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Bioinformatics Analysis Identifies Potential Hub Genes, Therapeutic Agents, and Crucial Pathways in the Pathogenesis of Refsum's Disease

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Abstract: Refsum's disease is an infrequent, inherited, autosomal recessive condition. The purpose of this study was to investigate prospective therapeutic drugs to cure Refsum's disease and to determine the critical genes/pathways through bioinformatics analysis. Using the text mining method, Refsum's disease-related genes were discovered using MCODE plugin, and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were used to examine modules. A protein–protein interaction (PPI) network was created by STRING and visualized in Cytoscape. Drug–gene interactions were investigated using the drug–gene interaction database. Using the text mining of gene (TMG) method, 64 distinct genes in Homo sapiens linked to Refsum's illness. There were 71 edges and 48 nodes in a PPI network. Thirteen hub node genes were discovered using a cluster analysis of filtering nodes. The genes were then significantly elevated in protein transmembrane transport, protein import to the peroxisome matrix, and fatty acid beta-oxidation, according to the enrichment analysis. We discovered that two already-approved medications could target 2 of the 13 chosen genes. The findings revealed the hub genes for Refsum's illness to be SLC27A2, AGXT, GNPAT, CRAT, HSD17B4, AMACR, CAT, SCP2, PEX13, PEX10, PEX1, PEX7, and PEX2. Additionally, ibuprofen and alcohol were named as prospective therapeutic agents for the management and therapy of Refsum's illness. Only bioinformatics tools were used in this study; further, in vitro and in vivo studies are required because phenotypic variations will change among individuals based on the gene's expressivity.

Keywords: Refsum's disease, drug discovery, text mining, hub genes, MCODE

1. Introduction

The peroxisomal biogenesis spectrum of illnesses encompasses the unusual autosomal recessive condition called as Refsum's disease. The numerous membrane-bound intracellular organelles, that is, peroxisomes, are used to catalyze a number of processes in cellular metabolism and biosynthesis. Refsum's illness is primarily caused by the presence of faulty genes for particular enzymes. Refsum's illness is a fairly uncommon condition. The prevalence of Refsum's illness is unknown with precision. Sigvald Refsum first identified the rare autosomal recessive syndrome known as Refsum's disease (hereditary motor and sensory neuropathy type IV) in 1945 [1]. Bone malformation retinitis pigmentosa, sensory neuropathy, hard hearing, and ataxia are the hallmarks of adult Refsum's illness [2–5].

Peroxisomes are membrane-bound intracellular organelles that perform diverse roles in cellular metabolism, including alpha oxidation, beta-oxidation, and catabolism of fatty acids (branched-chain), some of the amino acids along with ethanol, as well as biosynthesis of bile salts and cholesterol present in the brain's white matter. Refsum's disease is an autosomal recessive condition characterized by abnormalities in peroxisomal biogenesis that affect both the beta-oxidation and alpha-oxidation pathways [6]. In comparison to the time- and money-consuming process of developing novel pharmacological remedies, repurposing an existing medication to address a disease other than its original purpose may be a more efficient and affordable option [7]. Text mining of biomedical literature is a helpful method for creating hypotheses since it can unveil novel connections among genes and disorders [8, 9]. Together with biological awareness and advanced analytical techniques, text mining can be used to obtain new information on the potential for repurposing existing medications [10]. This study sets out to explore therapeutic targets and innovative drug therapies for Refsum syndrome using biological databases, other analytical tools, and the available published literature.

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2. Methods

2.1. Array of key genes employing text mining analysis

Text mining analysis has been carried out by maneuvering pubmed2ensembl (http://pubmed2ensembl.ls.manchester.ac.uk) to find genes associated with Refsum's illness. This database connects 150k Ensembl genes from 50 species to approximately 2 million articles in PubMed publications [11, 12]. For the purpose of data extraction, it also pinpoints relationships between the literature and genes. The search terms "Refsum's disease" and "Refsum Peripheral Neuropathy" have been implemented from 100,000 relevant document IDs to generate a catalog of important genes related to Refsum's disease. To minimize overlapping genes associated with other eye diseases, numbness, and peripheral neuropathy, the search terms employed were limited. "Homo sapiens (GRCh37)" was carefully chosen as the species dataset, and "filter on MEDLINE: PubMed ID" was used to limit the results of interest. The text mining of genes (TMGs) were retrieved as the intersection of gene hits from the three sets, and the unduplicated genes were then removed. The approach flowchart and an outline of the study are shown in Figure 1.

2.2. Evaluation of biological processes as well as pathway enrichment

Using GeneCodis, a web-based platform, the TMGs produced via text mining have been examined for biological process (BP) annotations [13]. The genes involved in the enrichment process were further examined for functional connections utilizing the TMGs as input and genes with markedly enriched BPs associated with Refsum's illness have been chosen [14]. The TMGs were utilised to create a network of functional interactions between genes using GeneMania (version 3.5.2). The automatically chosen network weighting function, max resultant genes = 20, and max resultant characteristics = 10 were the advanced statistical options used. The generated network included genes that are most similar to the original list along with functional annotations from Gene Ontology (GO).

2.3. Network construction and module analysis for protein–protein interactions

Using STRING (version 11.5), (https://string-db.org/) a database containing almost 24,600,000 proteins and more than 3,100,000,000 interactions from 5,090 different species, protein–protein interaction (PPI) network analysis was used to create a network of 48 restorative genes based on the Gene Ontology or GO. Nodes possessing a significant betweenness centrality are considered to be vital as they are comparable to heavily trafficked crossroads on major routes or bridges, whereas nodes with a higher degree are known to be crucial because they may coincide with pathogenic genes. By using the Cytoscape, various hub genes were located in the network and, further hub genes and major gene modules (clusters) from the PPI network were explored using the built-in plugin Molecular Complex Detection (MCODE, version 2.0.2) [15–19]. "Degree cut-off = 2", "node score cut-off = 0.005", "k-core = 2," and "max depth = 100" were the cut-off values.

