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Integrating Transcriptomic and Structural Insights: Revealing Drug Repurposing Opportunities for Sporadic ALS

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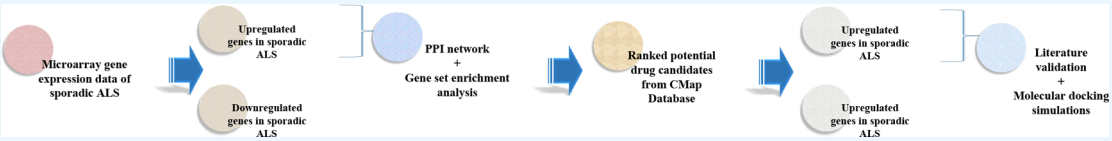

ABSTRACT: Amyotrophic lateral sclerosis (ALS) is a progressive and devastating neurodegenerative disorder characterized by the loss of upper and lower motor neurons, resulting in debilitating muscle weakness and atrophy. Currently, there are no effective treatments available for ALS, posing significant challenges in managing the disease that affects approximately two individuals per 100,000 people annually. To address the urgent need for effective ALS treatments, we conducted a drug repurposing study using a combination of bioinformatics tools and molecular docking techniques. We analyzed sporadic ALS-related genes from the GEO database and identified key signaling pathways involved in sporadic ALS pathogenesis through pathway analysis using DAVID. Subsequently, we utilized the Clue Connectivity Map to identify potential drug candidates and performed molecular docking using AutoDock Vina to evaluate the binding affinity of short-listed drugs to key sporadic ALS-related genes. Our study identified Cefaclor, Diphenidol, Flubendazole, Fluticasone, Lestaurtinib, Nadolol, Phenamil, Temozolomide, and Tolterodine as potential drug candidates for repurposing in sporadic ALS treatment. Notably, Lestaurtinib demonstrated high binding affinity toward multiple proteins, suggesting its potential as a broad-spectrum therapeutic agent for sporadic ALS. Additionally, docking analysis revealed NOS3 as the gene that interacts with all the short-listed drugs, suggesting its possible involvement in the mechanisms underlying the therapeutic potential of these drugs in sporadic ALS. Overall, our study provides a systematic framework for identifying potential drug candidates for sporadic ALS therapy and highlights the potential of drug repurposing as a promising strategy for discovering new therapies for neurodegenerative diseases.

1. INTRODUCTION

Amyotrophic lateral sclerosis (ALS), commonly referred to as Lou Gehrig's disease, is a fatal neurodegenerative disorder affecting both upper and lower motor neurons.[1] This disease is characterized by progressive muscle weakness, impaired speaking, and ultimately death within 2−5 years after diagnosis. ALS occurs on a global scale, with an approximate occurrence rate of 2 cases per 100,000 person-years and a prevalence ranging from 6 to 9 cases per 100,000 individuals.[2,3] Despite extensive research, there is currently no cure for ALS, and there is an urgent need to explore new therapeutic opportunities for this devastating disease. ALS can be classified into two types: familial ALS and sporadic ALS. Sporadic ALS accounts for approximately 90% of all cases and is believed to be caused by environmental factors and individual lifestyle, making it more challenging to develop effective treatments.[4] In contrast, familial ALS is present in less than 10% of cases and has a genetic component that may provide a more precise target for therapeutic intervention. Mitochondrial dysfunction, oxidative stress, excitotoxicity, and impaired DNA damage repair are some of the major pathways implicated in the disease.[5] The development of sporadic ALS is influenced by several factors such as genetics, environment, and lifestyle. C9orf72, SOD1, FUS, TARDBP, and UBQLN2 are some of the genes implicated in the pathogenesis of sporadic ALS, and they are involved in various pathways, including protein homeostasis, RNA processing, and cytoskeletal dynamics, that contribute to the disease through distinct molecular mechanisms.[6−8] Aberrant protein aggregation, oxidative stress, inflammation, and impaired axonal transport are among the potential mechanisms contributing to sporadic ALS's pathogenesis. However, the precise molecular mechanisms underlying these processes and their selective vulnerability to motor neurons remain poorly understood.[9,10] Thus, the urgent need for effective therapies and the identification of new targets for intervention is a critical goal in ALS research due to the rapid progression and poor prognosis of sporadic ALS.[11,12]

