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Original article

Neuronetworks: Analysis of brain pathology in Mucopolysaccharidoses – A systems biology approach

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ABSTRACT

Mucopolysaccharidoses (MPS) are rare lysosomal storage diseases characterized by defects in the activity of lysosomal hydrolases that degrade glycosaminoglycan, with progressive multisystemic involvement. Neurological damage is present in several MPS types. The relationship between the accumulation of glycosaminoglycans and the neurological disorder presented in these diseases remains unknown. For this purpose, we analyzed distinct types of MPS using publicly available transcriptomic data with a systems biology approach to search for clues about the pathophysiological mechanisms involved in the brain pathology of MPS. The most relevant proteins in the networks and ontology terms related to neurological damage in MPS were identified and compared among diseases. We performed the clustering analysis for GSE111906 (MPSI), GSE95224 (MPSII), GSE23075 (MPSIIIB), GSE15758 (MPSIIIB), and GSE76283 (MPSVII). Regarding biomarker discovery analysis, the top 10 genes were ranked according to the maximal clique centrality. Different ontologies were present in the different types of MPS. Ontologies were also present in all the MPS types, like axon guidance, Calcium signaling, PI3K-Akt signaling pathway, and Wnt signaling pathway. We hypothesize that these pathways are deranged because glycosaminoglycans play an essential role in the extracellular matrix composition, helping to regulate several processes. Systems biology approaches may help to understand the mechanisms of neuropathology in the different types of Mucopolysaccharidoses.

1. Mucopolysaccharidoses with neuropathic impairment

Mucopolysaccharidoses (MPS) are lysosomal storage diseases (LSD) characterized by defects in the activity of glycosaminoglycan degrading enzymes [54]. Neurological damage is present in MPS I and II (severe cases), III (all subtypes), and VII [83]. In all these MPS, the major brain-accumulated glycosaminoglycan is heparan sulfate (HS), although MPS I, II, and VII also accumulate dermatan sulfate. HS interacts with many molecules and is involved with multiple ligands, receptor interactions and signaling [21,56]. For this reason, HS storage elicits a wide range of pathogenic cascades, leading to disturbances in neurons and glial cells [53]. Impaired HS degradation induces the neuropathological progression, and symptoms like optic atrophy, retinopathy, hearing impairment, seizures, cognitive and behavioral symptoms, neurodevelopmental delays, and sleep disturbances [27,70]. HS also interacts with many cytokines, such as FGF, TGF-β family, HGF, VEGF, hedgehog (Hh) and Wnt [87], leading to neuroinflammatory processes [40,85,5].

Networks facilitate the representation and modeling of biological data [6,28]. A network view of neurological diseases facilitates accurate quantitative characterizations and biomarkers discovery of complex nervous system disorders with graph theory approaches [8,47,77], as they help identify genes (nodes) with functional relevance. For LSD, specifically MPS, this approach is gaining momentum, especially with metabolomic and proteomic studies [68,80,19], as the mechanisms underlying neurological impairment in MPS are not completely understood [42,61]. Here we analyzed different types of MPS using publicly available transcriptomic data to search for clues about the pathophysiological mechanisms involved in the brain pathology of MPS, beyond the primary storage. The selected studies for this work include

Abbreviations: MPS, Mucopolyssacharidoses; GEO, Gene Expression Omnibus; FDR, False Discovery Rate; MCC, Maximal Clique Centrality; GO, Gene Ontology; GAGs, Glycosaminoglycans.

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Table 1
Study design. General information about tissues and comparisons used in the networks and number of differentially expressed genes.

GEO	Tissue or cells	Comparison	Genes up	Genes down	Total DEGS
GSE111906	IPS neural	Hurler – Hurler Scheie – Scheie x control (MPSI x control)	1473	527	2000
GSE95224	Midbrain/diencephalon/hippocampus	Ids-KO x WT	489	511	1003
GSE23075	neural stem cells	Patient x control	1441	559	2000
GSE15758	Neurons of medial entorhinal cortex and lateral entorhinal cortex	Naglu+/- x Naglu-/-	1017	156	1173
GSE76283	hippocampus	Mutant x normal	405	780	1185

MPSI Hurler (OMIM#607014); MPS II (OMIM#309900); MPS IIIB (OMIM#252920), and MPS VII (OMIM#253220), which were the ones with available data.

2. Methods

2.1. Download and analysis of transcriptome data

The transcriptome datasets were retrieved from Gene Expression Omnibus (GEO – [22,7]) with the accession numbers GSE111906 (MPS type I, human neural iPS), GSE95224 (MPS II, mouse cerebral cortex and midbrain/diencephalon/hippocampus), GSE23075 (MPS IIIB, human neural stem cells cultivated in neurosphere's), GSE15758 (MPS IIIB, mouse neurons of medial entorhinal cortex and lateral entorhinal cortex), and GSE76283 (MPS VII, mouse hippocampus).

We performed gene expression analysis in edgeR v.3.28.1 [65], in the case of RNA-seq datasets. We used limma package v.3.44.3 for the microarray datasets [64], with the appropriate functions according to the GeneChip requirements. More information about the datasets may be found in our database for differential expressed genes in Mucopolysaccharidoses, MPSBase (https://www.ufrgs.br/mpsbase/). Transcriptome data was analyzed as differentially expressed genes (DEG), compared to normal tissue in each dataset, filtered by False Discovery Rate (FDR) adjust method, as summarized in Table 1. We performed the gene enrichment analysis with the top 2000 differentially expressed genes for each dataset.

2.2. Network design

Gene network was primarily employed in metasearch engine STRING v.11.0 [79], using up to 2000 differentially expressed genes for each dataset. This cut-off value was determined as this was the maximum number of DEGs for all datasets when using a fold-change of 2 and FDR 0.05. We used confidence network edges with high confidence scores, aiming to obtain only experimental data. Text mining interactions were excluded from the analysis. Only the query proteins were considered, without first and second shell interactors. The analyses were performed in Cytoscape v.3.8, with curated plugins [73].

2.3. Clustering and centrality metrics

For clustering analysis, the Molecular Complex Detection – MCODE v.1.6.1 was used to determine the densely connected regions in the networks [3]. The analysis was based on vertex weighting by the local neighborhood density and outward traversal from a locally dense seed protein to isolate the highly clustered regions [3,18]. We chose the following parameters: node score cut-off = 0.2; fluff = 0.5, and no haircut.

To identify candidate genes for biomarker discovery we used Cytohubba v.0.1 [17] with the local based method Maximal Clique Centrality (MCC), when $MCC(v) = \sum_{C \in S(v)} (|C| - 1)!$ where S(v) is the collection of maximal cliques that contain v, and $(|C| - 1)!$ is the product of all positive integers less than |C|.

To identify the most topologically relevant nodes in the network, we used Centiscape v.2.2 [72], and we chose five parameters of centrality: one for the network - (Diameter) and four for the nodes: Betweenness, Closeness, Degree, and Stress. The network diameter influences how proteins communicate and influence the function of each other.

The degree is the simplest topological index, corresponding to the number of nodes adjacent to a given node, where "adjacent" means directly connected. The degree allows evaluating the regulatory relevance of the node.

The closeness in a protein-protein interaction network can be interpreted as the "probability" of a protein to be functionally relevant for many other proteins. A protein with high closeness, when compared to the average closeness of the network, may be essential to the regulation of other proteins [71].

Stress is calculated by measuring the number of shortest paths passing through a node. It means the importance of a protein and its capability of holding together communicating nodes. The higher the value, the relevance of the protein increases in connecting regulatory pathways [71].

