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ORIGINAL ARTICLE

Network Analysis to Identify Key Genes and Drug Targets for Amyotrophic Lateral Sclerosis using *in silico* Approaches

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Abstract

Objectives: The current study describes a comprehensive analysis using bioinformatics to identify the key genes and molecular pathways associated with Amyotrophic lateral sclerosis (ALS), and to evaluate potential therapeutic targets for disease management. **Methods:** ALS-associated genes were identified using GeneCodis, and text mining was performed. Pathways linked to ALS were found using KEGG. A protein-protein interaction (PPI) network for the Target Multi-Gene (TMG) set was created in Cytoscape and STRING. MCODE was utilized to group key modules. The DAVID tool was used to discover gene ontology and conduct KEGG analysis on key genes. ClueGO and ShinyGO illustrated the roles and operations of clustering gene keys within a large module. The DGIdb assessed drug interactions between key genes to uncover viable ALS therapeutics. **Findings:** A total of 47 genes linked to ALS were identified. The gene functional enrichment study revealed 41 elevated gene GO terms, as well as 12 pathways from KEGG. A single gene unit was constructed using the MCODE-based PPI network structure, featuring 18 nodes and 146 edges. We identified eight FDA-approved drugs that could target seven of the 21 key genes, which may provide a theoretical basis for advanced personalized therapy. **Novelty:** This study revealed a total of 21 key genes, 12 pathways, and 8 drug candidates that could provide the basis of novel targeted therapies for targeted treatment and management of ALS.

Graphical Abstract: Schematic representation of evaluating and identifying key genes, molecular pathways, and drug targets

Keywords: Amyotrophic lateral sclerosis; Pathways; Protein-protein interaction; Drug-gene interaction; Key genes

1 Introduction

Patients with ALS have progressive, adult-onset, localized muscular weakness and atrophy, which spreads with the disease. Most typically, weakness begins in the limb muscles, primarily in distal muscles rather than proximal muscles. Symptoms such as dysarthria, dysphagia, dysphonia, and muscle weakness are present in 30% of cases. The symptoms and worsening rate of ALS vary with age at onset. ALS is persistently progressing in most people, with a median lifespan of around three years following symptoms, and death is usually due to respiratory failure. Around fifty percent of ALS patients may develop extra-motor symptoms that cause motor problems, and 10 to 15% will be diagnosed with FTD, also known as frontotemporal dementia. In between 35 and 45 percent of cases, there will be moderate behavioral alterations. FTD is a neurological disorder that involves the temporal region degeneration that appears as cognitive anomalies, mental agility weaknesses, and difficulties with speech [(1)]. The improved El Escorial, as well as Awaji criteria, are commonly used during the clinical diagnosis of patients suffering from ALS. Both requirements utilize different radiological results in their diagnosing models. So, the role of radiography in the progression of ALS patients is primarily to figure out additional potential causes for the individual's clinical picture; some clinical imaging has been associated with upper motor neuron dysfunction, which is an important feature of ALS for the diagnosis and understanding of pathogenesis. People with ALS depend on magnetic resonance imaging (MRI) and neuroimaging. According to investigations, iron gets accumulated in the precentral gyrus of people with ALS.

In both ALS (40%) and FTD (25%) patients, hexanucleotide (G4C2) expansion in the Chromosome 9 open reading frame 72 (C9orf72) gene is followed by mutations in the superoxide dismutase 1 (SOD1) gene, TARDBP gene, encoding TAR DNA-Binding Protein-43 (TDP-43) gene, and fused in Sarcoma (FUS) gene. Interestingly, genetic mutations in ALS patients affect genes such as C9orf72 (7% of cases), TARDBP, SOD1, FUS, VCP, p62, PFN-1, MATR3, OPTN, UBQLN2, CHCHD10, TBK1, TUBA4A, NEK1, C21orf2, and CCNF [(2)],[(3)]. Approximately 5-10% of ALS cases exhibit autosomal dominant inheritance. Genomic analysis has facilitated the identification of disease-causing mutations, which have also been found in patients without a family history of the condition. Overall, known pathogenic mutations can be detected in around 15% of ALS cases. Many of these mutations occur in genes encoding RNA-binding proteins (RBPs), with the most common being TDP-43 and FUS. RBPs control transcription, DNA reaction, mRNA splicing, RNA equilibrium, microRNA production, translation, transport, and stressed granule formation. TDP-43 is a protein that results in the abnormal accumulation of itself in the cytoplasm. TDP-43 protein aggregates were found in 97% of ALS patients [(4)]. The current study utilizes biological databases and *in silico* techniques to explore putative molecular processes and characterize the genes responsible for the fatal heterogeneous illness ALS, thus identifying new therapeutic targets. Through this approach, we identified 21 hub genes and associated pathways that play critical roles in ALS pathophysiology. Additionally, our analysis uncovered 26 drug-gene interactions, providing potential avenues for therapeutic intervention and drug repurposing. These findings enhance our understanding of ALS mechanisms and pave the way for targeted treatment strategies.

2 Methodology

2.1 Identifying Important Genes through Text Mining Investigation

The PubMed database was utilized during text-mining research to discover the 47 genes that are associated with ALS, uncovering connections among the genes, as well as studies in science for collecting data. In the future, it is a resource that is freely available to everyone to access and hyperlinks further two billion papers plus fifteen thousand Ensemble genes spanning 50+ organisms. It utilized search phrases with the objective of compiling a set of the most essential genes. The query criteria have been altered to prevent genetic overlapping connected with several visual disorders. The "Homo" genus data was chosen. This search output has been limited to "filtering using the MEDLINE database: PubMed ID" with "sapiens (GRCh37)". Following the elimination of all the genes that weren't duplicated, the text mining genes (TMGs) have been found to have the junction of two distinct sets' genetic matches. Figure 1 displays the methodology and a quick summary of the study design.

[Pubmed2ensembl was used for text mining to find the genes linked to ALS. Genes enriched in biological processes and KEGG pathways from Gene Ontology (GO) were found using GeneCodis. Software packages STRING and MCODE were utilized to build a network of protein-protein interactions. With the use of DAVID, ClueGO, and ShinyGo, the KEGG pathways and GO biological process terms were examined. The drug-gene interaction database (DGIdb) was used to construct the drug list based on the gene-drug interaction.]