2.4. Drug–gene interactions

Using major module genes as prospective targets, the data were taken from the drug–gene interaction database (DGIdb, Version 4.2.0) (www.dgidb.org) [20]. Further, PubChem (https://pubchem.ncbi.nlm.nih.gov) has been used to obtain the chemical composition of discovered medications. It has thousands of macromolecular targets related to about 25,000,000 distinct chemical structures and 90,000,000 bioactivity conclusions.

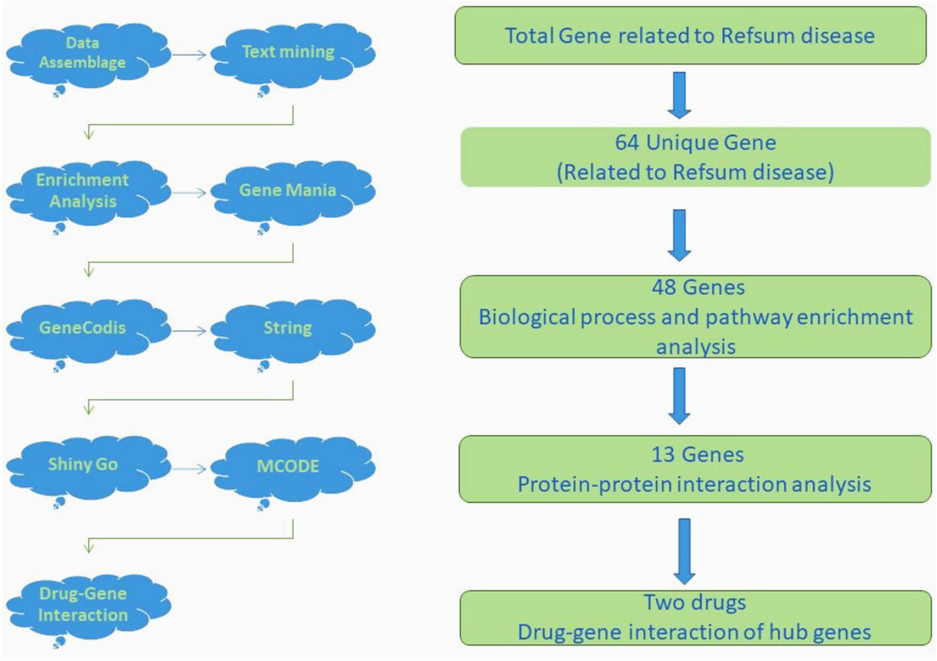

Figure 1. Flowchart summarizing the study's design and methodology

3. Results

3.1. Screening of potential genes

Using the TMG method, we discovered 64 distinct genes in Homo sapiens linked to Refsum's illness. The 64 TMGs evaluated by GeneMania are shown in Figure 2 along with their network, genetic relationships, pathways, and co-expression analysis. Based on their Kyoto Encyclopedia of Genes and Genomes (KEGG) profiles, 48 genes were chosen as potential genes for further investigation.

3.2. Enrichment analysis of TMGs

By employing GeneCodis, BP, KEGG, and GO, the expressions that are closely associated with the pathogenesis of Refsum sickness were discovered. The examination of the KEGG annotations revealed 48 highly enriched genes. The five utmost enriched functions involved 18, 3, 4, and 5 TMG, respectively and were "peroxisome" ($P = 2.61e-22$), "primary bile acid biosynthesis" ($P = 9.57e-03$), "cholesterol metabolism" ($P = 1.08e-02$), "insulin resistance" ($P = 1.69e-02$), and "fatty acid metabolism" ($P = 1.39e-01$). Overall, GO enrichment analysis identified 15 key pathways involving various TMGs.

The ten most significantly enriched pathways were "peroxisome organization" (P =4.28e-14), "fatty acid beta-oxidation" ($P = 3.83e-11$), "very long-chain fatty acid metabolic process" ($P = 2.84e-08$), "protein import into peroxisome matrix" ($P = 4.28e-08$), and "lipoprotein metabolic process" ($P = 4.69$ e-06), "fatty acid metabolic process" ($P = 7.52E-05$), "very long-chain fatty acid catabolic process"($P = 8.85e-05$), "lipoprotein