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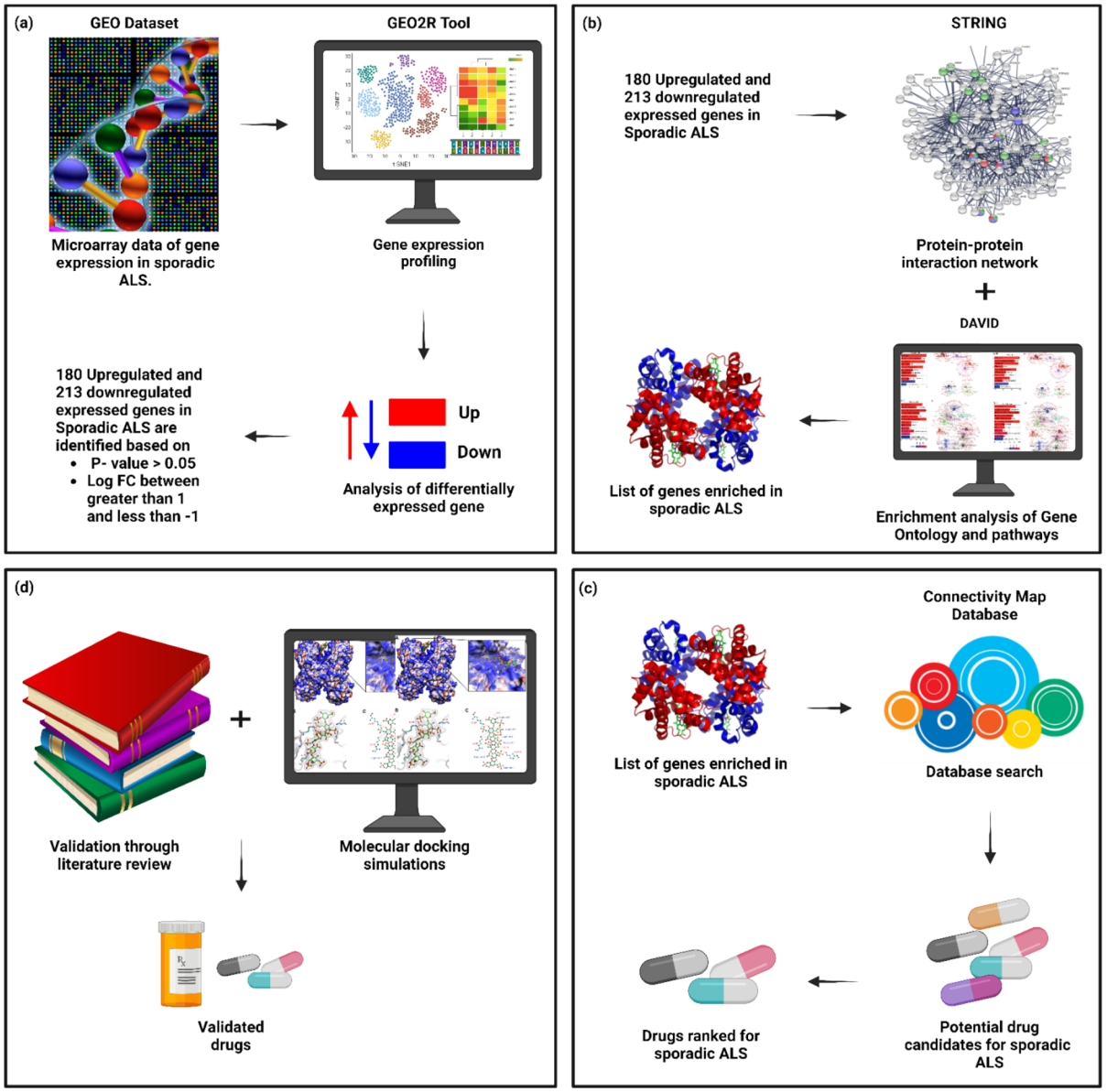