The betweenness is like stress but provides a more robust and informative centrality index. This is crucial to evaluate how it maintains the functionality and consistency of signaling mechanisms or a given biological function. Also, it may indicate the relevance of a protein as capable of holding together communicating hubs with a similar function [71,72]. We combined these parameters to provide biologically meaningful node identification and functional classification.

2.4. Functional enrichment analysis

The functional enrichment was quantitatively assessed (p-value) using a hypergeometric distribution. Multiple test correction was also implemented by applying the FDR algorithm [9] at a significance level of $p < 0.05$. We used the Biological Network Gene Ontology (BiNGO) plugin v.3.0.4 [49] and CluePedia: A ClueGO plugin for pathway insights using integrated experimental and *in silico* data v.2.5.7 [11,12], with the terms or pathways consulted in the database of GO Immune System, KEGG, Reactome, and Wikipathways [2,38,39,37]. A summary of the methodology is presented in Fig. 1.

3. Results

3.1. Clustering analysis and topological findings

We performed the clustering analysis for the five datasets: GSE111906 (MPS type I), GSE95224 (MPS II), GSE23075 (MPS IIIB), GSE15758 (MPS IIIB), and GSE76283 (MPS VII). The number of nodes varied from 2000 (GSE111906) to 1003 nodes (GSE95224). Differences between the number of genes in the input dataset and in the final output network are explained by the exclusion of nodes with no interactors. The GSE111906 network shows six clusters; whereas GSE95224 is divided into seven clusters as well as GSE23075, and GSE15758 and GSE78283 had five clusters each

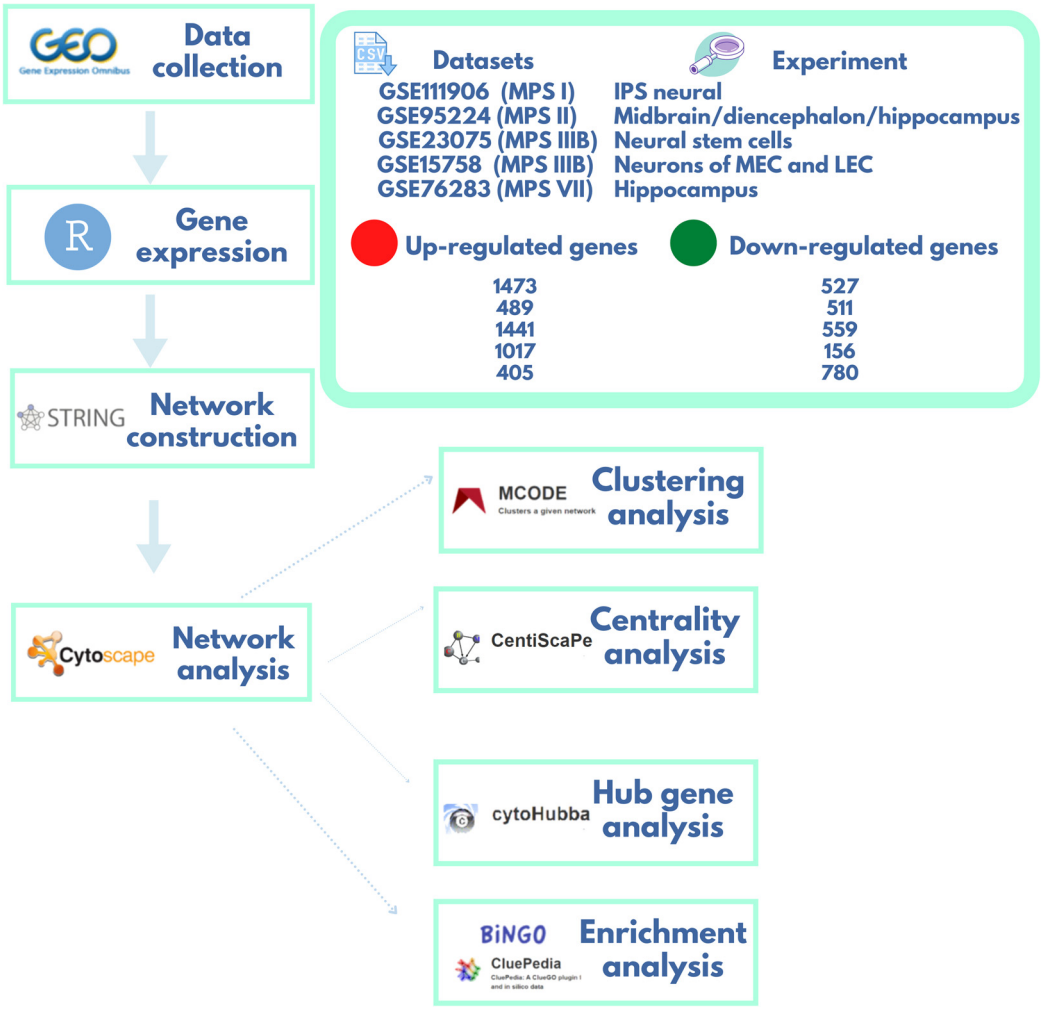

Fig. 1. Workflow of the analysis.

Table 2
Mean of topological results of the networks using Centiscape.

Topology	GSE111906	GSE95224	GSE23075	GSE15758	GSE76283	Mean
Diameter	11.0	14.0	10.0	12.0	14.0	12.2
Degree	9.70	7.72	13.74	6.09	8.65	9.18
Betweenness	2642.41	895.56	2426.36	431.46	1349.17	1548.992
Closeness	2.93E-05	0.013	3.09E-11	0.004	0.025	8.41E-03
Stress	25798.37	6006.27	39002.52	2177.88	9036.39	16404.286
Nodes	862	262	917	243	495	555.8
Edges	4181	1071	6304	740	2141	2887.4

GSE111906: MPS I, GSE95224: MPS II, GSE23075: MPS IIIB, GSE15758: MPS IIIB, GSE76283: MPS VII.

(Fig. 2). We summarize the centiscape topological network indices and the five datasets' statistical results in Table 2.

According to centiscape centralities, the hub genes were ordered by degree, as shown in Table 3. Results indicate that the GSE111906 hub genes are markedly associated with integrin, and signaling pathways like ERK, G-protein coupled receptor (GPCR), and RET. The top 5 genes ordered by degree for GSE95224 are related to clathrin-mediated endocytosis, ERK, MAPK, TGF-Beta and signaling by GPCR. For the GSE23075 the top 5 genes are related to the immune system and MAPK cascade involved in innate immune response, and signaling pathways like ATM, FGFR, insulin, and TLR4. The GSE15758 top hub genes were related to rRNA processing in the nucleus and cytosol, signaling by ERK and the ribosome. The top 5 genes in GSE76283 are related to ATM pathway, adaptive and innate immune system, antigen processing and presentation, G-protein mediated regulation, and Calcium pathways.