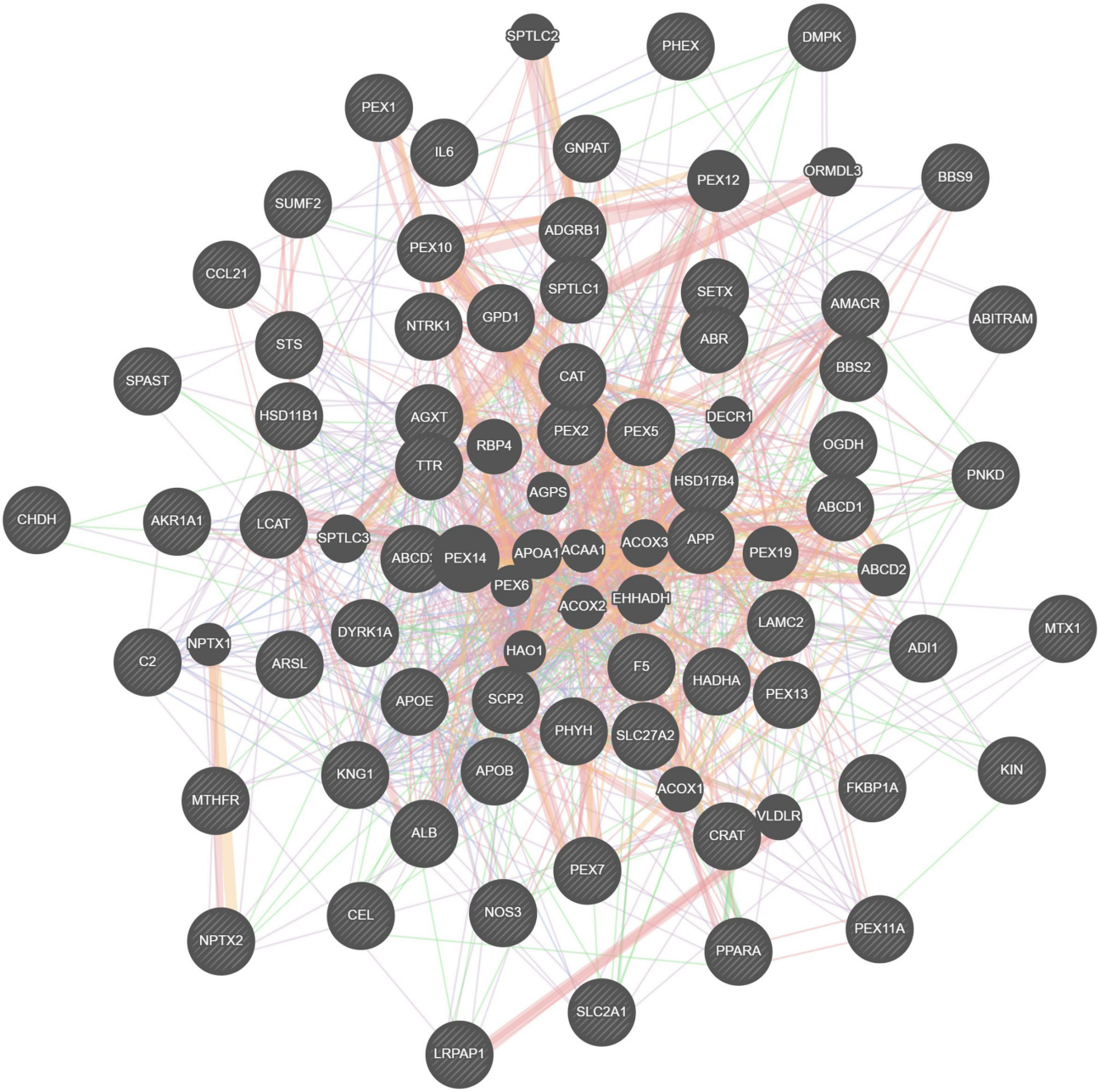

Figure 2. Network of protein–protein interactions among all TMGs associated with Refsum

Table 1. Top 15 enriched Gene Ontology biological process (BP) terms attributed to the text mining genes

BP	Query set genes	Overall genes in genome	P value	Genes
Peroxisome organization	9	24	4.28e-14	ABCD1, ABCD3, PEX1, PEX1, PEX11A, PEX2, PEX5, PEX7, SCP2
Fatty acid beta-oxidation	9	50	3.83e-11	ABCD1, ABCD3, HADHA, HSD17B4, PEX2, PEX5, PEX7, SCP2, SLC27A2
Very long-chain fatty acid metabolic process	6	22	2.84e-08	ABCD1, ABCD3, HSD17B4, PEX2, PEX5, SLC27A2
Protein import into peroxisome matrix	5	11	4.28e-08	PEX1, PEX10, PEX2, PEX5, PEX7
Lipoprotein metabolic process	5	26	4.69e-06	APOB, APOE, APP, LCAT, PPARA
Fatty acid metabolic process	8	205	7.52e-05	AMACR, CRAT, GNPAT, HADHA, HSD17B4, PHYH, PPARA, SLC27A2
Very long-chain fatty acid catabolic process	3	6	8.85e-05	ABCD1, ABCD3, SLC27A2
Lipoprotein biosynthetic process	3	6	8.85e-05	APOB, APOE, LCAT
Fatty acid alpha oxidation	3	7	1.37e-04	PEX13, PHYH, SLC27A2
Bile acid biosynthetic process	4	25	1.40e-04	ABCD3, AMACR, SEP2, SLC27A2
Lipid metabolic process	13	809	2.49e-04	AKRIA1, APOB, APOE, CEL, CRAT, HADHA, HSD11B1, HSD17B4, LCAT, SCP2, SLC27A2, SPTLC1, STC
Protein targeting to peroxisome	3	10	3.51e-04	PEX1, PEX5, PEX7
Fatty acid beta-oxidation using acyl CoA oxidase	3	12	5.90e-04	PEX1, PEX5, PEX7
Microtubule based peroxisome localization	2	2	1.86e-03	PEX1, PEX13
Cholesterol metabolic process	5	105	2.00e-03	APOB, APOE, APP, CAT, LCAT

biosynthetic process" (8.85e-05), "fatty acid alpha oxidation" ($P = 1.37e-04$), and "African trypanosomiasis" ($P = 2.34e-01$). Table 1 demonstrates various GO terms, and the KEGG analysis of the TMGs' numerous enriched molecular pathways is shown in Table 2.

3.3. PPI network construction, modular analysis, and key genes identification

For the 48 target genes, STRING was employed to build a PPI network with a high confidence score of > 0.900. The degree of the gene's node border's color (a node with a light green border indicates the smallest degree of association, while one with a dark green border indicates the strongest degree of association) reveals its function in the development of the eye. The network (Figure 3) contained 48 nodes and 71 edges. By utilizing a cluster analysis of filtering nodes, 13 hub node genes were recognized among 48 nodes (Table 3). The hub genes acknowledged are SLC27A2, AGXT, GNPAT, CRAT, HSD17B4, AMACR, CAT, SCP2, PEX13, PEX10, PEX1, PEX2, and PEX7.

Based on GO similarity, the hub genes REVIGO analysis identified nine clusters, the majority of which were associated with fatty acid beta-oxidation, membrane organization, peroxisome organization, starvation response, and pexophagy (Figure 4). Two modules were produced by the MCODE-based modular analysis. A total of 13 genes are included in the PPI network; module 1 (SLC27A2, AGXT, GNPAT, CRAT, HSD17B4, AMARC, CAT, SCP2) contained 8 genes displaying 71 edges, and module 2 (PEX13, PEX10, PEX1, PEX7, PEX2) contained 5 genes with 203 edges (Supplementary Figure 1 and Supplementary Figure 2). The pathway enrichment analysis performed by employing KEGG, and the ShinyGo platform revealed that the genes in module 1 were related to the catabolic processes of organic acids, monocarboxylic acids, fatty acids, and bile acids (Supplementary Figure 3). Protein targeting to peroxisomes, peroxisomal membrane transport, protein localization to peroxisomes, and intracellular protein transmembrane transport were all substantially correlated with the module 2 genes (Supplementary Figure 4). Altogether, the enrichment analysis showed that these genes were significantly enriched in fatty acid beta-oxidation, protein import to peroxisome matrix, and protein transmembrane trafficking.