2.2 Evaluation of Biological Component and Network Enrichment analysis

By combining data from several sources, such as the Gene Ontology (GO) a set of terms that explain the products of genes via the Biological Process (BP), Molecular Function (MF), and Cellular Component (CC) and KEGG pathways, which offer

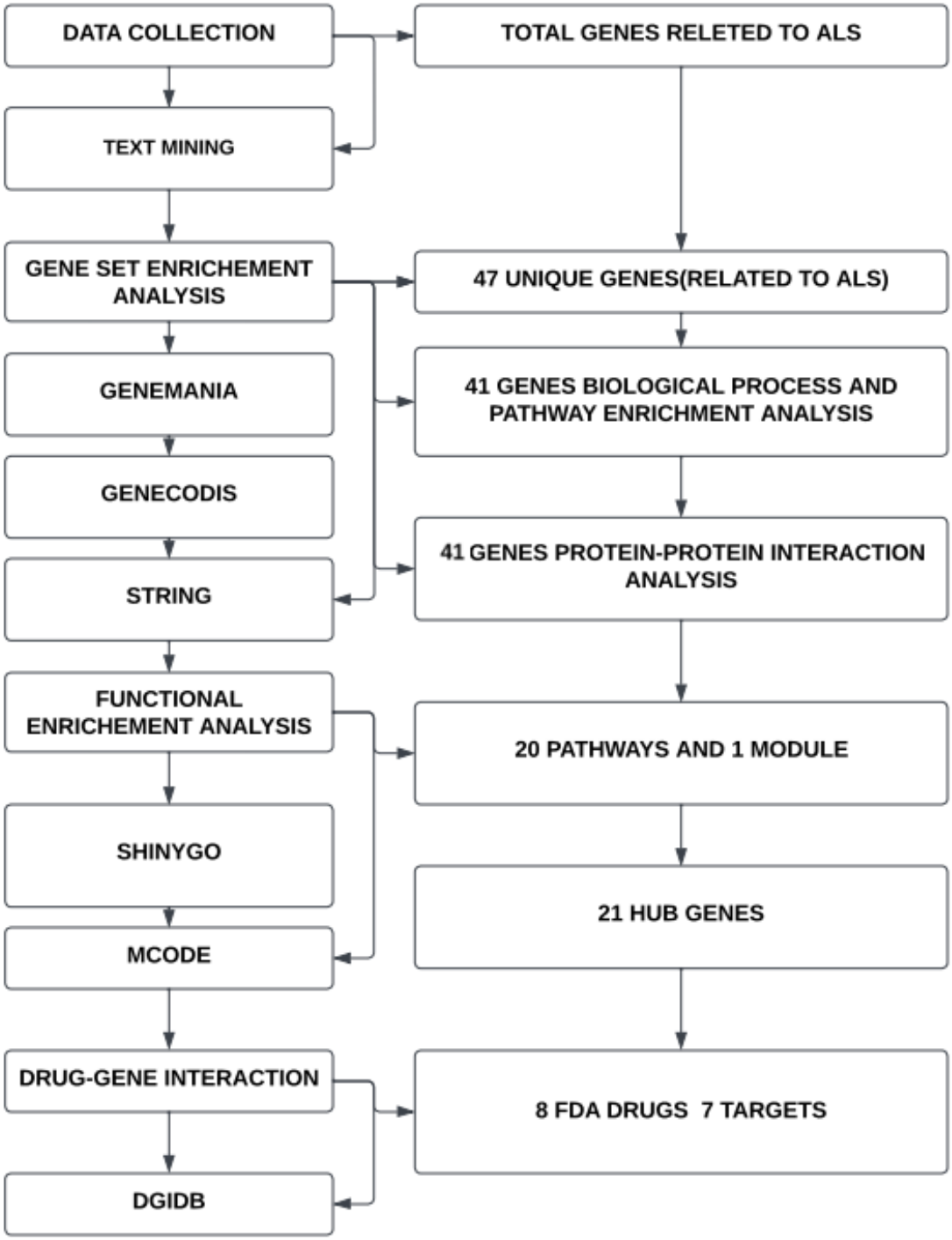

Fig 1. An overview of the methodology flowchart and study design

information on renowned biological process pathways, and InterPro motifs, are evaluated by GeneCodis to analyze the lists of genes operationally. The input data set consisted of TMGs, and genes exhibiting biological processes linked to neuron cellular homeostasis that were highly enriched were chosen based on an adjusted P-value. The GO is a broad classification system that includes CC, MF, and BP. For the current analysis, the BP category is specifically used based on the P-value. KEGG pathway analysis was also carried out based on the P-value, and the findings were examined further. The Cytoscape plugin (version 3.10.1) and GeneMania (version 3.5.2) were used to create gene-to-gene interacting systems using TMGs.

2.3 Protein-Protein Interaction

The STRING (12.0) has been used to build a network using PPI with 87 GO ranked genes in order of priority. Around 24.6 million peptides, in addition to more than 3.1 billion relationships representing 5,090 distinct organisms, are included in the web-based database STRING[5]. The association degree (k), centrality of betweenness (BC), closeness centralization (CC), Eigenvector Connectivity (EC), and eccentricity are the key metrics for nodes in network theory. The primary advantage of PPI network examination is its ability to support a broad spectrum of biological processes, including data and path understanding. Additionally, it offers confidence scores derived from data from experiments, preserved genome areas, gene-fusion events, overlap events, co-expression data, database information, text mining, and homology, among other sources. Key proteins, which are high-degree nodes in the PPI system, are significant molecules because they can connect to genes that bring severe illnesses. Because obstructions, or clusters with lots of BC, resemble routinely frequented regions, they are more likely to contain important genes.

The molecular interactions system was then visualized, and key genes were identified using the Cytoscape software, which integrates the expression of genes, biology systems, and genotypes. In this study, the key nodes obtained a high score based on the networks scale-free feature and were used for centrality analysis by assessing the network topology. Using the sub-network of these essential proteins as the backbone justified additional investigation[6].

The Cytoscape software visualizes the connections between the expression of genes, biological systems, and genotypes. In this study, central nodes emerged with an elevated score based on the network's scale-free characteristic and were used for central assessment by examining the network design routes of signaling involved in amyotrophic lateral sclerosis. Additionally, a built-in Cytoscape plugin, Molecular Complex Detection (MCODE, version 2.0.0), was applied to distinguish the major gene modules (clusters) and gene keys from the PPI network. The threshold values were "degree cutoff=2," "nodescore cutoff=0.2," "k-core=2," and "the maximum depth=100."

Revigo is a web server that summarizes complicated lists of Gene Ontology (GO) concepts by identifying a representative subset of the GO terms using a straightforward clustering technique based on semantic similarity metrics. ShinyGO is a user-friendly, graphical enrichment analysis application that helps researchers extract relevant insights from gene lists. It relies on an extensive annotated collection obtained from Ensembl and STRING-db, which includes 59 plants, 256 animals, 115 archaeal, and 1678 bacterial taxa. The ShinyGO platform was utilized to identify if the genes are substantially enriched or not[7].

2.4 Drug-Gene Interaction

The Drugs-Gene Interactions Directory (DGIdb) is an online resource that gathers data from several sources to demonstrate drug-gene interactions and gene drug activity. They use DGIdb (Version 3.0) to examine drug-gene interactions in significant module genes that could serve as potential targets for current medications or chemicals[8]. The molecular structures for the observed substances have been obtained via the PubChem database. It contains twenty-five million distinct chemical arrangements and ninety million biological activity outcomes for numerous macromolecular domains.

3 Results and Discussion

3.1 Determination of Unique Genes

Using the TMG approach, we identified 47 distinct genes in Homo sapiens that are linked to ALS. The network of genetic linkages is depicted in Figure 2. A co-expression study, as well as routes between the 47 TMGs, were investigated by GeneMania. Based on their GO and molecular networks, 41 genes were chosen for enrichment analysis.

[Blue denotes co-localization, green denotes genetic interactions, pink denotes physical interactions, and orange denotes predicted interactions.]