Figure 1. Overview of sporadic ALS drug repurposing. (a) Microarray data of gene expression in sporadic ALS were obtained from the GEO database. The GEO2R tool was utilized for gene expression profiling, revealing 180 upregulated and 213 downregulated expressed genes in sporadic ALS. The differentially expressed genes were identified based on p-value <0.05 and logFC >1 and <−1. These genes represent potential biomarkers and therapeutic targets for sporadic ALS. (b) The upregulated and downregulated genes were analyzed using the STRING database to generate a protein−protein interaction (PPI) network and identify key genes associated with sporadic ALS. Additionally, the up- and downregulated genes were separately uploaded to the DAVID bioinformatics tool to obtain functional annotations and enriched genes in sporadic ALS from KEGG and Reactome pathways. By combining the results from the PPI network and the enriched genes list, we obtained a list of key genes in sporadic ALS. (c) The list of enriched up- and downregulated genes was uploaded to the Connectivity Map database to identify potential drug candidates for sporadic ALS. (d) From the resulting 50 negatively scored drugs, 40 were eliminated following a literature survey, and the remaining 10 were ranked for drug repurposing analysis. Additionally, 10 key genes were selected from the enriched gene list through literature validation. These 10 selected drug candidates and 10 key proteins are subjected to molecular docking analysis to identify potential drug candidates for Sporadic ALS.

The conventional approach to drug discovery, known as *de novo* drug discovery, is a complex and time-intensive process involving the development of new drugs from scratch.[13] It encompasses various stages, including target identification, lead compound discovery, and optimization, which necessitate significant investments of time and financial resources.[14] Nonetheless, this method holds promise for creating novel compounds with tailored specificity for targeting sporadic ALS and potentially offering disease-modifying effects. However, *de novo* drug discovery encounters inherent challenges, such as notable failure rates in clinical trials, limited translation of preclinical findings into human efficacy, and the substantial costs associated with research and development endeavors.[15] On the other hand, drug repurposing presents a promising alternative approach to *de novo* drug discovery. It involves the utilization of existing drugs for new indications, bypassing the initial stages of compound development.[16,17] Drug repurposing offers several advantages, including faster development timelines and lower costs compared to traditional drug discovery approaches and the potential to identify new therapeutic

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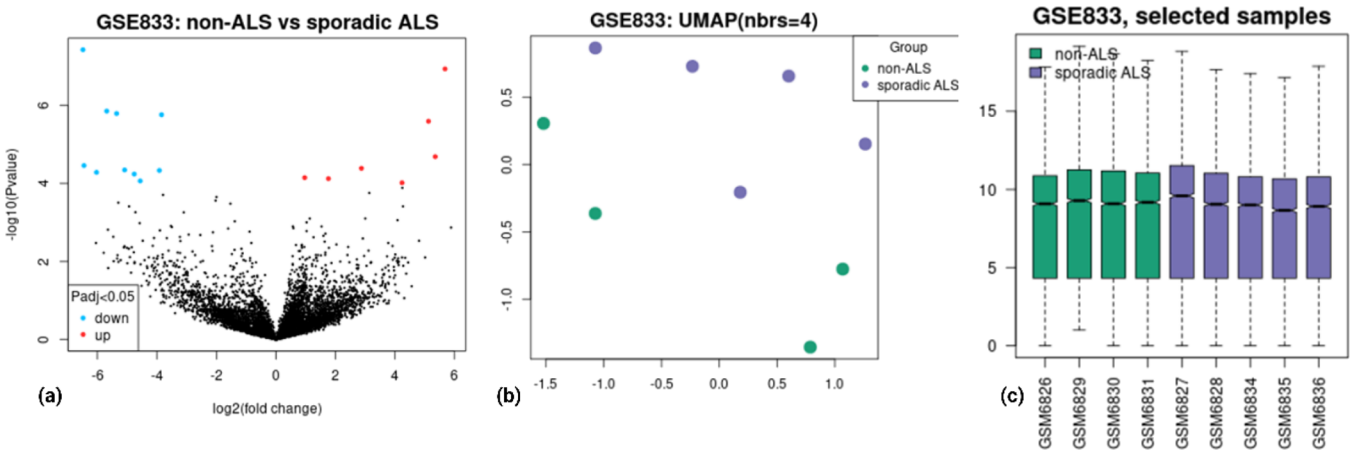

Figure 2. GEO2R results for sporadic ALS gene expressions. (a) Scatter plot showing the differential gene expression analysis of non-ALS vs sporadic ALS using the GSE833 data set. (b) Clustering results of the total number of non-ALS and sporadic ALS groups using the GSE833 data set. (c) Represents the total number of non-ALS and sporadic ALS.

