaBLE's enriched terms appear more specific due to their emphasis on pathways directly implicated in disease mechanisms such as inflammation, protein homeostasis and immune signaling, processes central to the progression of neurodegenerative conditions. For instance, NF-κB pathways are crucial drivers of neuroinflammation, promoting cytokine release and neuronal damage, a hallmark of Alzheimer's disease [11]. Similarly, macroautophagy plays a pivotal role in clearing misfolded proteins such as beta-amyloid and alpha-synuclein, whose toxic accumulations aggravates neurodegeneration [12]. Furthermore, chronic microglial activation, dysregulated cytokine signaling, dysregulated post-translational modifications and cellular response to bacterial molecules collectively amplify neuroinflammation, leading to the formation of pathological protein states and linking environmental triggers to neurodegeneration. These specific processes contrast with the broader cellular functions captured in the DIAMOnD's enriched terms, which, while related, lack the direct connection to the pathological mechanisms underlying neurodegenerative diseases. This distinction highlights the greater specificity of DiaBLE's putative disease-genes in identifying processes that critically drive disease progression.

A total of 110 enriched terms from the checked biological knowledge resources were identified as overlapping between the original disease genes and DiaBLE's putative disease genes. These include several terms directly related to neurodegenerative diseases, such as "Cellular Response to Amyloid-Beta" [13], "Pathways of Neurodegeneration" and "Regulation Of Neuron Death" which represent central processes in these conditions.

Figure 4: Comparison of Gene Ontology Biological Process significant terms for DIAMOnD and DiaBLE



4. Drug repurposing

The first method of ranking identified as top three drugs:

- DOXORUBICIN HYDROCHLORIDE, an anticancer medicine used to treat different types of cancer;
- CISPLATIN, a chemotherapy drug based on platinum used for solid tumors such as ovarian and lung carcinoma;
- EMAPALUMAB-LZSG, a monoclonal antibody targeting INFγ, used as a treatment for patients with primary hemophagocytic lymphohistiocytosis (HLH), a severe inflammatory disease.

Upon reviewing the DGIdb file, it was observed that some gene interactions for the top three identified drugs were reported multiple times. To handle this, duplicate drug-gene interactions were included in the ranking only when they represented distinct interaction types. In contrast, this second method identified the same first two drugs but a different third one:

- CELECOXIB, a nonsteroidal anti-inflammatory (NSAID) that treats pain and inflammation in conditions like osteoarthritis and rheumatoid arthritis.