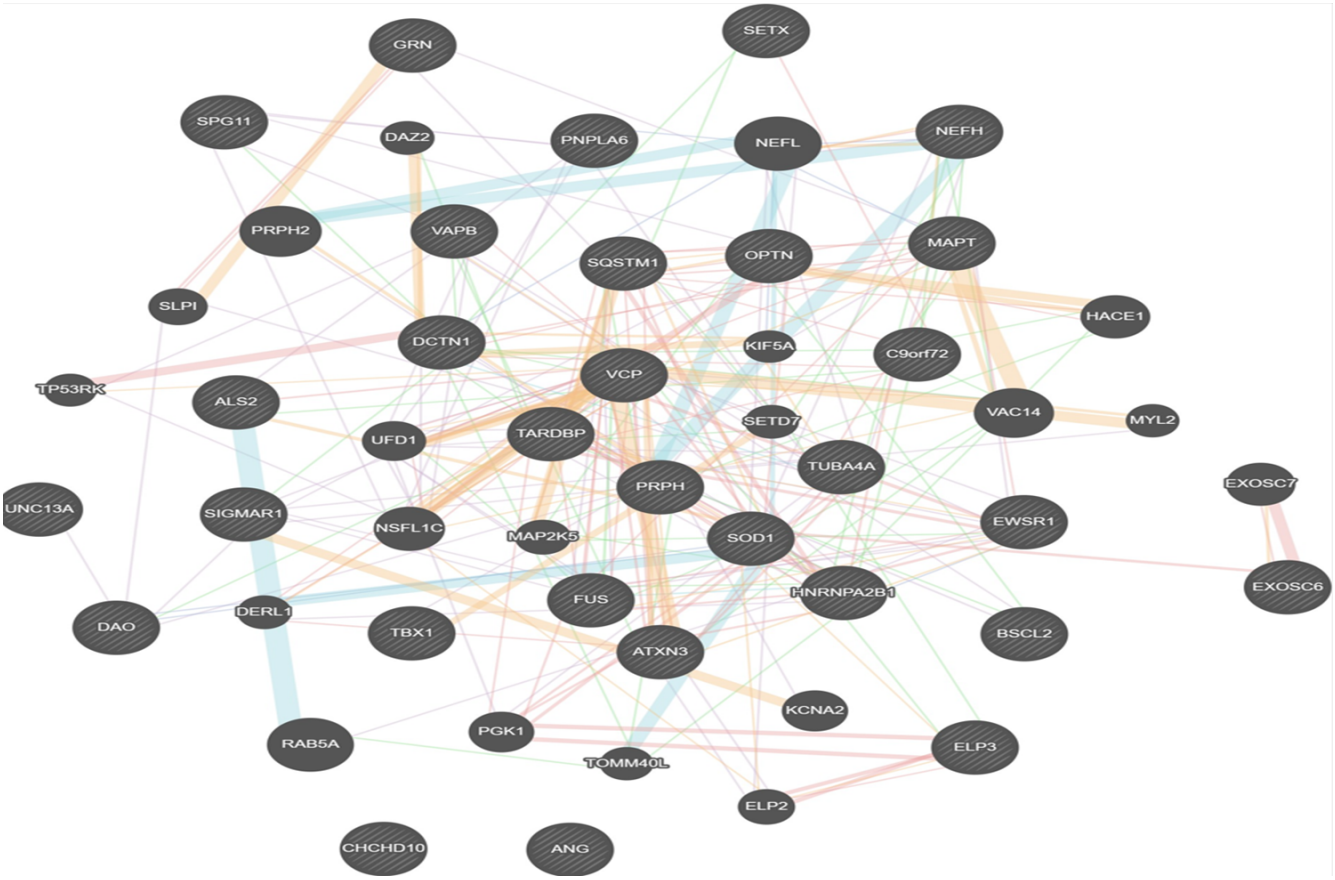

Fig 2. The image illustrates the network, genetic interactions, co-expression, and pathways analyzed with the Cytoscape plugin Genemania.

3.2 Enrichment Investigation of TMG's

The most enriched terminology contributing to ALS was identified using GeneCodis, GO biological process (BP), and KEGG. The GO and BP annotations analysis identified 41 significantly enriched genes. The 15 most enriched functions were "autophagy" (P=1.7956E-12), "neuron cellular homeostasis" (P=2.01601E-11), "stress granule assembly" (P=1.223236E-10), "Axonal transport" (P=3.8025E-10), "neuromuscular junction development" (P=8.690704E-10), "maintenance of synapse structure" (P=1.352569E-8), "neurofilament cytoskeleton organization" (P=1.352569E-8), "Regulation of autophagosome assembly" (P=8.549776E-8), "Macroautophagy" (P=8.67832681E-8), "Gene expression" (P=8.67832681E-8), "Axon development" (P=1.897212249E-7), "late endosome to lysosome development" (P=2.3951236E-7), "Viral release from host cell" (P=3.6772096E-7), "Positive regulation of superoxide anion generation" (P=3.67754919184E-7), "Muscle cell cellular homeostasis" (P=5.4110736E-7), and "RNA metabolic process" (P=5.4110736E-7). Overall, 10 major pathways were discovered by KEGG enrichment analysis. The five most significantly enriched pathways were "Amyotrophic lateral sclerosis" (P=0.0004E-23), "Pathways of neurodegenerative multiple diseases" (P=0.0001E-14), "Mitophagy animal" (P=0.029013), "Transcriptional misregulation in cancer" (P=0.033039), and "mRNA surveillance pathway" (P=0.0499386). Depicts the top 15 enriched GO terms, whereas the top 10 enriched KEGG molecular pathways of the TMGs.

3.3 PPI Network Analysis

STRING was used to construct a PPI network for the 47 text-mining genes with a high confidence score >0.900 (Figure 3). The network consisted of 18 nodes and 143 edges. Cluster analysis was performed using the MCODE plugin in Cytoscape to identify highly interconnected regions. The parameters used were a degree cutoff of 2, node score cutoff of 0.2, k-core value of 2, and a maximum depth of 100. Based on this analysis, 21 key genes were identified. The key genes were ANG, SETX, TARDBP, FUS, SOD1, UBQLN2, ATXN2, OPTN, C9orf72, GRN, PFN1, FIG4, SPG11, CHMP2B, NEFH, MAPT, VCP, DCTN1, SQSTM1,

CHCHD10, and KIFAP3 (Figure 4). The key genes were validated using the ALS Online Database (ALSoD), which classifies genes based on their association with ALS into categories such as definitive ALS genes, clinical modifiers, strong evidence, moderate evidence, and tenuous evidence. Among the ALS key genes, TARDBP, FUS, SOD1, UBQLN2, OPTN, C9orf72, PFN1, VCP, and CHCHD10 are classified as definitive ALS genes due to their strong evidence and established roles in ALS pathology. ATXN2 is recognized as a clinical modifier, as intermediate-length expansions in this gene increase the risk of developing ALS. Genes such as ANG, FIG4, CHMP2B, and SQSTM1 are categorized under moderate evidence, suggesting their associations with ALS have been observed. Other genes, including SETX, GRN, SPG11, NEFH, MAPT, DCTN1, and KIFAP3, fall under the tenuous evidence category, as their links to ALS are less robust or based on limited studies (Table 1).