options for sporadic ALS.[18] Moreover, repurposing drugs already approved for other diseases can help accelerate the development of new ALS treatments. In recent years, drug repurposing has emerged as a promising strategy for the treatment of sporadic ALS.[19] For example, riluzole, a Food and Drug Administration (FDA)-approved drug for ALS treatment, has been repurposed for other neurodegenerative diseases, including Alzheimer's and Parkinson's diseases.[20] Other compounds with potential therapeutic activity in ALS have been identified, including dexpramipexole and ambroxol, which target pathways involved in ALS pathogenesis, such as oxidative stress, mitochondrial dysfunction, and inflammation.[21−23] Therefore, the exploration of drug repurposing for the treatment of sporadic ALS represents a promising avenue for the identification of new therapeutic options to combat this devastating disease.[24]

The present investigation aimed to identify potential drug candidates for the treatment of sporadic ALS by analyzing gene expression data. To this end, a data set of sporadic ALS genes was obtained from the GEO Database and subjected to statistical analysis to classify the genes as upregulated and downregulated. For further analysis, key interacting genes were identified from the up- and downregulated sporadic ALS gene lists using the STRING database. Additionally, the same gene list was submitted to the DAVID bioinformatics tools to uncover the functional annotation of each gene, including associated pathways and gene ontology terms, with particular emphasis on Reactome and KEGG pathways. After scrutinizing the relevant literature, we identified key pathways and genes involved in sporadic ALS progression. Furthermore, we performed a comparative study using the key genes obtained from the STRING database, and we selected the up- and downregulated genes separately from both KEGG and Reactome pathways for further drug repurposing research. These genes were further analyzed by submitting the upregulated and downregulated gene sets to the Clue.io web server to obtain drug perturbagens from the Connectivity Map database. The top 50 small-molecule drugs with the highest negative scores were selected, as they were deemed to have the ability to reverse the expression of disease-causing genes in sporadic ALS. Furthermore, we short-listed 10 drugs and 10 key genes from the gene list after a literature survey linked to sporadic ALS for molecular docking studies with AutoDock Vina. The top 50 small-molecule drugs with the highest negative scores were short-listed for their ability to potentially reverse the expression of disease-causing genes in sporadic ALS. Finally, a literature survey was conducted to shortlist 10 drugs and 10 key genes for molecular docking studies using AutoDock Vina.[25] The docking studies identified Cefaclor, Diphenidol, Flubendazole, Fluticasone, Lestaurtinib, Nadolol, Phenamil, Temozolomide, and Tolterodine as potential repurposing candidates. Among these, Lestaurtinib demonstrated high binding affinity toward multiple proteins, suggesting its potential as a broad-spectrum therapeutic agent for sporadic ALS. Moreover, our analysis revealed NOS3 as the gene that interacts with all the short-listed drugs, suggesting its possible involvement in the mechanisms underlying the therapeutic potential of these drugs. An overview of the study is given in Figure 1.

2. MATERIALS AND METHODS

2.1. Data Acquisition and Statistical Comparison of Gene Expression Levels. The transcriptomic data of sporadic ALS samples obtained from microarray analysis were retrieved from the GEO database (GSE833). The study included analysis of postmortem gray matter total RNA extracted from the lumbar spinal cord of five sporadic ALS patients (average age of death, 56 years) and four non-ALS controls (average age of death, 60.5 years). The analysis employed Affymetrix HuFL GeneChip probe arrays comprising 7070 probe sets, representing approximately 6800 human genes. Information regarding the data set's gene expressions is provided in Figure 2 and Supplementary Figure 1.[26]

The GEO2R tool in the GEO database was utilized to perform the initial differential gene expression analysis of the retrieved data set.[27,28] The data set was stratified into non-ALS and sporadic ALS samples for the purpose of analysis. The resultant data from the GEO2R tool were obtained and subsequently processed in Microsoft Excel. A p-value threshold of less than 0.05 was applied to the GEO2R-generated files, and the Log2 fold change range was restricted to values greater than 1 and less than −1. Following the preliminary analysis, differentially expressed genes (DEGs) were ranked based on the Log2 fold change, and all the upregulated and downregulated genes were subsequently identified for further analysis (shown in Figure 1a).