Regarding the biomarker discovery analysis, the top 10 hub genes were ranked according to the maximal clique centrality (MCC, Table 4). For the GSE111906 (MPS I), integrin, clathrin-mediated endocytosis, and signaling pathways like ERK, GPCR, and RET were found. Other pathways are related to the immune system and cell cycle. The GSE95224 (MPS II) cytohubba top genes are related to ERK, cell adhesion molecules, neuropeptide hormone activity (*PENK*), neuroactive ligand-receptor interaction, and signaling by GPCR. For GSE23075 (MPS IIIB), top genes comprise the ontologies acetylation and gene expression, chromatin regulation, lysosome, mRNA splicing - major pathway, and immune processes such as activated TLR4 signaling and innate immune system. For GSE15758 (MPS IIIB), top 10 genes were related to the activation of the mRNA upon binding the cap-binding complex and eIFs, and subsequent binding to 43S, rRNA processing in the nucleus and cytosol, RNA binding, and viral mRNA trans-

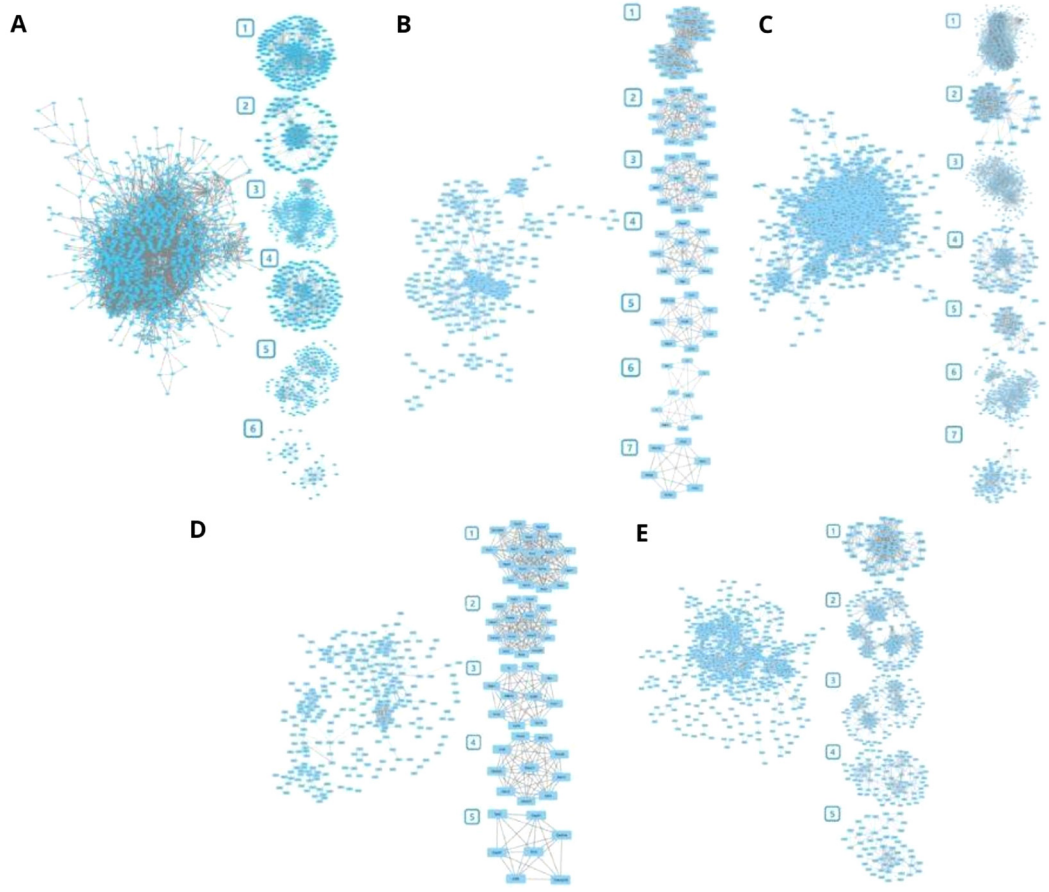

Fig. 2. MCODE clustering results. A = GSE111906, MPS I; B = GSE95224, MPS II; C = GSE23075, MPS IIIB, D = GSE15758, MPS IIIB, E = GSE76283, MPS VII.

Table 3
Centiscape node centralities per dataset. Top 5 genes ordered by degree.

Dataset	Gene	Betweenness	Closeness	Degree	Stress
GSE111906	*ITGB1*	3.771	4,04E+11	61	548478.0
	PTK2	3.839	4.0E-4	60	402604.0
	FN1	1.338	3,90E+11	54	162538.0
	EGFR	34.908	4,10E+11	52	395534.0
	PXN	18.542	3,96E+11	51	247706.0
GSE95224	*GNG7*	10937.61	12.300	56	56742.0
	AVP	5248.4	11.547	38	20482.0
	AGT	3.743	11.933	38	27098.0
	CCL2	25.67	1.183	37	23542.0
	MCHR1	25.67	1.183	37	23542.0
GSE23075	*CDK1*	29.232	4,36E+12	77	587714.0
	NCBP2	12.902	4,12E+12	75	245610.0
	HSPA8	32.193	4,17E+12	72	410880.0
	GTF2F1	62.15	3,81E+12	71	110116.0
	MAPK1	30.701	4,00E+12	67	459904.0
GSE15758	*RPS15A*	15.176	2.0	20	9732.0
	RLP7	15.09	17.825	20	2218.0
	RPS11	13.013	1.996	20	8612.0
	GNB2	25.466	29.239	20	4518.0
	RPS9	5.423	18.867	19	5616.0
GSE76283	*CDC20*	7.936	5,79E+11	40	71366.0
	CDK1	6.472	6,29E+11	37	73588.0
	LYZ1	6.554	6,99E+11	36	53346.0
	SERPINA3N	94.928	6,81E+11	35	46248.0
	GNB5	67.025	6,80E+11	34	60460.0

GSE111906: MPS I, GSE95224: MPS II, GSE23075: MPS IIIB, GSE15758: MPS IIIB, GSE76283: MPS VII.

Table 4
Genes in top 10 nodes ranked by maximal clique centrality (MCC).

GSE111906	GSE95224	GSE23075	GSE15758	GSE76283
TGOLN2	*GNG7*	*GPKOW*	*RPS15a*	*SERPINA3N*
P4HB	*AGT*	*RBM5*	*RPS11*	*LYZ1*
FN1	*CD6*	*HNRNPUL1*	*RPL23*	*LYZ2*
QSOX1	*MCHR1*	*SRSF11*	*RPS14*	*TOLLIP*
CKAP4	*AGTR2*	*DHX9*	*RPS9*	*PYCARD*
CDC20	*PENK*	*NCBP2*	*RPL26*	*HMHA1 (ARHGAP45)*
FBX07	*GAL*	*PAPOLA*	*RPS25*	*GNS*
KBTBD6	*SSTR3*	*CTSF2*	*RPL37a*	*CYB5R3*
KBTBD7	*SST*	*HNRNPA0*	*RPS3a1*	*GALNS*
SOCS3	*CD5*	*SMNDC1*	*RPL10a*	*CTSC*

GSE111906: MPS I, GSE95224: MPS II, GSE23075: MPS IIIB, GSE15758: MPS IIIB, GSE76283: MPS VII.

lation. Finally, the GSE76283 (MPS VII) top 10 genes provided by cytohubba were present in the ontologies cytochrome P450, glycosaminoglycan degradation, innate immune system, NF-KappaB Pathway, signaling by GPCR, and transport to the Golgi and subsequent modification. Fig. 3 shows the nodes with the best ranks, according to Maximal Clique Centrality and its first neighbors.

Processes related to Calcium signaling, GPCR and immune pathways are present in all datasets and were identified by both cytohubba and centiscape.