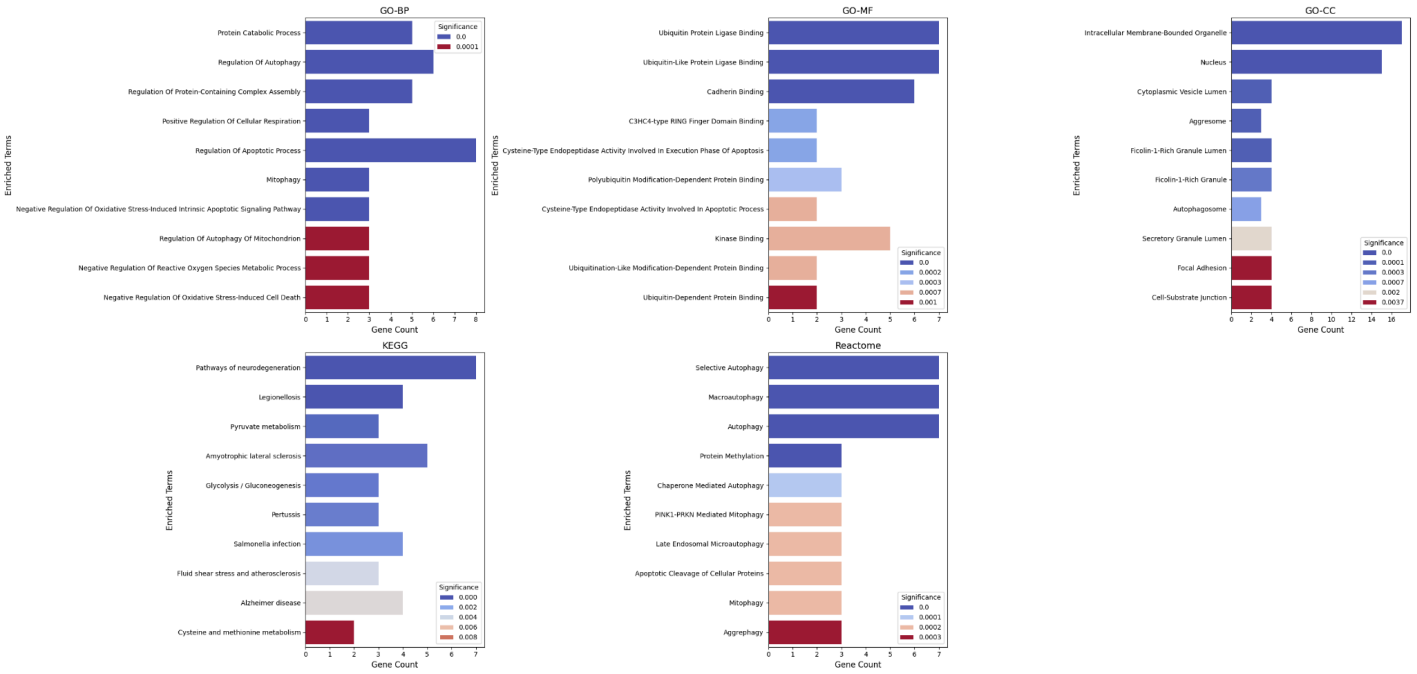
The clinicaltrials.gov data reveals the absence of trials for DOXORUBICIN HYDROCHLORIDE, CISPLATIN, and EMAPALUMAB-LZSG, likely due to pharmacological and biological limitations. Both **doxorubicin** and **cisplatin** are designed to target proliferating cells, a characteristic not typically associated with neurodegenerative conditions. Additionally, these drugs exhibit poor blood-brain barrier penetration and significant toxicity, including neurotoxicity, making them unsuitable for chronic central nervous system disorders [14]. These drugs likely emerged from the algorithm due to their involvement in molecular processes common to neurodegenerative diseases, such as DNA damage and ROS induction due to their interaction with genes like CASP3 and XIAP, which are implicated in apoptosis mechanisms. While inflammation is a common feature in neurodegeneration, **emapalumab**'s non-specific action could disrupt the complex immune balances of the Central Nervous System [15]. In contrast, CELECOXIB emerges as a potentially interesting drug with eight clinical trials, several of which are completed, although their results remain inconclusive. One study, for instance, investigated celecoxib's potential role as an NSAID in preserving cognitive function in elderly adults with a family history of Alzheimer's (ClinicalTrials.gov ID NCT00007189), concluding that celecoxib, when taken for 1

to 3 years, does not protect against cognitive decline in these individuals [16]. Nevertheless, multiple preclinical studies in mice suggest that **celecoxib** could be a promising target for neurodegeneration like Alzheimer's and Parkinson's disease due to its anti-inflammatory effects, a crucial element in these conditions [17].

5. PROCONSUL results

From the top 20 putative genes obtained with PROCONSUL (run with temperature of the softmax function equal to 1), it is interesting to note that the genes shared between this group of top genes from PROCONSUL and the one from chosen DiaBLE (*Appendix Table 4*), have been reported in several studies as being linked to neurodegenerative diseases.
Alterations in some of these genes have been associated with dysregulation and dysfunction of essential cellular processes in disorders like Alzheimer's and Parkinson's [18]. Moreover, specific polymorphisms in these genes have been implicated in hallmark features of conditions like ASL [19]. These findings suggest that these genes may play significant roles in the development and progression of neurodegenerative diseases and could potentially be considered disease-associated genes.
The Enrichment analysis was also performed on the top 20 putative genes identified with PROCONSUL (*Figure 4*). It highlighted enrichment in important processes associated with neurodegenerative diseases, particularly Alzheimer's and Parkinson's, such as protein catabolic processes [20], mitophagy [21], autophagy [12] and oxidative stress [22]. Additionally terms were related to ubiquitin regulation for protein degradation [23]. Finally, there are also other significant terms directly related to other neurodegenerative conditions such as Amyotrophic Lateral Sclerosis [19].
These results suggest that the identified genes are relevant to understand neurodegenerative disease.