Table 1. Validation of ALS Key Genes Using ALSoD

Gene symbol	Gene name	Category
ANG	angiogenin	Moderate evidence
SETX	senataxin	Tenuous
TARDBP	TAR DNA binding protein	Definitive ALS gene
FUS	FUS RNA binding protein	Definitive ALS gene
SOD1	superoxide dismutase 1	Definitive ALS gene
UBQLN2	ubiquilin 2	Definitive ALS gene
ATXN2	ataxin 2	Clinical modifier
OPTN	optineurin	Definitive ALS gene
C9orf72	C9orf72-SMCR8 complex subunit	Definitive ALS gene
GRN	granulin precursor	Tenuous
PFN1	profilin 1	Definitive ALS gene
FIG4	FIG4 phosphoinositide 5-phosphatase	Moderate evidence
SPG11	SPG11 vesicle trafficking associated, spatacsin	Tenuous
CHMP2B	charged multivesicular body protein 2B	Moderate evidence
NEFH	neurofilament heavy	Tenuous
MAPT	microtubule associated protein tau	Tenuous
VCP	valosin containing protein	Definitive ALS gene
DCTN1	dynactin subunit 1	Tenuous
SQSTM1	sequestosome 1	Moderate evidence
CHCHD10	coiled-coil-helix-coiled-coil-helix domain containing 10	Definitive ALS gene
KIFAP3	kinesin associated protein 3	Tenuous

The REVIGO analysis of the key genes identified four groups according to GO ontology which were associated with ALS: selective autophagy, neurofilament cytoskeleton organization, RAB protein signaling, and gene expression pathway (Figure 5). The pathway enrichment analysis was done using KEGG and the ShinyGO platform; the genes were associated with autophagy, catabolic process, cellular catabolic process, cellular component, or biogenesis (Figures 6 and 7). The higher the enrichment score, the more vital the enrichment of the gene set associated with specific gene ontology terms or pathways. Enrichment scores higher than $P > 0.005$ indicate that the pathway is more significant. The distinctive characteristics of the 21-key gene list have been compared with the full genome (Figure 8), and Chi-squared tests have been used to evaluate whether the 21 key genes demonstrate specialized features when compared to the entire genome utilizing the ShinyGO platform (Figure 9). In general, the analysis of enrichment revealed that these genes remained significantly enriched in causing ALS.

3.4 Drug-Gene Interaction

For the drug-gene association study, we identified 21 key genes as potential drug targets (Table 2). Overall, seven different gene targets are identified. Among them, eight are FDA-approved drugs, and the genes having interactions with drugs are SOD1, TARDBP, FUS, and C9orf72. VCP and MAPT were the exceptions.

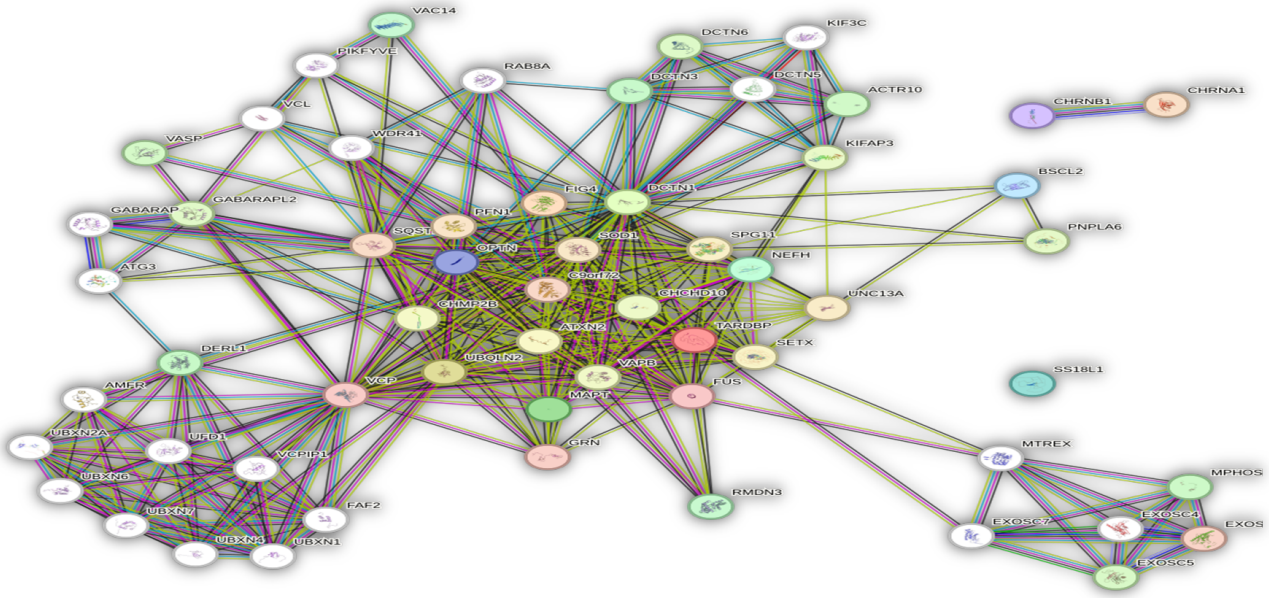

Fig 3. The protein-protein interactions for all forty-seven text-mining genes have been performed via string

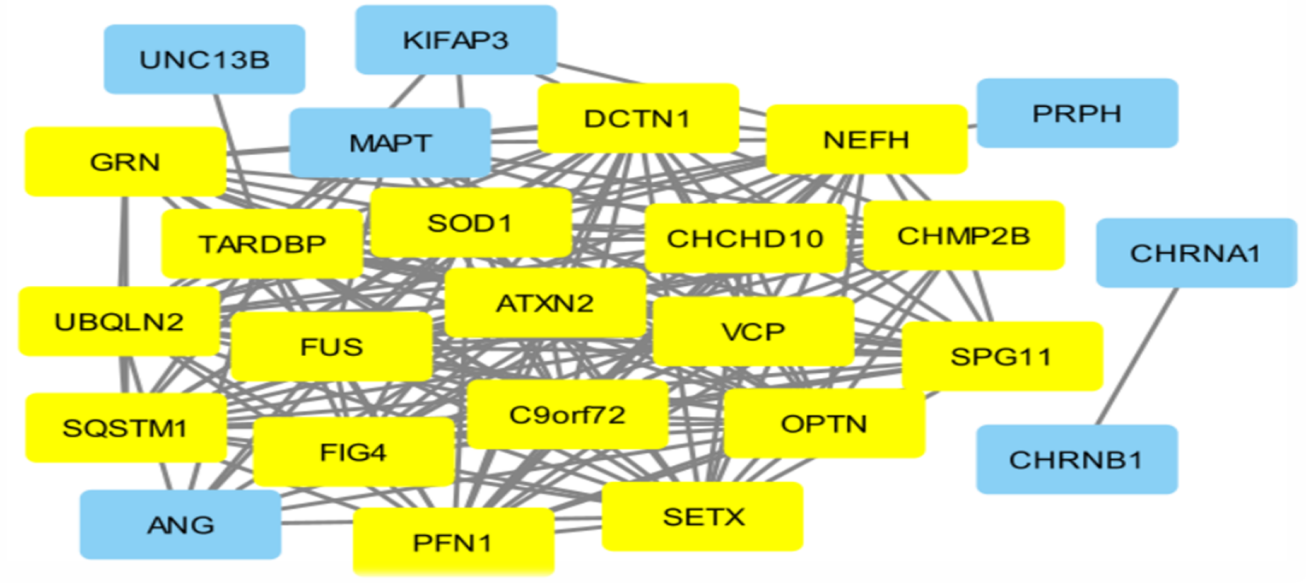

Fig 4. One module was inferred which contains 18 nodes and 146 edges. Also, the key genes were discovered using Cytoscape