3.2. Gene-set enrichment results

We identified the over-represented gene ontology (GO) pathways related to the nervous system and functions that were significantly enriched in the different gene sets. Fig. 4 presents the top 10 enriched GO results, and the number of genes present in the three GO categories. The analysis reveals that some components, like Cytoplasm (GO Cellular Component) and Binding (GO Biological Process) appear in datasets from all MPS types. Others, like Cell (GO Cellular Component) and Membrane (GO Cellular Component)

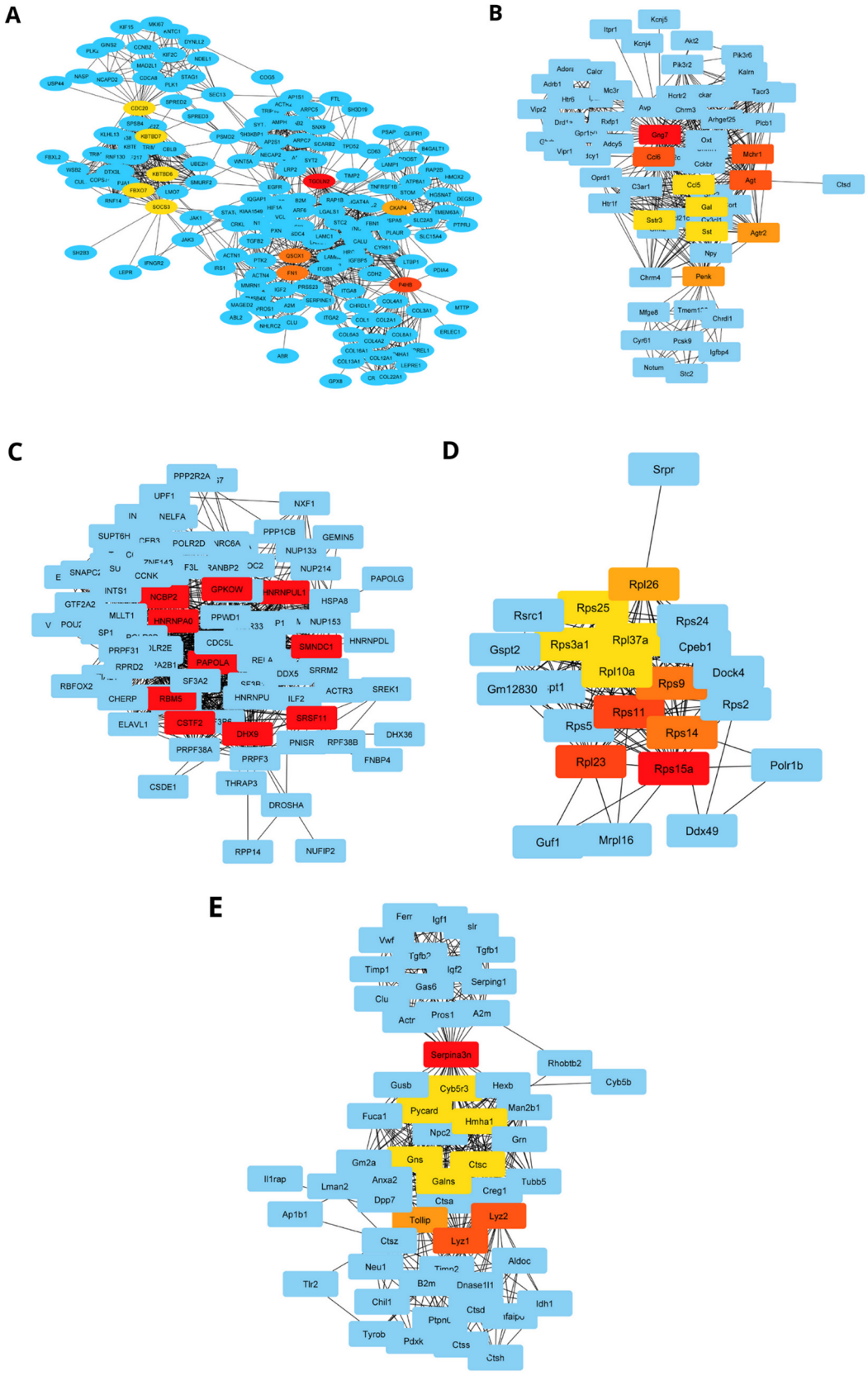

Fig. 3. Analysis of gene hubs with Cytohubba, using the MCC algorithm. A = GSE111906, MPS I; B = GSE95224, MPS II; C = GSE23075, MPS IIIB, D = GSE15758, MPS IIIB, E = GSE76283, MPS VII.

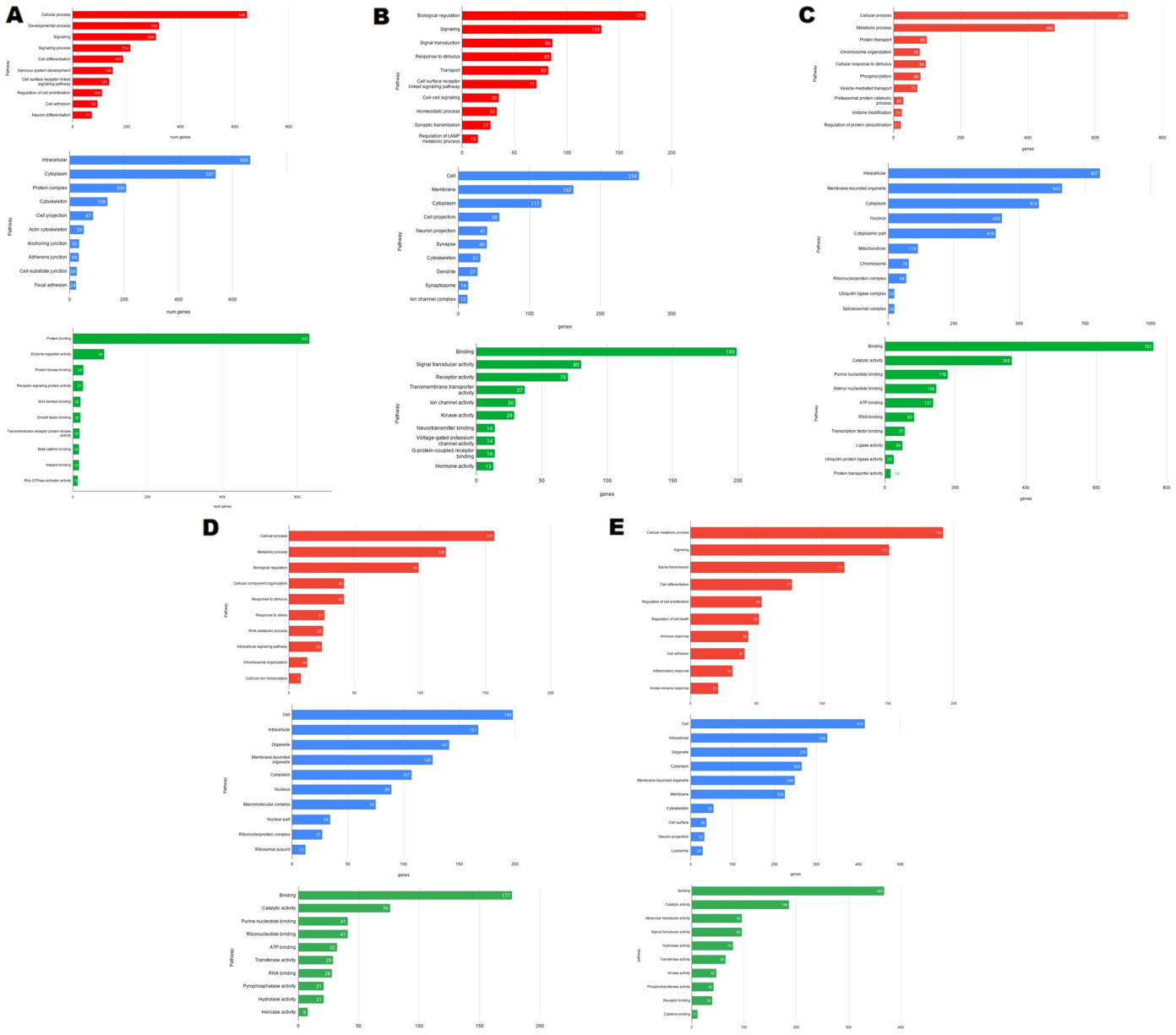

Fig. 4. Enrichment results of Gene Ontology. Red = Biological Process; Blue = Cellular Component; Green = Molecular Function.

are absent only in the dataset from MPS I, whereas Cytoskeleton (GO Cellular Component) and Signaling (GO Molecular Function) are absent only in both MPS IIIB datasets.