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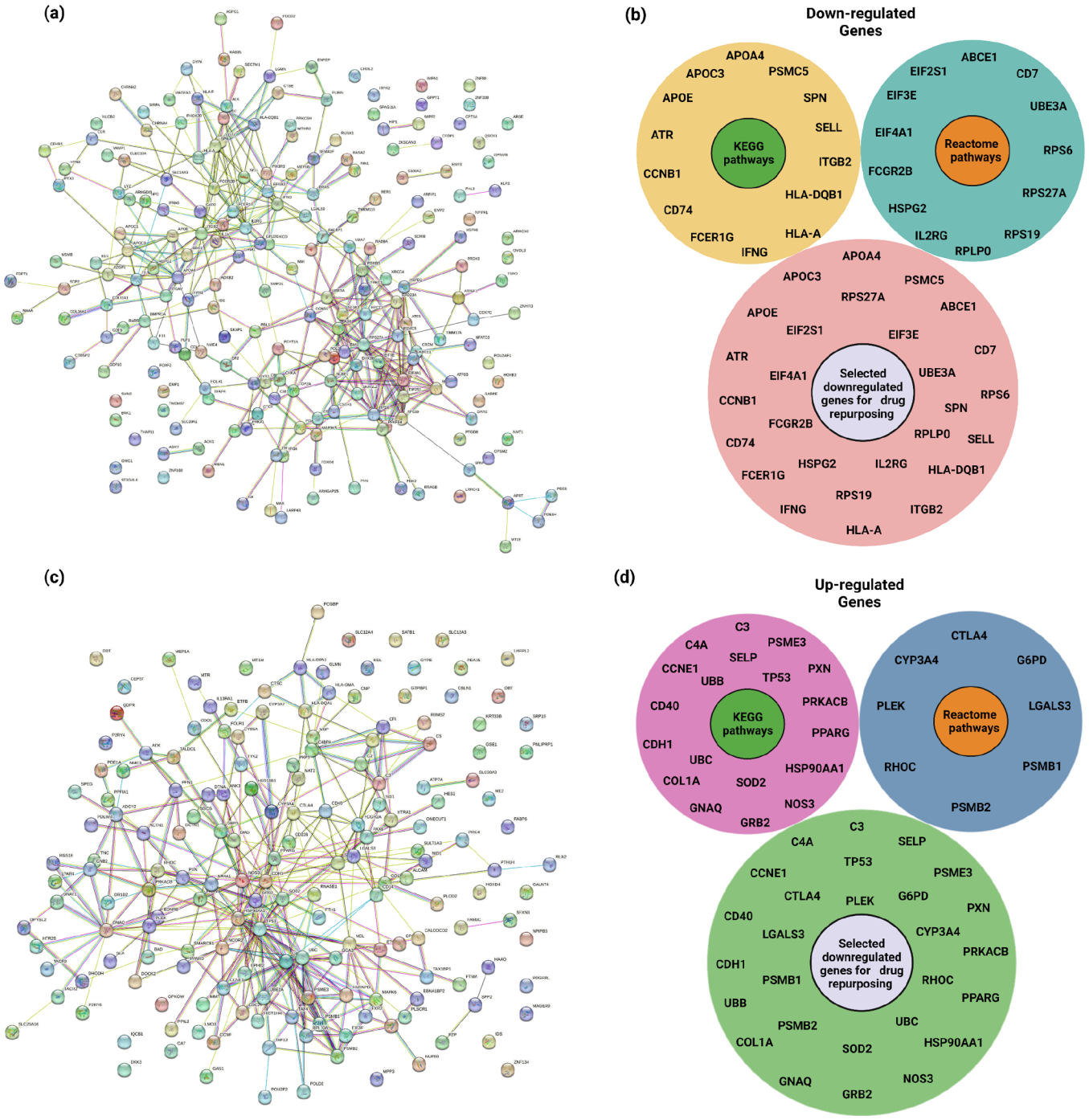

Figure 3. Key upregulated and downregulated genes in sporadic ALS and STRING results. Protein−protein interaction networks were generated using the STRING database for (a) upregulated and (c) downregulated genes separately in sporadic ALS. (b) Screened a combined total of (b) 27 upregulated and (d) 27 downregulated genes from Reactome and KEGG pathways, using the key interacting genes list generated from the STRING database.