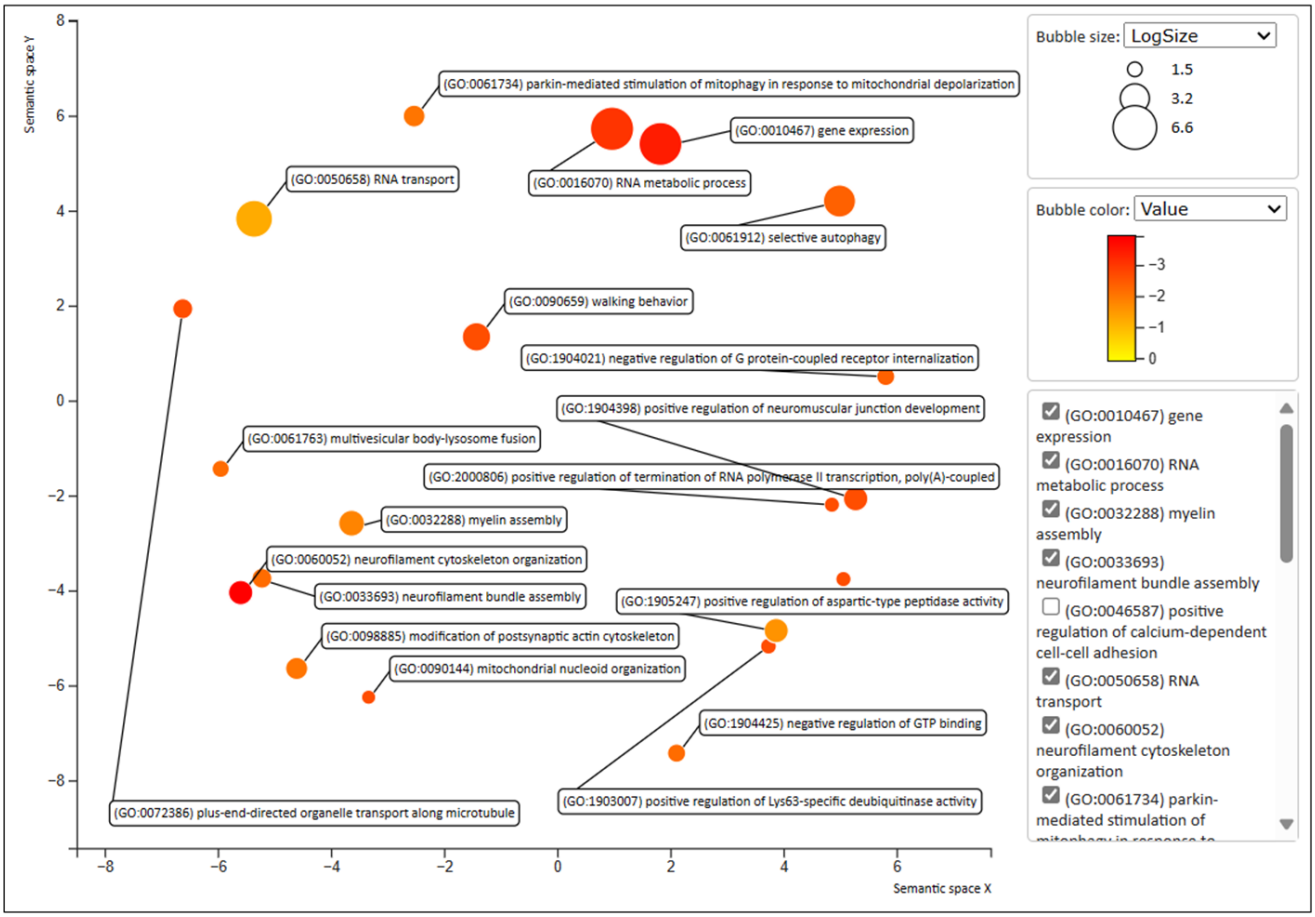

Fig 5. The image displays ALS-related enhanced GO keywords. We utilized REVIGO web server to perform pathway functional enrichment analysis

4 Discussion

ALS is a neurological condition characterized by the degeneration of both lower motor neurons and upper motor neurons. Typically, ALS progresses gradually in most individuals, with an average lifespan of three years following the onset of symptoms; death is often due to respiratory failure. Approximately 50% of ALS patients experience extra-motor symptoms that lead to motor impairments, while 10 to 15% are later diagnosed with FTD. Therefore, it is essential to identify the molecular processes contributing to ALS[(9)]. Our results indicate that the molecular causes of ALS intersect across multiple distinct signaling networks, creating a wider range of possibilities for therapy and prognostic biomarkers[(10)]. In the current study, 47 genes were identified through text mining that may be implicated in the development of ALS. The enhanced GO and BP terms associated with these genes are primarily related to autophagy, neuronal cellular homeostasis, stress granule assembly, axonal transport, neuromuscular junction development, maintenance of synapse structure, neurofilament cytoskeleton organization, regulation of autophagosome assembly, macroautophagy, gene expression, axon development, late endosome to lysosome development, viral release from host cells, and positive regulation of superoxide anion generation[(11)-(12)]. The PPI network and enrichment study identified 21 key genes. The study utilized bioinformatics methods to discover 47 genes linked to ALS and evaluate their biological pathways.

The PPI network analysis revealed 21 key genes that are consistent with previous ALS literature: ANG, SETX, TARDBP, FUS, SOD1, UBQLN2, ATXN2, OPTN, C9orf72, GRN, PFN1, FIG4, SPG11, CHMP2B, NEFH, MAPT, VCP, DCTN1, SQSTM1, CHCHD10, and KIFAP[(13)],[(14)],[(15)],[(16)],[(17)]. These genes are primarily involved in autophagy control, oxidative stress response, protein misfolding, mitochondrial dysfunction, and neuronal cell homeostasis. These processes are essential for maintaining cellular health and function, and disruptions in these pathways can contribute to the development and progression of ALS. Figure 10 illustrates the functional evaluation and networks for the primary genes in a single module, as shown by ClueGO.