3.4. Drug–gene interaction analysis of core genes

We chose 13 hub genes as possible therapeutic targets in the drug–gene interaction investigation. Overall, 2 of the 13 are possible gene targets, and drug–gene interactions are anticipated for 2 FDA-approved medications (ibuprofen and alcohol) (Table 4).

Table 2. Top enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with the genes using text mining

Refsum-KEGG pathway	Query set genes	Overall genes in genome	Corrected P value	Genes
Peroxisome	18	18	2.61e-22	ABCD1, ABCD3, AGXT, AMACR, CAT, CRAT, GNPAT, HSD17B4, PEX1, PEX10, PEX11A, PEX13, PEX2, PEX5, PEX7, PHYH, SCP2, SLC27A2
Primary bile acid biosynthesis	3	17	9.57e-03	AMACR, HSD17B4, SCP2
Cholesterol metabolism	4	50	1.08e-02	APOB, APOE, LCAT, LRPAP1
Insulin resistance	5	108	1.69e-02	IL6, NOS3, PPARA, SLC27A2, SLC2A1
Fatty acid metabolism	3	57	1.39e-01	HADHA, HSD17B4, SCP2
Complement and coagulation cascades	3	85	1.97e-01	C2, F5, KNG1
Glyoxylate and dicarboxylate metabolism	2	30	1.97e-01	AGXT, CAT
Antifolate resistance	2	30	1.97e-01	IL6, MTHFR
PPAR signaling pathway	3	74	1.97e-01	PPARA, SCP2, SLC27A2
Biosynthesis of unsaturated fatty acids	2	27	1.97e-01	HSD17B4, SCP2
Metabolic pathways	16	1516	1.97e-01	ADI1, AGXT, AKR1A1, AMACR, CAT, CEL, CHDH, HADHA, HSD11B1, HSD17B4, MTHFR, NOS3, OGDH, SCP2, SPTLC1, STS
African trypanosomiasis	2	36	2.34e-01	IL6, KNG1
Glycerophospholipid metabolism	3	98	2.32e-01	GNPAT, GPD1, LCAT
Glycine, serine, threonine metabolism	2	39	2.32e-01	AGXT, CHDH
HIF 1 signaling pathway	3	109	2.34e-01	IL6, NOS3, SLC2A1

4. Discussion

A hereditary motor sensory neuropathy type IV, Refsum's disease, is characterized by the phytanic acid accumulation in plasma, lipid-containing tissues, retinitis pigmentosa, anosmia, blindness, hard hearing, sensory neuropathy, and ataxia [21]. The molecular mechanisms of Refsum's disease must therefore be explored. Our analysis unveils that there are several signaling pathways in Refsum's disease that contribute to a wider variety of therapeutic targets and prognostic biomarkers. In this study, 64 genes were found that might contribute to Refsum's disease development.

The enriched GO and BP terms allocated to these genes were interrelated primarily with peroxisome organization, fatty acid beta-oxidation, protein import into the peroxisome matrix, fatty acid metabolic process, and lipoprotein metabolic process. The enrichment analysis and PPI network identify 13 hub genes, SLC27A2, AGXT, GNPAT, CRAT, HSD17B4, AMACR, CAT, SCP2, PEX13, PEX10, PEX1, PEX7, and PEX2, that were implied in protein import into peroxisome matrix, monocarboxylic acid catabolic process, peroxisome targeting sequence binding, and peroxisome. ClueGO is used to analyze and illustrate the functional pathways of key genes from modules 1 and 2 as displayed in Figure 5(a). The organization of functions and pathways within core genes is illustrated in Figure 5(b), while KEGG pathways and enriched GO terms are illustrated in Figure 5(c). Based on the assessment, eight genes such as SLC27A2, AGXT, GNPAT, CRAT, HSD17B4, AMACR, CAT, and SCP2 were involved in the process of small molecule metabolic process, lipid catabolic process, fatty acid beta-oxidation, and lipid modification. The unimpaired breakdown of phytanic acid is only probable if pristanic acid, the product produced by α-oxidation of phytanic acid undergoes degradation. The deficiency of pristanic acid resulting from pathogenic variants in AMACR blocks pristanic acid oxidation, leading to elevated plasma phytanic acid concentration [22, 23]. A key antioxidant