2.2. Construction of the Protein−Protein Interaction Network. The STRING database is a bioinformatics tool that provides a comprehensive platform for exploring known and predicted protein−protein interactions. The STRING database was utilized here to identify protein−protein interactions of upregulated and downregulated genes separately. The interactions in the database are categorized into three confidence levels based on predefined parameters: low (<0.4), medium (0.4−0.7), and high (0.7−0.9). Generally, interactions with the highest confidence score are preferred, as they have the most significant influence on the overall network and help identify essential biologically relevant genes. However, this study used a confidence level of 0.4 to generate the PPI network for previously unexplored targets, hypothesizing the discovery of potential novel targets to counteract sporadic ALS. After subjecting the target proteins to topological analysis to identify their interacting parameters, those with the highest node degree up to 8 were selected for further investigation in both the upregulated and downregulated gene sets (shown in Figure 1b).

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2.3. Pathway Enrichment Analysis using DAVID. The DAVID Functional Annotation Tool was used to perform pathway enrichment analysis separately for up- and downregulated genes obtained from the GEO database.[29] For sporadic ALS targets, we annotated with KEGG and Reactome pathways and Gene Ontology terms to gain insight into their biological and molecular functions. Based on a literature search, sporadic ALS-associated pathways were categorized for up- and downregulated genes separately and compared to the key interacting genes selected from STRING. Any genes that were not present in the PPI pairs were eliminated, and the remaining protein targets were considered for drug repurposing (shown in Figure 1b).

2.4. Analysis Using Connectivity Map. We employed the Connectivity Map (CMap) database[30] to identify potential small-molecule drug candidates. The upregulated and downregulated genes from the KEGG pathway and Reactome were combined, as shown in the figure, and were submitted to CMap via the CLUE web application (https://clue.io/cmap). The connectivity score, a drug-scoring algorithm used by CMap gene expression resources, was employed to elucidate the connection between the queried signature of disease-associated genes and a drug perturbagen. The connectivity score ranges from −100 to +100. The drugs were ranked based on negative scoring to positive scoring, and the top 50 negative-scored drug perturbations were recorded for further analysis. These drugs were hypothesized to reverse the expression of the diseased genes (shown in Figure 1c).[31]

2.5. Validating Prioritized Drug Candidates. Initially, 50 drugs selected from the Connectivity Map (CMap) database[30] were validated through a literature survey. These drugs were carefully evaluated based on three criteria, including prior studies in sporadic ALS, non-FDA approval, and toxicity concerns, and those that met any of these criteria were excluded. Consequently, 10 drugs were finalized for further molecular docking studies using AutoDock Vina. After a thorough literature review of the gene list, we selected 10 critical genes for docking.[25] To facilitate docking, the cocrystallized structure of sporadic ALS targets with their respective PDB IDs was obtained from the Protein Data Bank[32] and visualized using the Pymol visualization tool. The prepared protein structures were then processed in AutoDock Vina[33] by removing water and bound ligands, followed by adding hydrogen atoms and Kollman charges and saved in PDBQT file format.

The ligands were obtained by retrieving their 3D structures from PubChem[34] in the SDF format. These structures were then converted to the PDB format using PyMol and subsequently prepared using AutoDock Vina to convert them into the PDBQT format. Binding sites were created before docking using the known binding sites in the crystal structure and from the literature survey. The docking simulations were carried out with 1000 iterations, and the binding affinity values for each ligand−target interaction were recorded and analyzed. The obtained results were used to suggest potential drugs for preclinical and clinical validation for sporadic ALS treatment (shown in Figure 1d).

2.6. Statistical Analysis. To perform an initial analysis of gene expression profiles, we used the R-based tool GEO2R and considered the adjusted *p*-value and Log2 fold change values to identify differential gene expression. The positive and negative data samples obtained from both gene expression data sets are presented in Supporting Information Figure 1. Moreover, we employed Microsoft Excel to analyze and categorize the up- and downregulated genes.