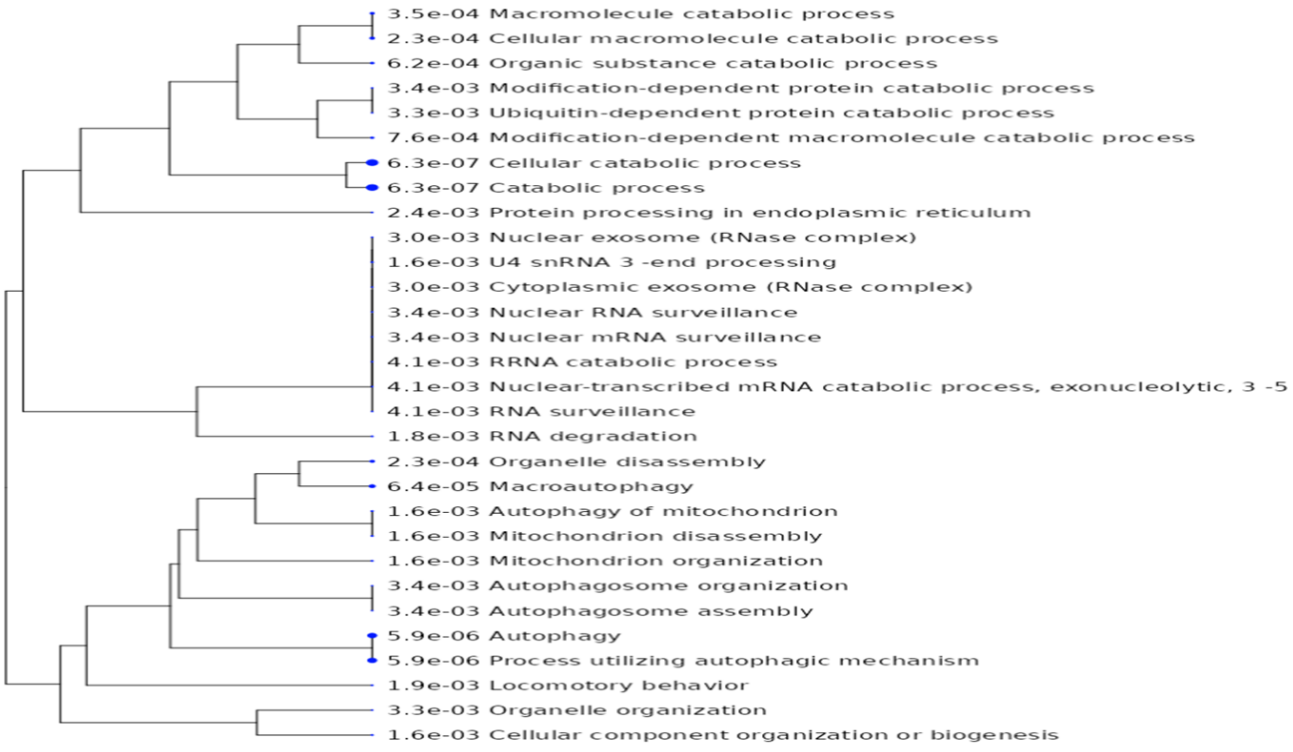

Fig 6. According to a tree visualization generated with the ShinyGo web server, the functional and pathway enrichment examinations in ALS were correlated with a high score of ($P > 0.005$)

According to these findings, the key genes are involved in processes such as autophagy, catabolic processes, cellular component biogenesis, and macroautophagy. The framework of autophagy is particularly crucial for combating and preventing pathogenic insults that could lead to neurodegeneration. The ALS-causing mutation in the C9orf72 gene involves the expansion of a hexanucleotide repeat (GGGGCC). Superoxide dismutase, commonly referred to as superoxide dismutase 1 (SOD1), is a human enzyme produced by the SOD1 gene on chromosome 21. Variations in the SOD1 gene have been linked to ALS, a neurodegenerative disease primarily affecting upper and lower motor neurons. Previous solid-state NMR reveals TDP-43, produced by the TARDBP gene, has also been associated with ALS, a fatal adult-onset disease of motor neurons[18].

Using the ALSoD, we further validated the key ALS genes. TARDBP, FUS, SOD1, UBQLN2, OPTN, C9orf72, PFN1, VCP, and CHCHD10 were confirmed as definitive ALS genes. ATXN2 was identified as a clinical modifier, linked to increased ALS risk. ANG, FIG4, CHMP2B, and SQSTM1 showed moderate evidence, while SETX, GRN, SPG11, NEFH, MAPT, DCTN1, and KIFAP3 had tenuous evidence, indicating weaker links to ALS. These findings emphasize the genetic heterogeneity of ALS and underscore the importance of prioritizing definitive genes while continuing to explore the roles of moderately and tenuously associated genes for a deeper understanding of ALS disease mechanisms.

The identified genes do not have equal significance in ALS, with C9orf72, SOD1, TARDBP (TDP-43), and FUS being the most critical due to their well-established roles in ALS pathology and their higher mutation frequencies. C9orf72 mutations are the leading genetic cause of ALS, accounting for a significant proportion of cases, while SOD1 was the first gene linked to ALS, with mutations leading to toxic protein aggregates. TARDBP is notable for TDP-43 protein aggregation, a hallmark of the disease, and FUS is significant in familial ALS cases. Other genes such as ANG, SETX, UBQLN2, ATXN2, OPTN, and GRN play important roles, albeit to a lesser extent, in processes like angiogenesis, DNA repair, protein degradation, and autophagy. The remaining genes (PFN1, FIG4, SPG11, CHMP2B, NEFH, MAPT, VCP, DCTN1, SQSTM1, CHCHD10, and KIFAP3) are less frequently implicated or less well-characterized in ALS.

The drug-gene interaction study identified 26 medications, categorized as antipsychotic agents, skeletal muscle relaxants, antihypertensive agents, and agents to mitigate EGFR inhibitor-induced dermopathy. Four candidate medications-hydrochlorothiazide, tetracycline, tubocurarine chloride, and pipecuronium bromide were highlighted due to their

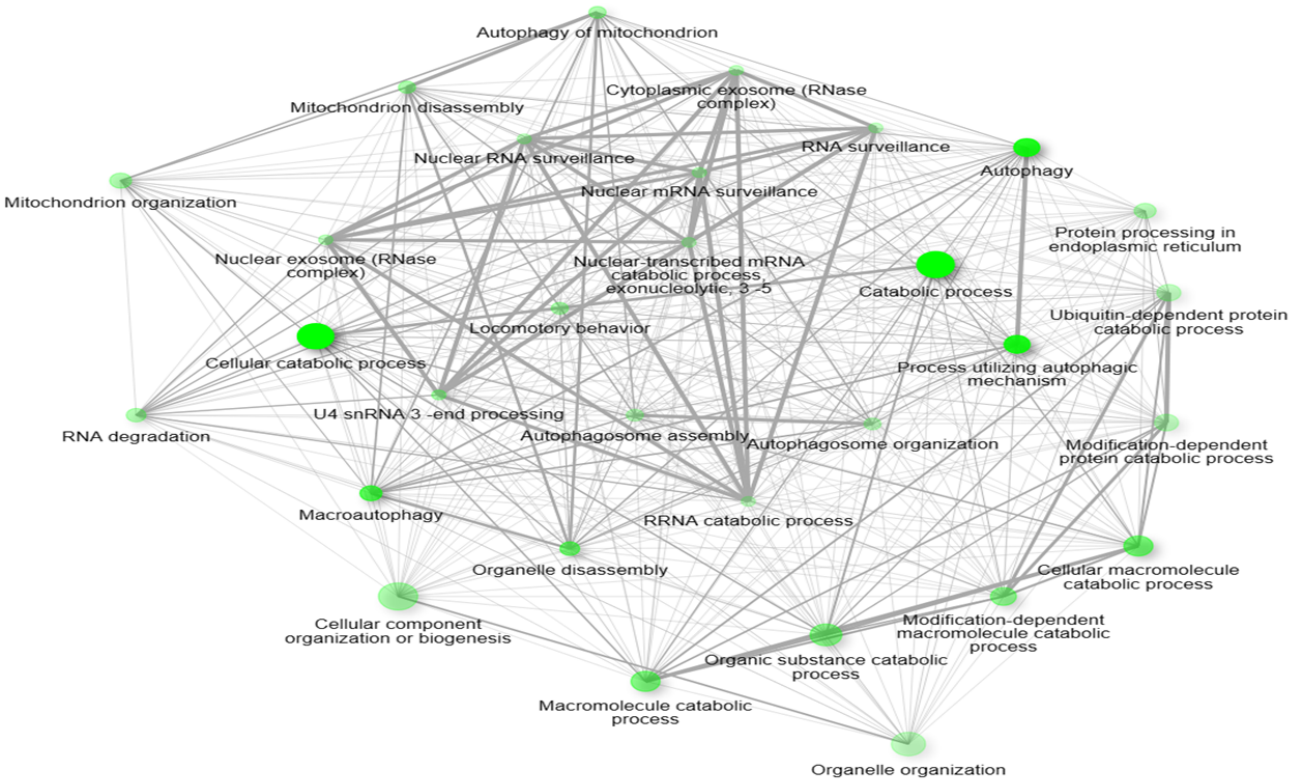

Fig 7. This image depicts network architecture of ALS which was done using SHINYGO