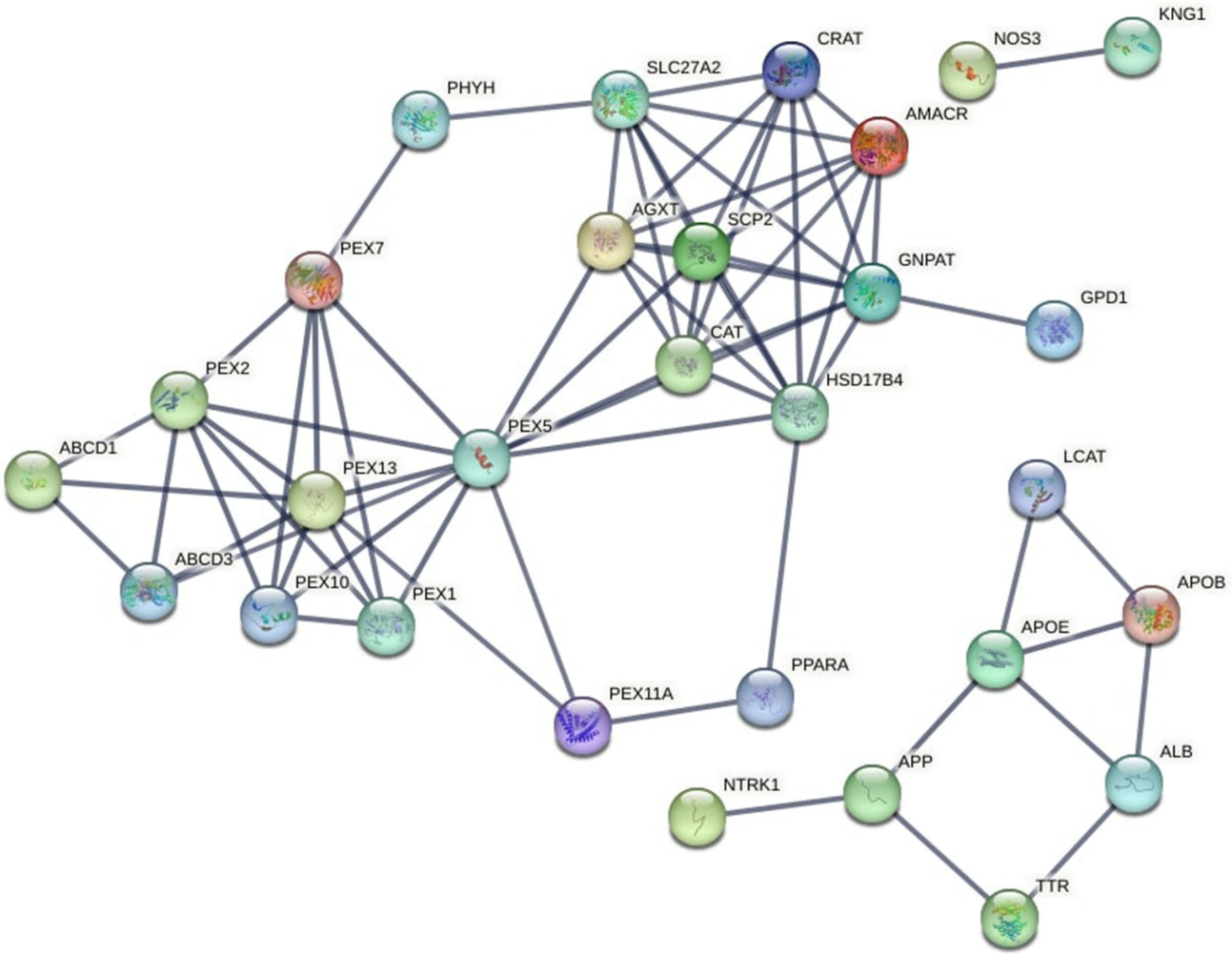

Figure 3. Text mining genes' identification and enrichment analysis

Table 3. Hub node genes in the network of protein–protein interactions

Genes	Degree	MCODE cluster	MCODE node status	MCODE score
SLC27A2	7	Cluster	Module1	7.0
AGXT	7	Cluster	Module1	7.0
GNPAT	7	Cluster	Module1	7.0
CRAT	7	Cluster	Module1	7.0
HSD17B4	7	Cluster	Module1	7.0
AMACR	7	Cluster	Module1	7.0
CAT	7	Seed	Module1	7.0
SCP2	7	Cluster	Module1	7.0
PEX13	4	Cluster	Module2	5.0
PEX10	4	Seed	Module2	5.0
PEX1	4	Cluster	Module2	5.0
PEX7	4	Cluster	Module2	5.0
PEX2	4	Cluster	Module2	5.0

enzyme of the body against oxidative stress is catalase, encoded by the CAT gene. Almost all aerobic cells have catalase enzymes in their peroxisomes that convert hydrogen peroxide to water and oxygen, preventing its toxic effects. Another gene from module 2, that is, PEX protein family, comprises of PEX1, PEX5, PEX7, and PEX10. Out of these four, the PEX7 gene plays a significant role in Refsum's disease. PEX7 assists the phytanoyl-coenzyme A (CoA) hydroxylase in transportation to peroxisome, which aids in the phytanic acid's degradation [24]. The PHYH gene imparts directions for making an enzyme called phytanoyl-CoA hydroxylase, a critical enzyme involved in α-oxidation. Phytanoyl-CoA hydroxylase is part of a process that breaks down phytanic acid in peroxisomes. Hence, enzyme is perilous for the normal function of cell structures called peroxisomes [25].

Another hub gene from module 1, that is, carnitine acyltransferases, effectuates the swapping of acyl groups among carnitine and CoA. These enzymes comprise carnitine acetyltransferase, carnitine octanoyltransferase, and carnitine palmitoyl transferases (CPTs). CPT-I and CPT-II are critical for the β-oxidation of long-chain fatty acids in the mitochondria by enabling their transport [17, 26]. The drug interaction analysis found two compounds of interest that are categorized as Non-Steroidal Anti-inflammatory Drugs NSAIDs and Central Nervous System CNS depressants.

The under-expression of the gene (AMACR) causes deficiency of the α-methylacyl-CoA racemase (AMACR) enzyme, which is