3. RESULT AND DISCUSSION

3.1. Identification of Drug Repurposing Candidates through Analysis of Gene Expression Data. In this study, we conducted an analysis of the GSE833 sporadic ALS data set obtained from the GEO database. Using the GEO2R analysis tool, we identified differentially expressed genes (DEGs) in sporadic ALS. The DEGs were selected based on adjusted *p*-values and log fold change (logFC), with a *p*-value threshold of 0.05 and logFC greater than 1 and less than −1. This rigorous filtering process allowed us to identify the most significant DEGs. Upon analyzing the expression levels of the upregulated and downregulated genes (the scatter plot shown in Figure 2a), we observed a total of 180 upregulated genes and 213 downregulated genes in sporadic ALS. These DEGs represent potential candidates for further investigation to unravel their role in the pathogenesis of sporadic ALS, as shown in Figure 1. Understanding the functions and mechanisms of these genes can provide valuable insights into the underlying molecular processes involved in sporadic ALS. To gain a deeper understanding of the functional implications of these DEGs, future studies should focus on the functional annotation of these genes. Functional annotation can help elucidate the key pathways and molecular mechanisms in sporadic ALS. By identifying these pathways and mechanisms, we can potentially uncover new targets for drug repurposing. Repurposing existing drugs for the treatment of sporadic ALS can offer a cost-effective and time-efficient approach, leading to the development of novel therapeutic strategies.

3.2. Identification of Key Genes through PPI Network Analysis. To identify key genes associated with upregulated and downregulated pathways in sporadic ALS, we constructed a protein−protein interaction (PPI) network (shown in Figure 3a,c).[35] The PPI network analysis aimed to uncover potential therapeutic targets for the disease. The downregulated gene set exhibited 209 nodes and 372 edges, while the upregulated gene set had 175 nodes and 348 edges. To identify previously untargeted genes, we set a medium confidence interaction score of 0.4 as the minimum requirement. This approach allowed us to identify novel targets with a lower stringency. From the PPI network analysis, we selected 30 key upregulated genes and 32 key downregulated genes based on a node degree of $\geq$ 8. These core targets were then used to identify key targets from pathways for drug repurposing. A detailed list of these key genes can be found in Table 2. The identification of these key genes provides valuable insights into the molecular mechanisms underlying sporadic ALS and offers potential avenues for therapeutic interventions. Overall, our PPI network analysis has successfully identified a set of potential drug targets that merit further investigation for the treatment of sporadic ALS.

3.3. Enrichment Analysis of Gene Ontology and Pathways in the Sporadic ALS Gene Data Set. To gain insight into the molecular mechanisms and pathways involved in sporadic ALS, we conducted an enrichment analysis of the gene ontology and pathways in the sporadic ALS gene data set. By utilizing the DAVID functional annotation tool,[36] we performed separate analyses for the upregulated and downregulated genes. To ensure the accuracy of the analysis, we excluded genes without a specific Entrez ID or an official gene name. For each gene set, we examined and recorded

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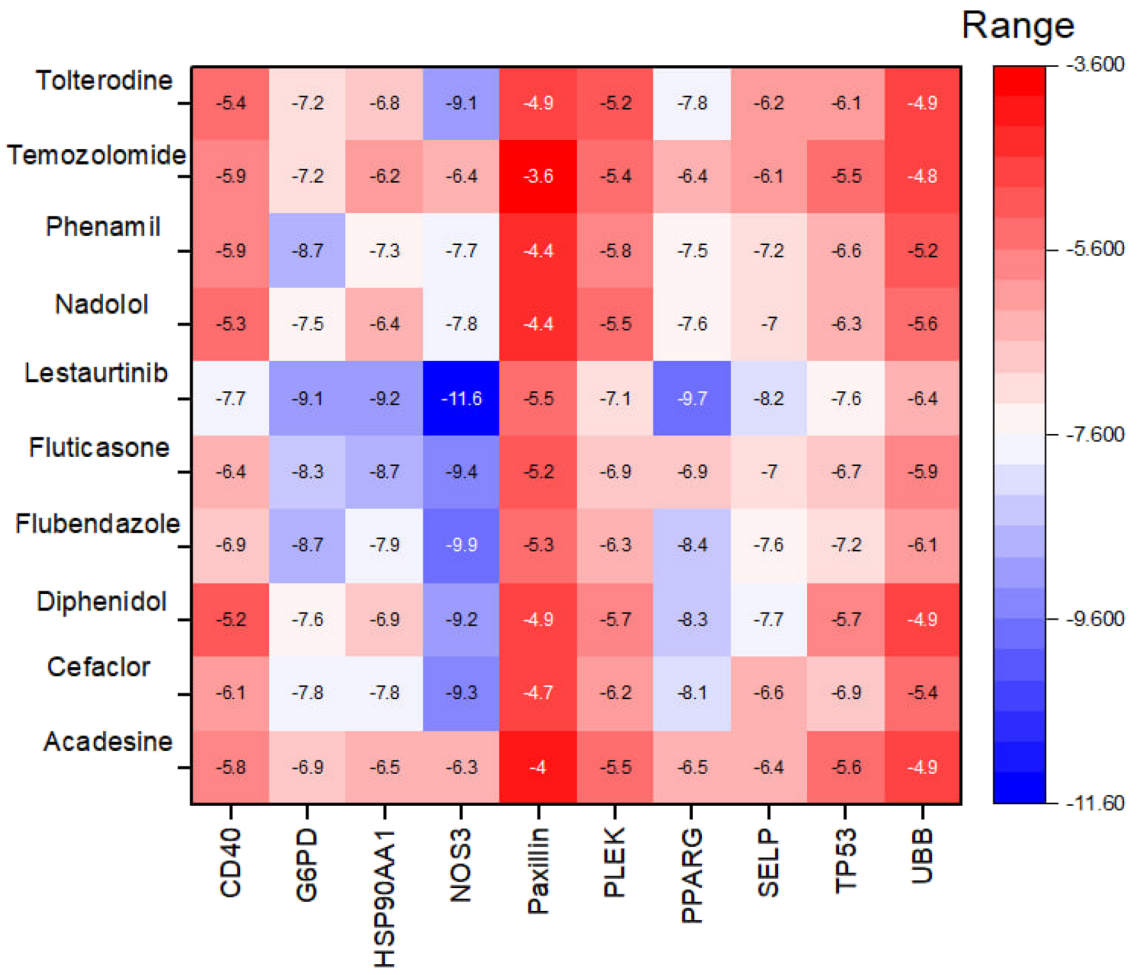