Figure 5: Significant terms for PROCONSUL algorithm



APPENDIX

Table 2: Main network metrics of disease LCC genes

Ranking	*Gene name*	*Degree*	*Betweenness*	*Eigenvector centrality*	*Closeness centrality*	*Ratio Betw/Degree*
1	MAPT	12	0.495278	0.473013	0.609756	0.041273
2	APP	12	0.426944	0.448481	0.581395	0.035579
3	SNCA	8	0.26111	0.387683	0.555556	0.032639
4	GSTM5	4	0.051389	0.149850	0.416667	0.012847
5	GSTM2	4	0.047222	0.136449	0.396825	0.011806
6	VIM	4	0.019167	0.269699	0.480769	0.004792
7	TARDBP	4	0.019167	0.269699	0.480769	0.004792
8	CRYAB	3	0.053889	0.164716	0.416667	0.017963
9	GSTM1	3	0.008056	0.141472	0.409836	0.002685
10	TBCD	3	0.000000	0.243918	0.471698	0.000000
11	SIRT1	2	0.153333	0.091417	0.409836	0.076667
12	KYNU	2	0.080000	0.017645	0.373134	0.040000
13	ATXN1	2	0.080000	0.074828	0.373134	0.040000
14	NGFR	2	0.022778	0.092486	0.384615	0.011389
15	NGF	2	0.001667	0.047920	0.3048780487804	0.000833
16	FUS	2	0.000000	0.138378	0.390625	0.000000
17	SPTAN1	2	0.000000	0.138378	0.390625	0.000000
18	GSTM4	2	0.000000	0.053343	0.320513	0.000000
19	PSEN1	2	0.000000	0.171687	0.438596	0.000000

20	MGST1	1	0.000000	0.088129	0.384615	0.000000
21	GSR	1	0.000000	0.083558	0.373134	0.000000
22	SOD2	1	0.000000	0.083558	0.373134	0.000000
23	TTC19	1	0.000000	0.083558	0.373134	0.000000
24	AGPAT3	1	0.000000	0.083558	0.373134	0.000000
25	GSTO1	1	0.000000	0.013942	0.274725	0.000000
26	IDO1	1	0.000000	0.003288	0.274725	0.000000

Table 3: Number of significant Enriched Terms at different levels of significance, for putative disease genes identified by Heat-Diffusion algorithm

Database	*Significance 5%*	*Significance 10%*	*Significance 15%*	*Significance 20%*	*Significance 25%*
GO-BP	0	0	0	0	17
GO-MF	2	3	4	5	95
GO-CC	1	1	2	2	2
KEGG	1	1	2	2	2
Reactome	0	0	0	8	83

Table 4: Common genes between the top 20 putative genes of PROCONSUL algorithm and the top 20 putative genes of DiaBLE algorithm

Common genes								
CASP3	*CASP7*	*EEF2*	*GSTM3*	*HSPB1*	*MAP1LC3B*	*MDH1*	*PINK1*	*SQSTM1*