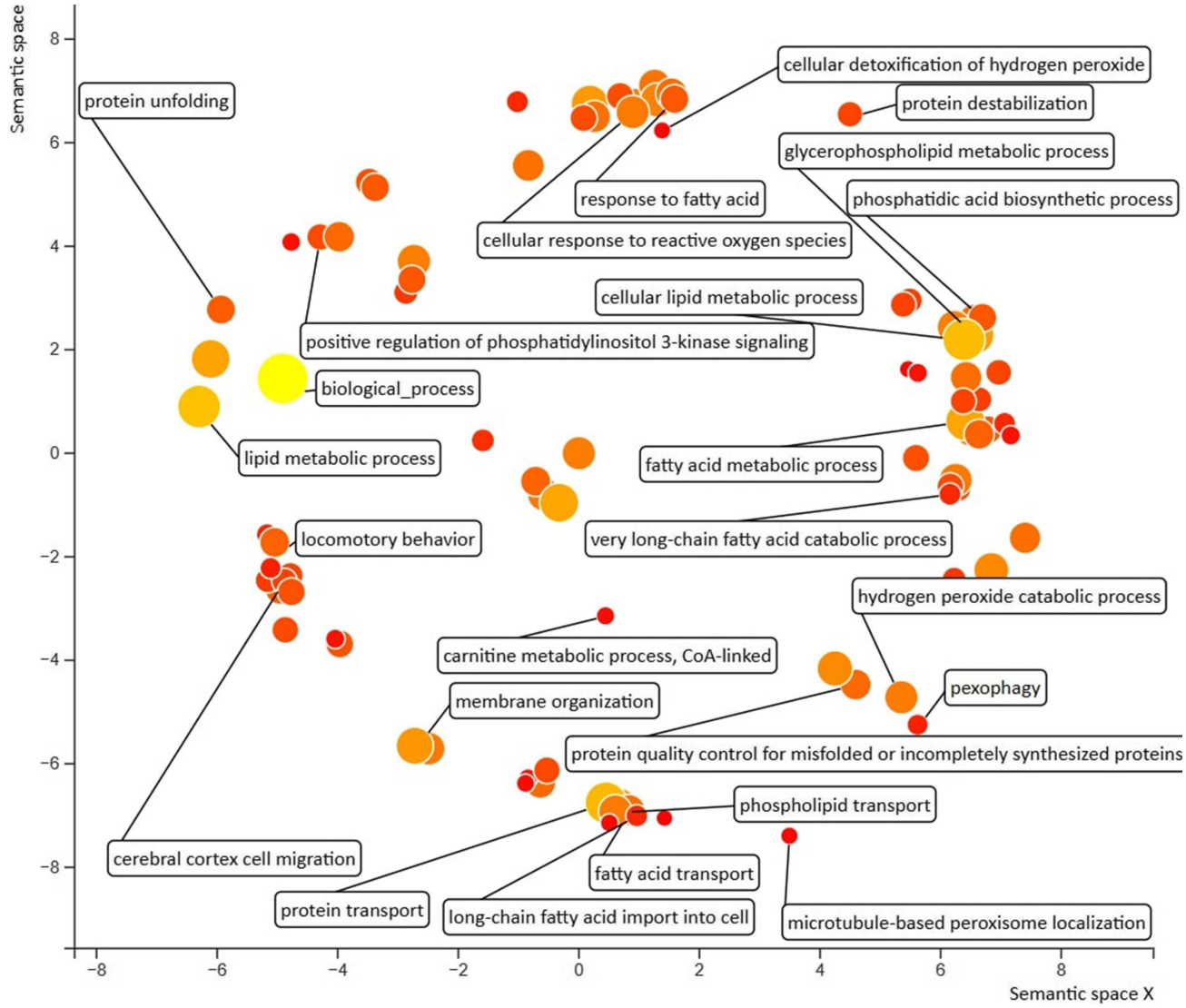

Figure 4. Gene Ontology (GO) terms of the 13 hub genes. The enriched GO keywords associated with vascular remodeling and eye morphogenesis are represented in the figure

Table 4. Information on the two FDA-approved medications that may target two of the 13 hub genes

Drug	Gene	Interaction score	Drug class	Approved	PubMed Id
Ibuprofen C13H18O2	AMACR	3.86	NSAIDs	Yes	25502615
Alcohol C2H5OH	CAT	1.44	CNS depressant	Yes	25514903

accountable for beta-oxidation of phytanic acid that leads to accumulation of phytanic acid and ultimately causes sensory-motor neuropathy, pigmentary retinopathy, upper motor neuron signs in the legs, and adrenomyeloneuropathy. So, one of the drugs that is ibuprofen activates the gene (AMACR) by promoting the action of PPAR alpha, which involves fatty acid oxidation and helps in phytanic acid oxidation in Refsum's disease that resolve ichthyosis, sensory neuropathy, ataxia, hearing loss, and anosmia. CAT gene encodes catalase enzyme, which helps in conversion of acyl CoA to tran-2-enoyl-CoA, which is an important step of beta-oxidation. The under-expression of the gene (CAT) inhibits the oxidation of phytanic acid, which results in sensory neuropathy and retinopathy that is indicative of Refsum's disease. So, another drug, that is, alcohol, oxidizes itself into acetaldehyde and helps in symptomatic relief of polyneuropathy and anosmia but it does not express the gene CAT.