Next, we analyzed the pathways present in Clinvar (only for the human datasets), GO Immune processes, KEGG, Reactome and Wikipathways. Figs. 5 and 6 show the enrichment results for the datasets of neurological transcriptomic analysis across the different databases. Since most results were redundant, we will focus the discussion on the KEGG results, which showed the largest number of pathways. Fourteen KEGG terms (36.84%) were present in all the MPS types, such as Axon guidance, Calcium signaling pathway, Focal adhesion, FoxO signaling pathway, Hippo signaling pathway, MAPK signaling pathway, Metabolic pathways, Neuroactive ligand-receptor interaction, Pathways in cancer, PI3K-Akt signaling pathway, Proteoglycans in cancer, Rap1 signaling pathway, Ras signaling pathway, and Wnt signaling pathway. Also, 17 KEGG terms (44.74%) appears in three types of MPS, such as Apelin signaling pathway, Apoptosis, Autophagy, Breast cancer, cAMP signaling pathway, Choline metabolism in cancer, Colorectal cancer, ErbB signaling pathway, Gastric cancer, Hepatocellular carcinoma, Lysosome, Melanogenesis, mTOR signaling pathway, Oxytocin signaling pathway, Phosphatidylinositol signaling system, Relaxin signaling pathway, and Th17 cell differentiation. Finally, 7 terms (18.42%) appear in two types of MPS: AMPK signaling pathway, cGMP-PKG signaling pathway, Chemokine signaling pathway, Estrogen signaling pathway, Phosphonate and phosphinate metabolism, Prostate cancer, and Tight junction. We hypothesize that these pathways are deranged because glycosaminoglycans play an essential role in the extracellular matrix composition, helping to regulate several processes.

To find out which genes are shared between the datasets from MPS types, we combined all the networks and obtained 14 genes shared between three types of MPS (Table 5). Of these genes, 1 (7.14%) is shared by datasets of MPS I, MPS II and MPS VII; 3 (21.43%) by MPS I, MPS II and MPS IIIB; 3 (21.43%) by MPS II, MPS IIIB and MPS VII, and 7 (50%) genes appear in MPS I, MPS IIIB, and MPS VII. We hypothesize that these genes shared between the different types of MPS may have functional relevance to the neurological aspects of these diseases.

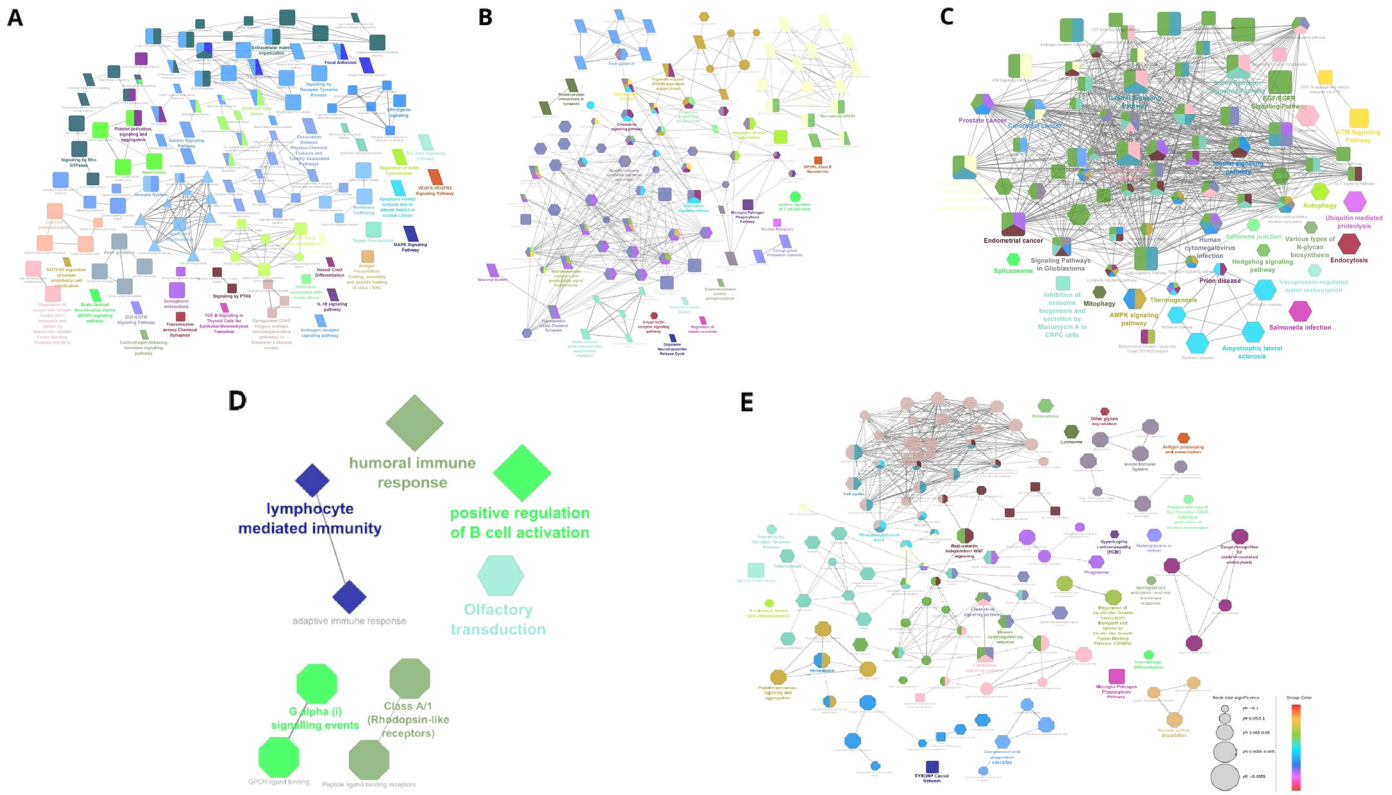

Fig. 5. ClueGO enrichment results. We used the databases of GO Immune processes, KEGG, Reactome, and Wikipathways. Node size significance is represented by the size. The colors indicate groups of related ontologies.

Table 5
Common genes between the MPS types.