Figure 6: Putative Disease Module with Top100 predicted genes from DIAMOnD

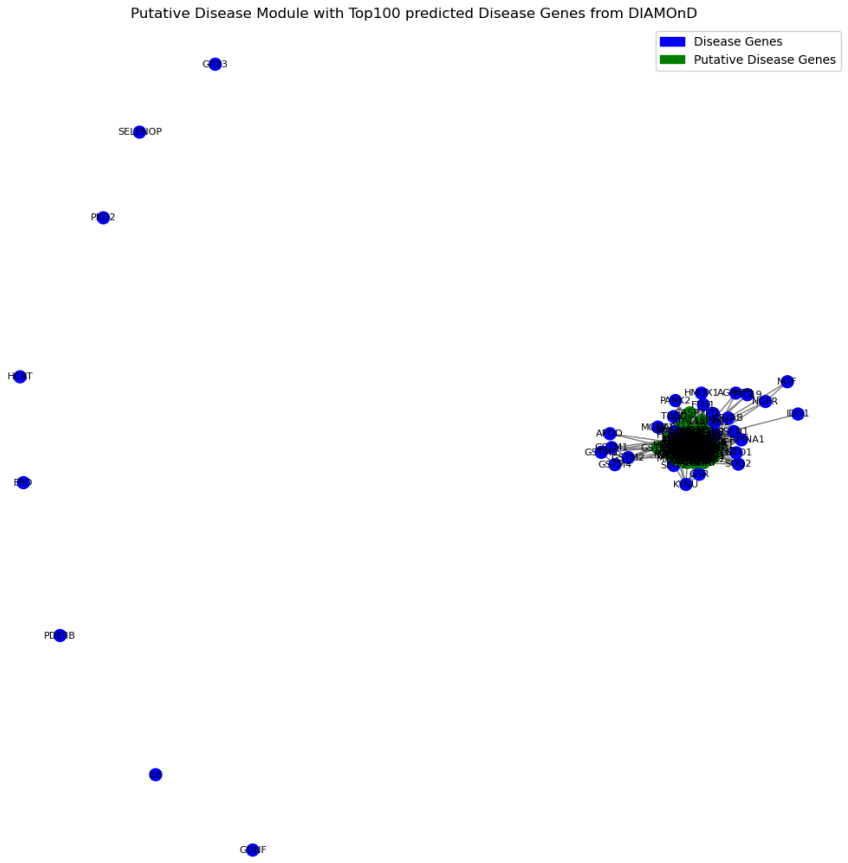
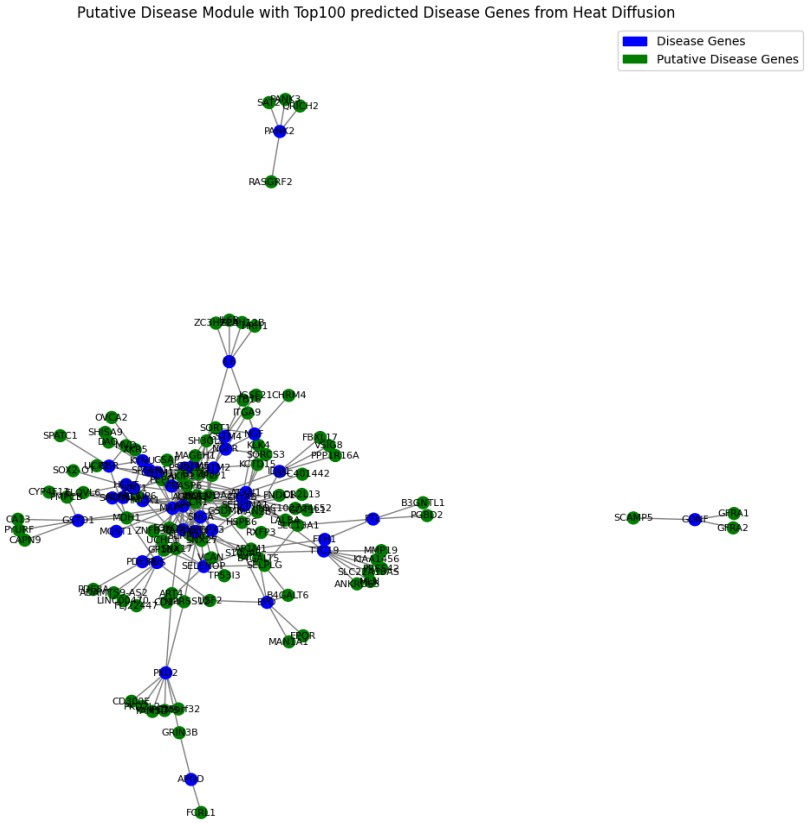


Figure 7: Putative Disease Module with top 100 predicted genes from DiaBLE

Figure 8: Putative Disease Module with top 100 predicted genes from Heat Diffusion



AUTHOR CONTRIBUTIONS

S.D.D., D.Z., S.N.C: data gathering; S.D.D., D.Z., S.N.C: algorithm implementation; S.D.D., D.Z., S.N.C: optional task; S.D.D., D.Z., S.N.C: cross-validation; S.D.D., D.Z., S.N.C: writing—original draft preparation, S.D.D., D.Z., S.N.C: writing—review & editing.

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