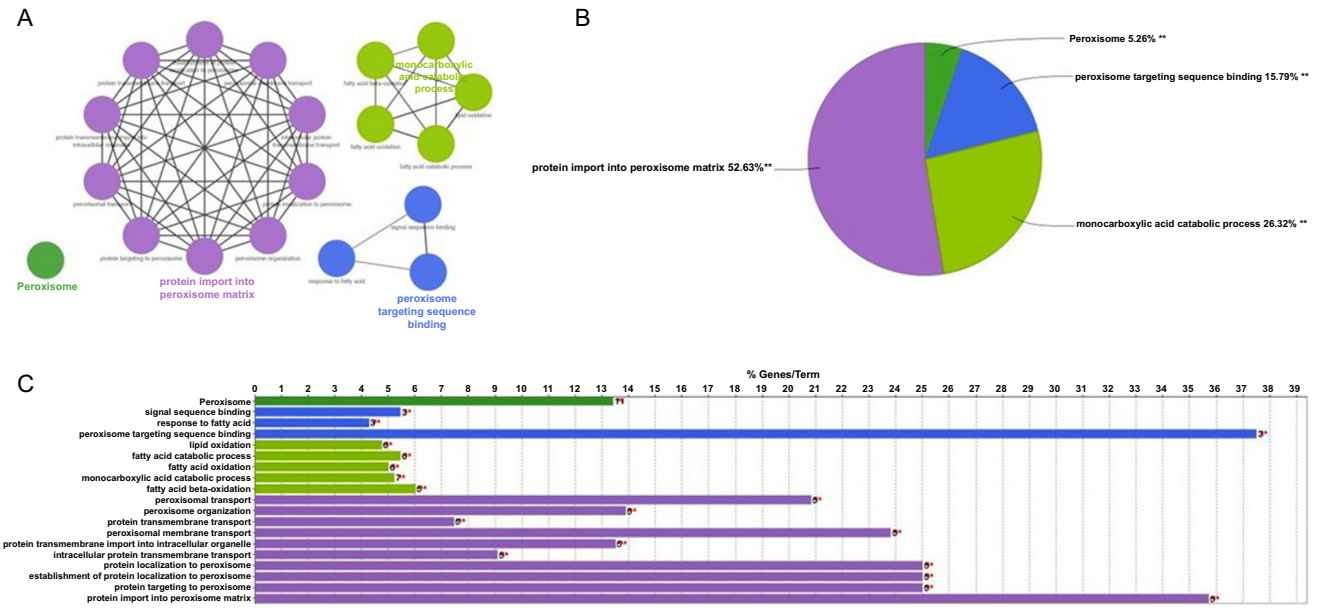

Figure 5. (a) Function analysis of the 13 core genes in module 1 and module 2. (a) Functions and pathways of the core genes were visualized using ClueGO. (b) Distribution of the functions and pathways among the core genes. (c) KEGG pathways and enriched GO terms, colors are assigned to each pathway. Corrected *P*-value of less than 0.01 was deemed statistically significant

5. Conclusion

So, from the above in silico analysis by employing different web servers and software, we conclude that the genes such as SLC27A2, AGXT, GNPAT, CRAT, HSD17B4, AMACR, CAT, SCP2, PEX13, PEX10, PEX1, PEX7, and PEX2 were identified as hub genes that might be concerned with the development of Refsum's disease. These genes seem to be principally allied with functions associated with protein import into the peroxisome matrix, lipid oxidation, response to fatty acid, peroxisomal transport, and transmembrane protein. As per the previous report, the under-expression of the genes (such as AMACR and CAT) manifests vision blindness, sensory neuropathy, deafness, ataxia, and ichthyosis. As Refsum's disease is rare and often autosomal, the present study provides additional insights into the disease when pathology is lacking or when pathway heterogeneity is evident. Hence, combining therapeutic approaches with medical intervention and gene identification may allow for the analysis of biological pathways unique to each case, while also identifying potential drug combinations based on gene products associated with Refsum's disease. Clearly, further research is needed in animal models as well as human patients, as individual variances in phenotype will rely on the expressivity of the corresponding gene product.

Ethical Statement

This study does not contain any studies with human or animal subjects performed by any of the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest to this work.

Data Availability Statement

The data that support this work are available upon reasonable request to the corresponding author.

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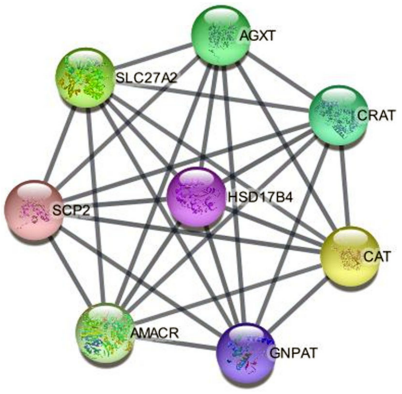
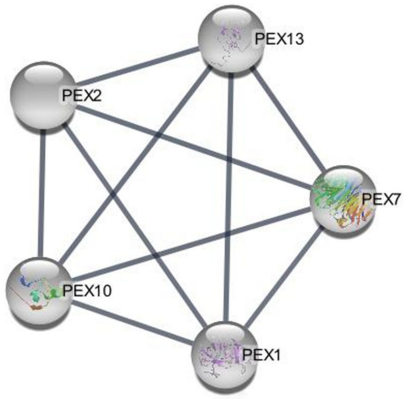
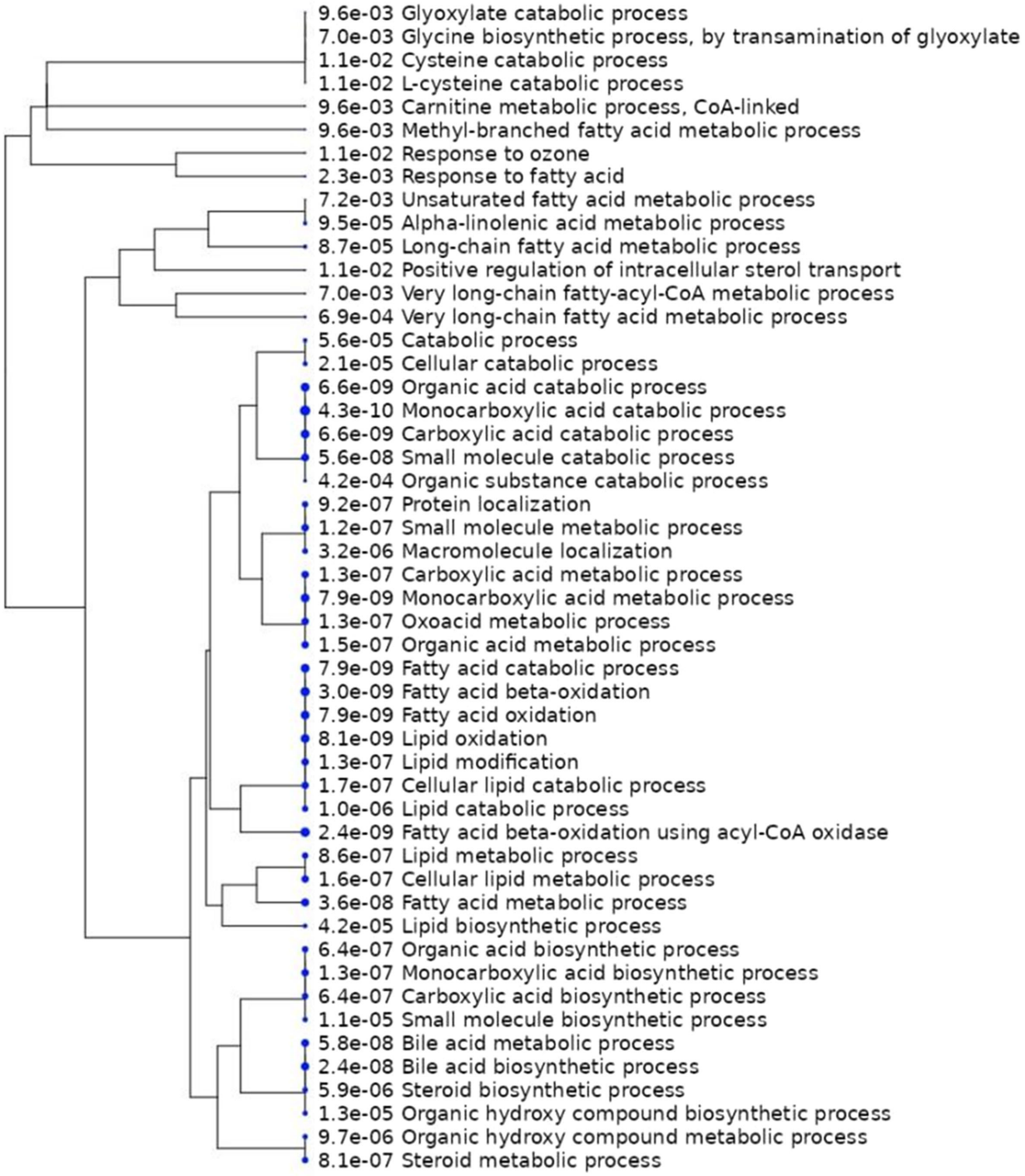
Supplementary Figures