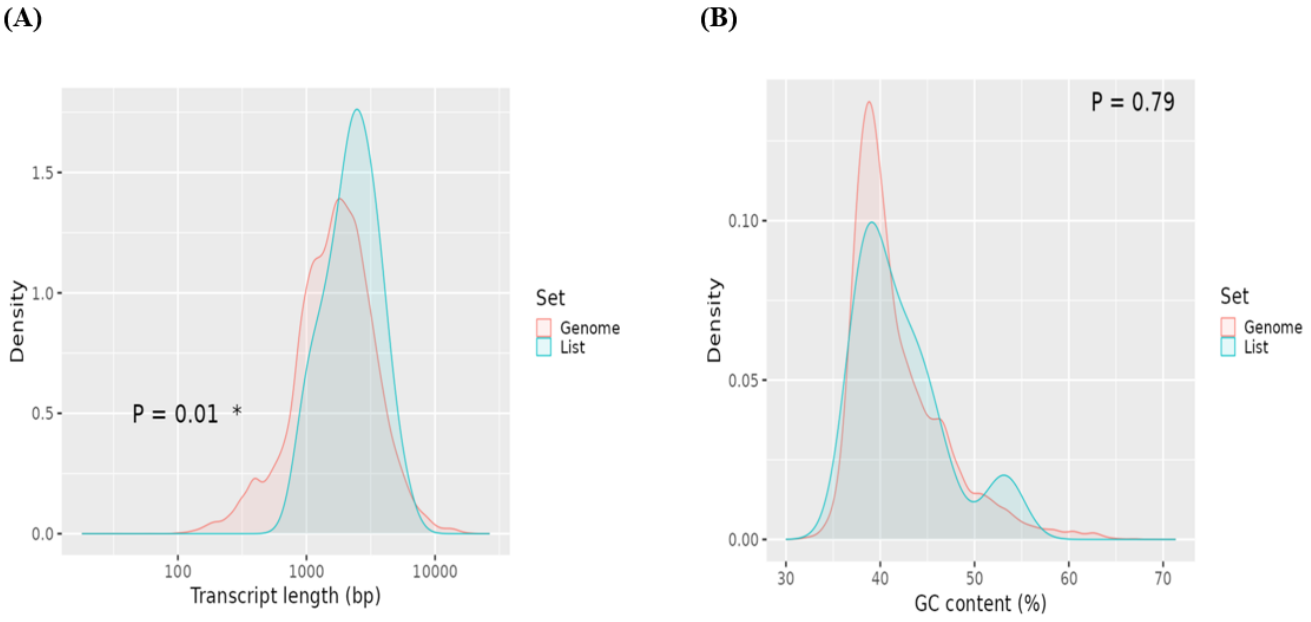

Fig 8. Characteristics of the 21 Key Genes List Compared with the Whole Genome (A) The X-axis (transcript length in base pairs, or bp) represents the length of transcripts in base pairs, while the Y-axis (density) shows the probability distribution of transcript lengths for the gene list compared to the whole genome. (B) The X-axis shows the GC content (as a percentage) of the transcripts in both the genome and the gene list, while the Y-axis shows the probability density of GC content in the gene list compared to the whole genome

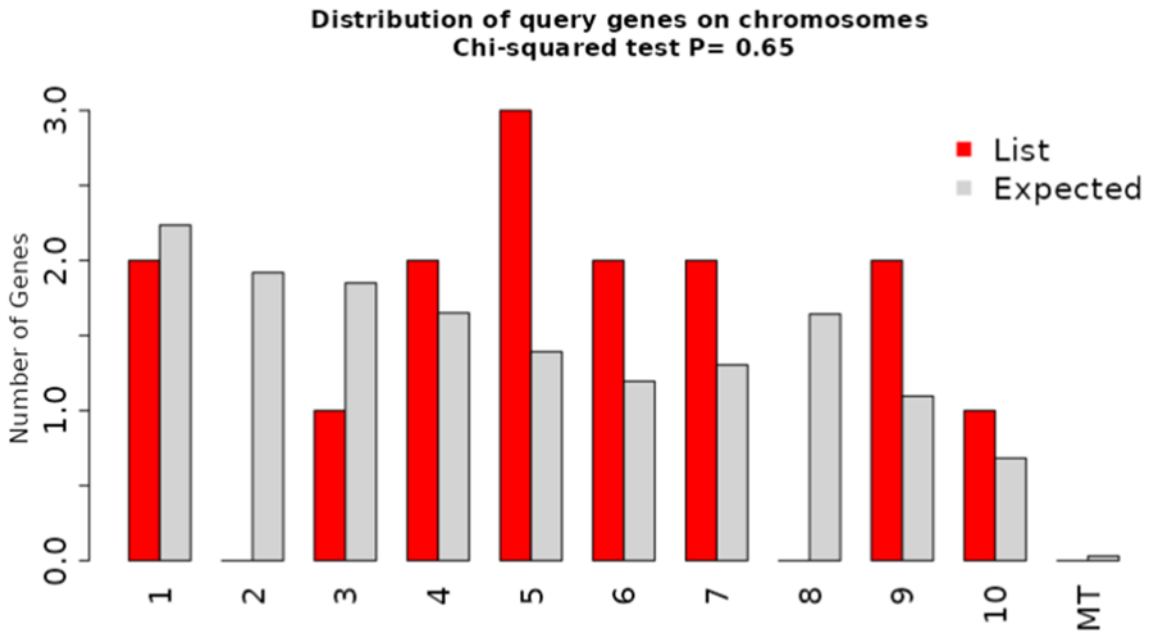

Fig 9. Chi-squared tests were executed to see if the 21 key genes (X-axis) have specialized characteristics when compared with the whole genome (Y-axis)