Gene symbol	Gene name	MPS type
ACTN4	Actinin Alpha 4	MPS I, MPS II, MPS IIIB
AXL	AXL Receptor Tyrosine Kinase	MPS I, MPS IIIB, MPS VII
B2M	Beta-2-Microglobulin	MPS I, MPS IIIB, MPS VII
CTSA	Cathepsin A	MPS I, MPS IIIB, MPS VII
CYB5R3	Cytochrome B5 Reductase 3	MPS I, MPS IIIB, MPS VII
CYR61	Cellular Communication Network Factor 1	MPS I, MPS II, MPS VII
FCGR2B	Fc Fragment Of IgG Receptor IIb	MPS II, MPS IIIB, MPS VII
HK1	Brain Form Hexokinase	MPS I, MPS IIIB, MPS VII
MRAS	Muscle RAS Oncogene Homolog	MPS I, MPS II, MPS IIIB
MSN	Moesin	MPS I, MPS IIIB, MPS VII
PENK	Proenkephalin	MPS II, MPS IIIB, MPS VII
PLCB1	Phospholipase C Beta 1	MPS II, MPS IIIB, MPS VII
PTK2	Protein Tyrosine Kinase 2	MPS I, MPS II, MPS IIIB
SYT11	Synaptotagmin 11	MPS I, MPS IIIB, MPS VII

4. Discussion

The neurological impairment in MPS has been described in several tissues like cortex, hippocampus, putamen, cerebellum, striatum, olfactory bulb, and thalamus [76]. Morimoto et al. [53] suggested that histopathological changes in hippocampus of MPS II mice correlate with neuronal cell dysfunction and loss of spatial learning ability. Villani et al. [84] described the hyperactivity and hippocampal-dependent learning deficits as well as hippocampal dependent memory impairment in MPS IIIB patients. Shapiro et al. [74] showed that children with MPS IIIA and B appear to develop autistic-like behaviors, primarily social and emotional abnormalities, around the age of 4 years, which are associated with a reduction in hippocampal volume. In the MPS VII mouse model, the role in synapse formation and dendritic spine maturation in the hippocampus was studied by Parente and colleagues [60]. Therefore, we hypothesize that hippocampal impairment is associated with neurodegeneration and neurological impairment in several types of MPS.

The network-based analysis was implemented using public transcriptomic datasets. To the best of our knowledge this is the first study to carry out such an analysis of Mucopolysaccharidoses with neurological manifestations using systems biology approaches. Our networks include human neural IPS cells (MPS I), mouse hippocampus (MPS II), mouse neurons (MPS IIIB, GSE15758), human neural stem cells in neurospheres (MPS IIIB, GSE23075), and mouse hippocampus tissue (MPS VII). We opted to use only one tissue type in each dataset for analysis, to improve the prediction performance, as suggested by Guan et al. [30].

We constructed various types of networks and topology analysis to identify critical molecular players and mechanisms involved in pathophysiology of neurological MPS types. As different diseases, with different datasets from various sources were analyzed, results will be discussed separately by the type of MPS.

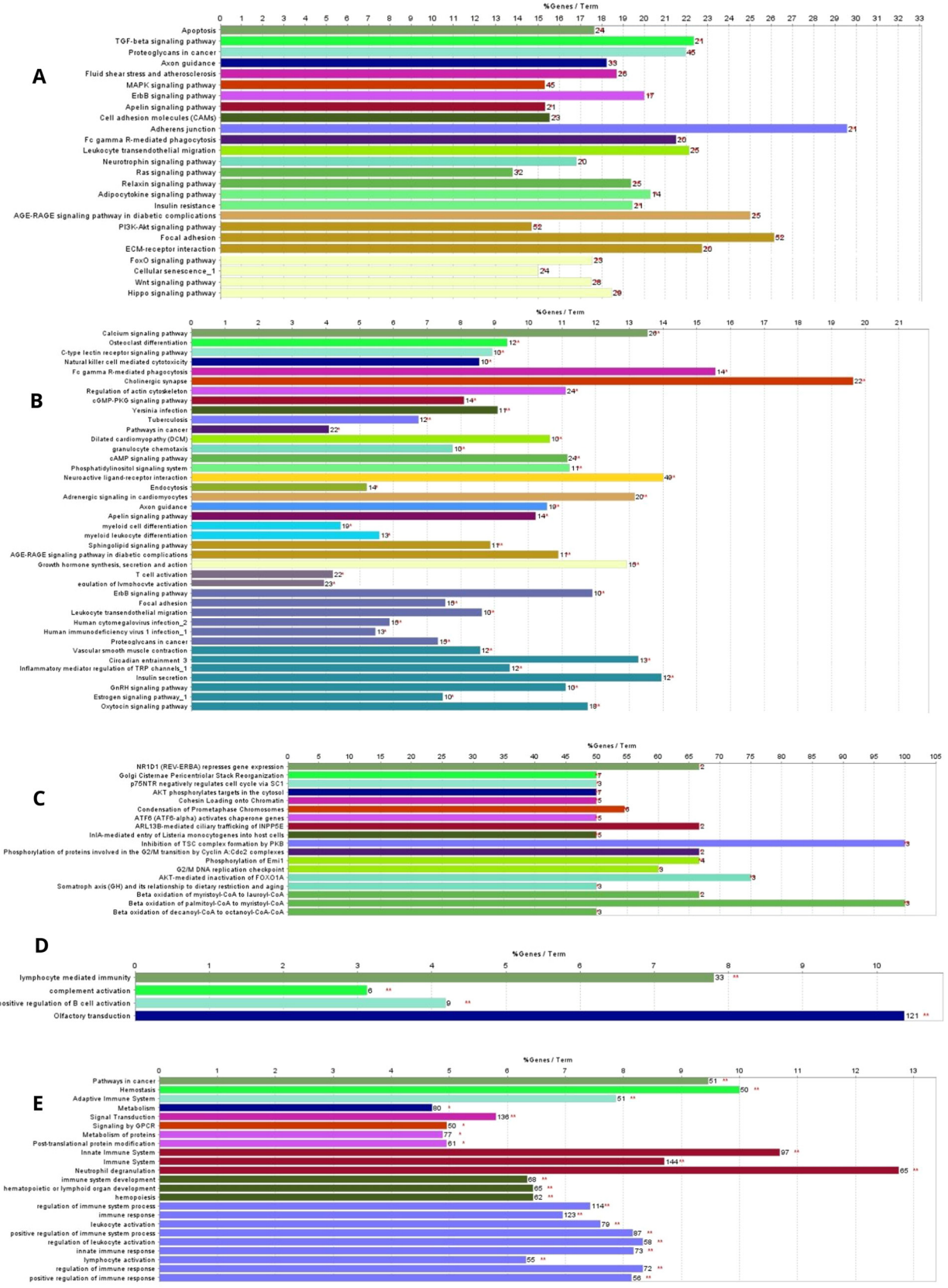

Fig. 6. Histogram of the ClueGO results with the % of genes per terms, and the number of genes presented in the networks. The colors indicate groups of related ontologies.

4.1. MPS type I

In the study of Swaroop and collaborators [78] the transcriptomic analysis revealed several deranged pathways in the patient-derived iPS neural stem cells, like GAG biosynthesis and degradation pathways, lysosomal function pathways, and autophagy. Golgi transport, endoplasmic reticulum stress, autophagy and vacuolum organization are also impaired. Those authors suggest investigating the TGFβ signaling pathway. In our analysis, the hub genes also participate in several signaling pathways related to the lysosome and extracellular matrix.