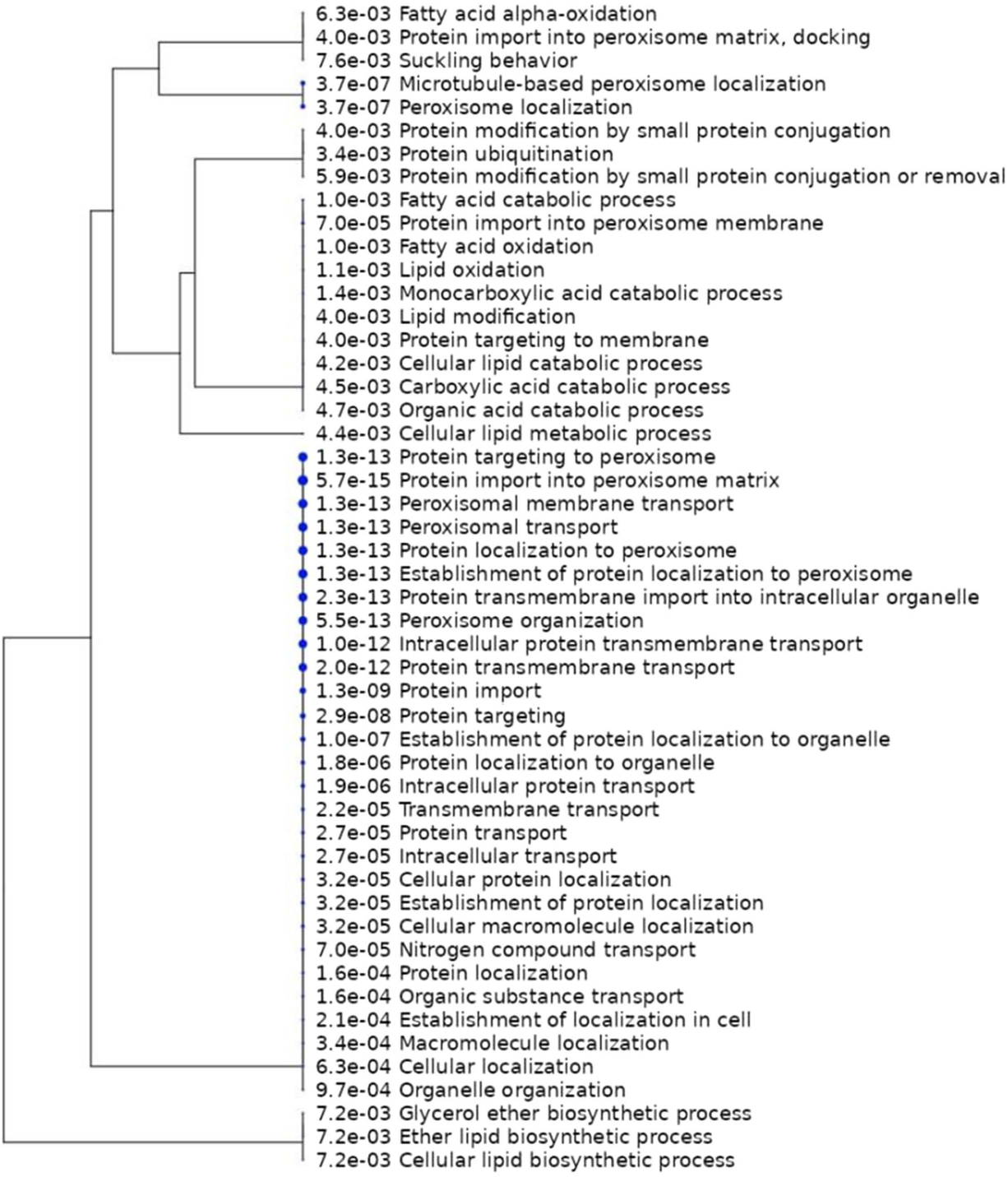
Supplementary Figure 1. Module 1 displaying with eight nodes



Supplementary Figure 2. Module 2 displaying with five nodes



Supplementary Figure 3. Significantly enriched GO terms in module 1



Supplementary Figure 4. Significantly enriched GO terms in module 2