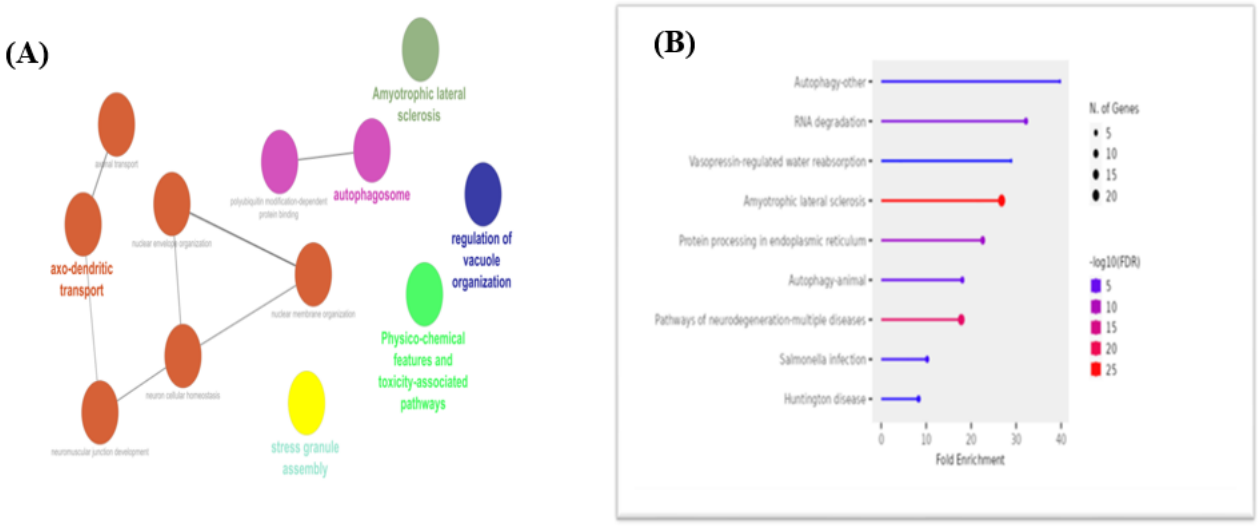

Fig 10. ClueGO was utilized for illustrating the (A and B) major genes' functions and pathways

Table 2. Details of the 26 Food and Drug Administration (FDA) approved drugs that potentially target 7 of the 21 key genes

Gene	Drug	Regulatory Approval	Interaction Score
ATXN2	Thioridazine	Approved	0.01085389
ATXN2	Methixene hydrochloride	Approved	0.05969641
ATXN2	Sertraline hydrochloride	Approved	0.00770276
MAPT	Masoprocol	Approved	0.0029140
MAPT	Inamrinone	Approved	0.0078850
MAPT	Hexachlorophene	Approved	0.0033511
MAPT	Vitamin K3	Approved	0.0046222
VASP	Hydrochlorothiazide	Approved	1.474501347
SOD1	Tetracycline	Approved	0.819167415
SOD1	Doxycycline anhydrous	Approved	0.670227885
SOD1	Oxytetracycline anhydrous	Approved	0.737250674
CHRNB1	Gallamine triethiodide	Approved	0.436889288
CHRNB1	Eculizumas	Approved	0.357454872
CHRNSI	Disatracurium besylate	Approved	0.491500449
CHRNB1	Mivacurium chloride	Approved	0.555333932
CHRN81	Rocuronium bromide	Approved	0.655333932
CHRNB1	Tusocurarine chloride	Approved	0.786400719
CHRNB1	Suconylcholine	Approved	0.393200359
CHRN81	Vecuronium bromide	Approved	0.561714799
CHANGS	Bapacuronium bromide	Approved	0.786400719
CHRN81	Metocurine icoide	Approved	0.655333932
CHANGS	Decamethonium bromide	Approved	0.786400719
CHRINBI	Pancuronium	Approved	0.491500449
CHRN81	Pipecuronium bromide	Approved	0.786400719
CHRINSI	Dokacurium chloride	Approved	0.561714799
CHRNB1	Atracurium besylate	Approved	0.655333932

exceptionally high drug interaction scores (Table 2). While hydrochlorothiazide may not be directly utilized for treating ALS, it can be administered to alleviate hypertension and edema in ALS patients. Tetracycline and its derivatives, such as minocycline, have been extensively studied for their potential therapeutic importance in ALS due to their anti-inflammatory and neuroprotective effects. Recent studies have suggested that tetracyclines, traditionally known as antibiotics, may have neuroprotective properties that could be beneficial in treating neurodegenerative disorders: ALS, Parkinson's, Alzheimer's, and multiple sclerosis disease[19]-[20]. Rose *et al.* (2024) used NMR spectroscopy to investigate the anti-inflammatory effects of tetracyclines in the context of Parkinson's disease[21]. The study found that certain tetracycline derivatives exhibited promising anti-inflammatory properties, which could be beneficial for treating neuroinflammation associated with Parkinson's disease. Tubocurarine chloride, a neuromuscular blocking agent derived from curare, is commonly used to provide muscle relaxation during surgery and mechanical ventilation. It works by inhibiting the activity of acetylcholine at the neuromuscular junction, resulting in muscle paralysis. Pipecuronium bromide is sometimes administered to ALS patients to facilitate muscle relaxation and allow for intubation and mechanical ventilation. The findings of this research could enhance our understanding of the biological pathways involved in ALS and contribute to the development of new treatments. Recent treatment through nanotechnology offers a promising breakthrough in ALS[22]. By integrating multi-omics data and leveraging network-based approaches, researchers can identify key biomarkers, therapeutic targets, and potential drug repurposing opportunities[23]-[24]. This holistic perspective not only enhances our understanding of ALS pathogenesis but also facilitates the development of personalized treatment strategies, ultimately aiming to improve patient outcomes and quality of life. Since this paper addresses an effective approach to discovering molecular pathways and therapeutic options for ALS using *in silico* studies, further research utilizing animal models is strongly encouraged to confirm the significance of the identified genes.

5 Conclusion

This study made a comprehensive *in silico* analysis to identify unique genes and potential drug targets associated with ALS. Utilizing the TMG approach, this study identified 47 distinct genes linked to ALS, which were further analyzed for co-expression and functional enrichment. The enrichment analysis highlighted 41 genes significantly associated with key biological processes, such as autophagy, neuronal cellular homeostasis, and axonal transport, demonstrating the complex molecular pathways involved in ALS pathology. The PPI network analysis revealed 21 key genes that play critical roles in ALS, including ANG, SETX, TARDBP, FUS, SOD1, UBQLN2, ATXN2, OPTN, C9orf72, GRN, PFN1, FIG4, SPG11, CHMP2B, NEFH, MAPT, VCP, DCTN1, SQSTM1, CHCHD10, and KIFAP.

Furthermore, the drug-gene interaction analysis revealed 26 potential therapeutic candidates, including tetracycline derivatives and neuromuscular blocking agents, which may offer new avenues for ALS treatment. Our findings emphasize the genetic heterogeneity of ALS, demonstrating that different genes contribute to disease progression with varying levels of significance. Importantly, C9orf72, SOD1, TARDBP, and FUS were identified as the most critical genes linked to ALS pathology. By integrating multi-omics data and leveraging computational approaches, this study provides valuable insights that could accelerate drug repurposing efforts and enhance biomarker identification for disease prognosis. However, further experimental validation, particularly through animal models**,** is necessary to confirm the biological significance of the identified genes and their potential for clinical application. These findings contribute to a comprehensive framework for understanding ALS at a molecular level, paving the way for innovative therapeutic strategies aimed at improving patient outcomes and quality of life.

6 Abbreviations

ALS - Amyotrophic lateral sclerosis
KEGG- Kyoto Encyclopedia of Genes and Genomes
PPI - protein-protein interaction
TMG - Target Multi-Gene
GGIdb - Drug Gene Interaction Database
STRING - Search Tool for the Retrieval of Interacting Genes/Proteins
MCODE - Molecular Complex Detection.
FDA - Food and Drug Administration
FTD - Frontotemporal dementia
MRI - Magnetic resonance imaging

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