The *TGOLN2*, the top-ranked hub gene, encodes an integral membrane protein related to the trans-Golgi network, which plays an essential role in vesicle formation and clathrin-mediated endocytosis. Other LSD also show disturbances in this pathway, as Pompe disease [26], Niemann-Pick A, Niemann-Pick C, Fabry, and Gaucher [63]. The other 10 top genes are related to adaptive immune system, interleukin-11 signaling pathway, regulation of mitotic cell cycle, RET signaling, and signaling by GPCR. Castaneda and colleagues [16] describe several LSD with early neuroimmune responses. HS accumulation in the brain may disturb immune regulators, many of which possess HS-binding motifs, as TLR4 and TLR2 [62,94]. Another deranged pathway in MPS I is the Oncostatin M signaling, that acts in neuronal proliferation and viability. This pathway also induces the mitogen activated protein kinases (MAPK) cascade [36]. The different MAPKs involved are extracellular signal-regulated kinases 1 and 2 (ERK1/2), p38 and c-jun N-terminal kinases (JNK). All these signaling pathways are deranged in the neurological MPS I network.

Regarding the enrichment analysis, several signaling pathways are disturbed. Some of these are related to proteins which interact with HS, such as RET, PDGF and integrins, and can contribute to the diversity of clinical symptoms secondary to the accumulation of GAGs. Perturbations in autophagy, axon guidance, and vesicle trafficking have been described as leading to neurodegeneration in many MPS types [24]. Another impaired pathway is the Relaxin signaling pathway, that mediates anti-fibrotic, angiogenic, anti-inflammatory, and anti-apoptotic processes, having organ protective effects across a range of tissues, including the brain. It promotes the activation of downstream signal transduction pathways, such as cAMP signaling, GPCR, VEGF, PI3K/Akt, p38 MAPK, and Notch1 [81]. The Notch1 pathway is related to synaptic plasticity [1] and may be involved in pathological modifications in stroke, Alzheimer's disease and CNS tumors [43]. Finally, neurotrophin signaling is related to hippocampal plasticity and neurodegeneration [33]. Other functions of neurotrophins are correlated with survival, proliferation and maturation of affected neurons in Alzheimer's disease and Parkinson's disease [69].

4.2. MPS type II

In the MPS II network, the top biological processes are signal transduction, transport, and synapse transmission Pará et al. [59], according to the study of Salvalaio et al. [67]. For cellular components, the ontologies are cell projection, membrane, cytoplasm, cytoskeleton, dendrite, and neuron projection. In addition, we found two new ontologies, synaptosome and ion channel complex. For molecular function ontologies, we found protein binding, voltage ion channel activity, hormone activity, kinase activity, and receptor binding. GPCR binding was found only in our top 10 ontologies enriched in the MPS II network.

The top enriched ontology present in the MPS II network is the Calcium signaling pathway, which is also impaired in Niemann-Pick C, Gaucher Type 1, Fabry, and Mucolipidosis type IV [25]. Also, the FC gamma receptors are part of the immunoglobulin superfamily that contribute to the immune system processes. The FcR activation induces the p38 and NF Kappa Beta cascade in neurons, glial cells, endothelial cells and in infiltrating leukocytes. The endothelial cell dysfunction leads to the instability of the blood-brain barrier permeability [57].

Regarding the gene hub analysis, the top ranked gene, G Protein Subunit Gamma 7 plays a role in the regulation of adenylyl cyclase signaling in certain regions of the brain [66]. The related pathways of this gene are G-protein complex, and EPO-induced MAPK pathway, inhibiting the mTOR pathway to induce autophagy and cell death, and also inhibiting cell division by the deregulation of actin cytoskeleton [45]. Other top ranked gene, the Proenkephalin (*PENK*), a component of synaptic vesicles, released into the synapse, helps to modulate the perception of pain, and is involved in brain processes relevant to hippocampal functions [14]. In neurological diseases, the aberrant expression of *PENK* is related to dementia in Parkinson disease [32], and with cognitive impairments in the murine model of Alzheimer's [51].

In MPS II, as in MPS VII, the neurological impairment may be severe, with rapidly progressing phenotypes or even mild to absent, presenting slowly progressing phenotypes [10]. Alzheimer disease shares several pathways deranged in MPS II, like neuroactive ligand receptors interaction pathway, calcium and insulin signaling. These pathways are molecular biomarkers for the cognitive decline in the hippocampus of Alzheimer [29]. We hypothesize that in MPS II these mechanisms also participate in cognitive function and could be biomarkers of neurological disease in MPS II.

4.3. MPS type IIIB

Two MPS IIIB datasets (GSE23075 and GSE15758) were included in our analysis and hence are discussed together. Lemonnier et al. [44] used patient-derived iPSC to model neuronal defects in MPS IIIB (GSE15758). The authors showed perturbations in pathways related to Golgi organization, cell migration, inflammation and neuritogenesis. In the MPS IIIB network, we found several processes related to immune response, such as activated TLR4 signaling, innate immune response, and complement activation. Several studies have described the role of immune system processes in the brain pathogenesis of MPS IIIB [20,40,62,34].

Regarding the hub genes for the GSE23075 network, the top genes participate in Acetylation and Gene expression processes, Innate immune system pathways, Lysosomal pathways, and mRNA splicing – major pathway. *GPKOW* encodes a putative RNA-binding protein which interacts directly with protein kinase -A and -X and is also found associated with the spliceosome. Mutations in this gene cause congenital microcephaly and growth retardation [15]. In the MPS IIIB network, this gene is also associated with neuron differentiation, and may serve as a biomarker for neuron and brain damage. Other hub gene, *CTSF*, a cysteine lysosomal protease, was identified with an altered expression in Alzheimer and Parkinson patients and is supposed to play an important role in the autophagic and endo-lysosomal pathway and in the ubiquitin-proteasome system in neurodegenerative diseases [75].

The most enriched pathways of GSE25075 reveal perturbations in the AKT signaling pathway, Oxytocin and Ras signaling pathway. The PI3K/AKT/mTOR signaling pathways are responsible for the regulation of signal transduction, apoptosis and several cellular processes in neurodegenerative diseases [88]. In MPS III, the accumulated HS in neurons and glial cells leads to neuronal apoptosis and microglia-mediated phagocytosis caused by oxidative stress and neuroinflammation [4]. The oxytocin signaling pathway and the oxytocin receptors (OTR) are GPCR which activate the MAPK/ERK1-2 and Ras pathways. In the brain, the oxytocin signaling mediates and controls social behaviors [13]. One of the many symptoms of MPS IIIB patients are impaired social skills and aggressive behavior, probably associated with speech delay and hear-

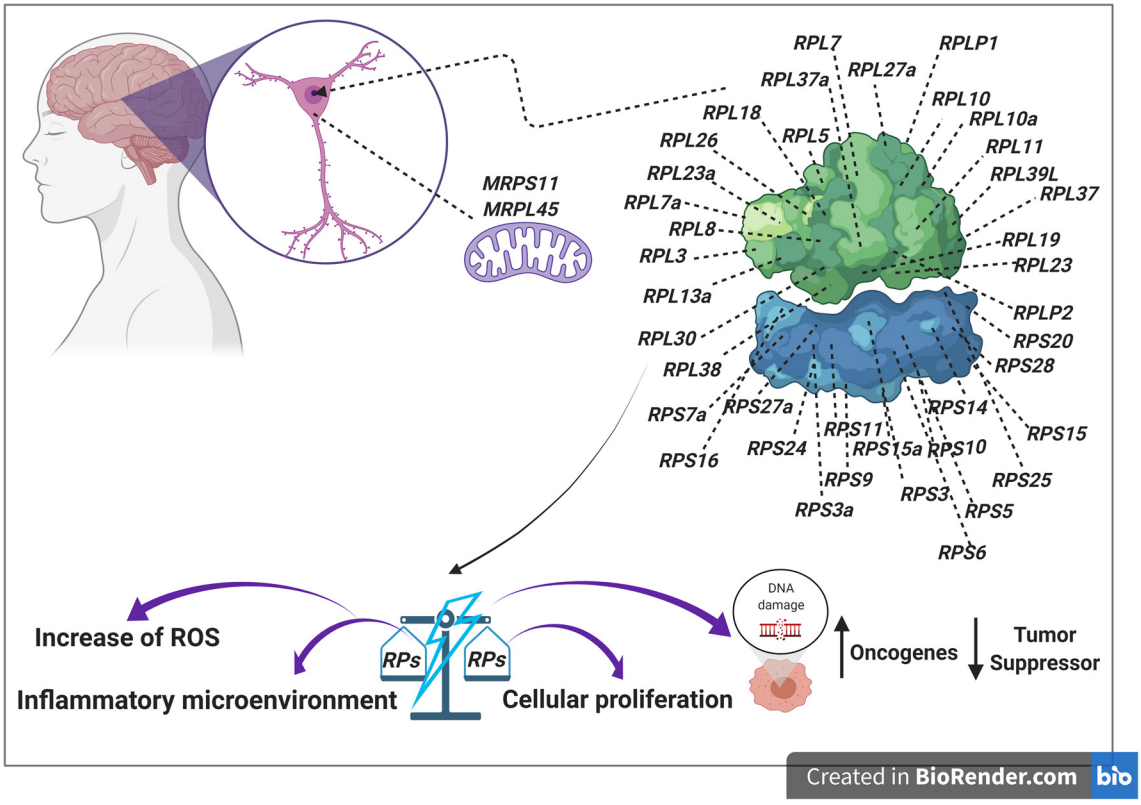

Fig. 7. Schematic representation of the localization of ribosomal proteins. On the right, the genes represented in this figure are differentially expressed in the datasets analyzed in our study. On the left, functional groups of the ribosomal proteins identified in the transcriptomes and the related functions. Below this, diagrammatic representation of the consequences of ribosomal protein perturbations in cells. Perturbations in the ribosomal proteins due to increase of reactive oxygen species (ROS), creation of an inflammatory response, disturbances in the proliferation, and DNA damage. This damage leads to increase of concentration of oncogenes, and decrease levels of tumor suppressor genes. (Figure created in Bio Render, https://biorender.com/.)

ing loss [23]. The perturbation of oxytocin signaling pathway and downstream pathways may contribute to explain the behavioral aspects of MPS IIIB.

The hub genes of GSE15758 belong to the same protein family, the Ribosomal Protein S and L, that encodes ribosomal 40S and 60S subunits. Altered ribosomal proteins lead to defects in ribosome biogenesis, resulting in ribosomal stress, and activation of the p53 signaling pathway, leading to p53-dependent cell cycle arrest and apoptosis [89]. Ribosomal Proteins (RPs) play a role in several biological processes, like regulation of programmed cell death, modulation of DNA repair, modulation of cell migration, regulation of angiogenesis [89,92]. The activation of p53 pathway mediated by RP causes decreased body size, skeletal anomalies, and central nervous system malformations in the murine model. Besides, cardiovascular and metabolic diseases are also associated with perturbations in RPs [89].

Indeed, in the original work, MPS IIIB in mice is described as a tauopathy [58]. Hyperphosphorylated tau was found in neurons of the medial entorhinal cortex, and in the dentate gyrus. The authors suggest that lysozymes induce the hyperphosphorylation of tau, but this mechanism is not well understood. In fact, HS plays an important role in protein aggregation in neurodegenerative diseases, like Alzheimer's and Parkinson [50]. Another important relationship is between the tau and ribosomes. Koren and colleagues [41] propose that tau pathology impacts translation, thus disturbing synaptic plasticity, cellular metabolism, and memory formation. These tau-mediated impairments in translation could explain the role of tau in neurological diseases, including MPS IIIB.

Zhou et al. [95] described the involvement of ribosomal proteins in various pathways and phenotypes. They suggest that ribosomal stress provokes accumulation of ribosome-free ribosomal proteins, which act in ribosome-independent functions like tumorigenesis, immune signaling, and development. Fig. 7 summarizes the functions and biological pathways of ribosomal proteins in the literature, focusing on the genes present in all datasets analyzed in our study.

4.4. MPS type VII

Parente and collaborators [60] showed that the top differentially expressed genes participate in apoptosis, immune response, GPCR signaling pathway, and vesicle mediated transport. We also found these pathways in our analysis of the MPS VII network. Furthermore, the analysis of hub genes demonstrated Serpin Family A Member 3, and Lysozyme genes as hub genes of the MPS VII network. Upregulation of *SERPINA3N*, member of the serine protease inhibitor class, induces neuroinflammation and astrocyte activation, and causes hippocampal neuron loss, epilepsy-like seizures, and memory deficits in mice [86]. Lysozymes show a protective role against amyloid-β in patients with Alzheimer disease [31].

GO analysis reveals pathways common to the MPS II network, like Insulin and Calcium signaling. These pathways are deranged in Alzheimer and are related with neurodegeneration [29]. The insulin growth factor regulates cell growth, mitochondrial processes, autophagy, oxidative stress, synaptic plasticity, and cognitive function [35]. Lysosomes can store Calcium and participate in the Calcium signaling. Medina et al. [48] demonstrated that lysosome controls autophagy via calcineurin-mediated induction of *TFEB*, a master transcriptional regulator of lysosomal biogenesis and autophagy.

Vesicle mediated transport in neurons is associated with the SNARE complex, and plays an important role in synaptic plas-

ticity, in addition to mediating the exocytosis of neurotransmitter receptors [82]. Another pathway related to the MPS VII network is FC gamma receptor signaling pathway. These receptors act as a crosslink between the adaptive and innate immune system, generating signals to the lysosomes and proteasomes to initiate molecule degradation [52,55].

Regarding the GPCR signaling pathway, a ligand of these receptors is apelin, a neuropeptide responsible for diverse physiological and pathological processes [46]. The apelin signaling is deranged in the MPS VII network. The Apelin-13 activates the PI3K/Akt and ERK1/2 signaling pathways to ameliorate brain lesions in mouse [90], and has a neuroprotective effect against apoptosis activating AMP-kinase pathway in an ischemia animal model [91]. These pathways are also deranged in the MPS VII network and suggest clues about using Apelin to treat the neurological damage, not only in MPS VII, but in all the MPS with neurological symptoms.

5. Conclusion

Glycosaminoglycans play an essential role in the extracellular matrix composition, helping to regulate several processes, and interact with a wide range of receptors important to cell communication, recognition, and maintenance of cell integrity. We hypothesize that the primary accumulation of GAGs leads to perturbation of several biological processes and pathways, as demonstrated in this work.

Network analysis can be useful to discover the impaired pathways underlying the several processes deranged in the mucopolysaccharidoses. Besides, the detection of subnetworks in disease conditions can provide valuable insight into disease etiology or therapeutic responses [93]. Systems biology approaches may help us to understand the mechanisms of neuropathology in the several types of Mucopolysaccharidoses, and to discover novel biomarkers and treatments for the neurological symptoms of these diseases.

Declaration of competing interest

The authors declare that there is no conflict of interest that could be perceived as prejudicial to the impartiality of the reported research.

Data availability

The datasets used in this study were available in the Gene expression Omnibus, https://www.ncbi.nlm.nih.gov/geo/. All the code used is available at https://github.com/Kur1sutaru/system_biology_mps.

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