Figure 4. Heatmap illustrating docking scores of repurposed drugs against 10 proteins associated with Sporadic ALS. The *y*-axis indicates the candidate-predicted drugs, while the *x*-axis shows the host sporadic ALS protein targets. The Docking score bar, ranging from dark blue to red, indicates the maximum to the minimum value, with dark blue representing the highest score of −11.6 and red representing −3.6. The heat map demonstrates the binding affinities of the drugs, with the highest binding affinities ranging from 9.0 to −11.06 kcal/mol. Lestaurtinib, cefaclor, fluticasone, and others have shown the highest binding affinities, indicating their potential as drugs for repurposing in the treatment of sporadic ALS.

information related to molecular function, biological process, and pathways, with a particular focus on pathways relevant to sporadic ALS (pathways are shown in Supplementary Tables 3 and 4). The pathways identified through the enrichment analysis were recorded separately for both the up- and downregulated genes, utilizing KEGG and Reactome pathway information. The identification of these pathways was further validated through a thorough review of the literature. To visualize the common genes obtained from both Reactome and KEGG pathways, we constructed a diagram (Figure 3b,d). Additionally, we selected genes obtained exclusively from the Reactome pathways for further analysis separately for the upregulated and downregulated genes. Genes present in the pathways but not included in the key interacting gene list from the STRING database[37] were excluded from our analysis. As a result of the enrichment analysis, we identified 27 upregulated genes and 27 downregulated genes that were considered for drug perturbation analysis (Figure 3b,d). These genes play important roles in the molecular pathways associated with sporadic ALS, and understanding their involvement can provide valuable insights into the disease mechanisms. In summary, our enrichment analysis of the sporadic ALS gene data set revealed significant pathways and molecular functions associated with sporadic ALS. By identifying these pathways and genes, we have expanded our knowledge of the molecular landscape of sporadic ALS and have established a foundation for further investigations, such as drug perturbation analysis, to explore potential therapeutic interventions for this devastating disease.

3.4. Connectivity Map Analysis Results and Literature-Based Validation. To identify potential drug candidates for the treatment of sporadic ALS, we conducted a connectivity map analysis using the 27 upregulated and 27 downregulated genes identified in our study. The results were filtered based on the enrichment score ranging from −99 to +99. Negative scores indicate drug molecules that have the potential to generate a gene signature negatively associated with or capable of reversing the observed gene expression changes in sporadic ALS. We selected the top 50 candidate compounds with negative scores, as presented in Table 1. These candidate compounds are hypothesized to counteract the effects of sporadic ALS by reversing the disease-associated gene expression signature. To further validate these candidate compounds, we conducted a comprehensive literature survey using three criteria: previously studied in sporadic ALS, chemical compounds, inhibitors or molecules, non-FDA

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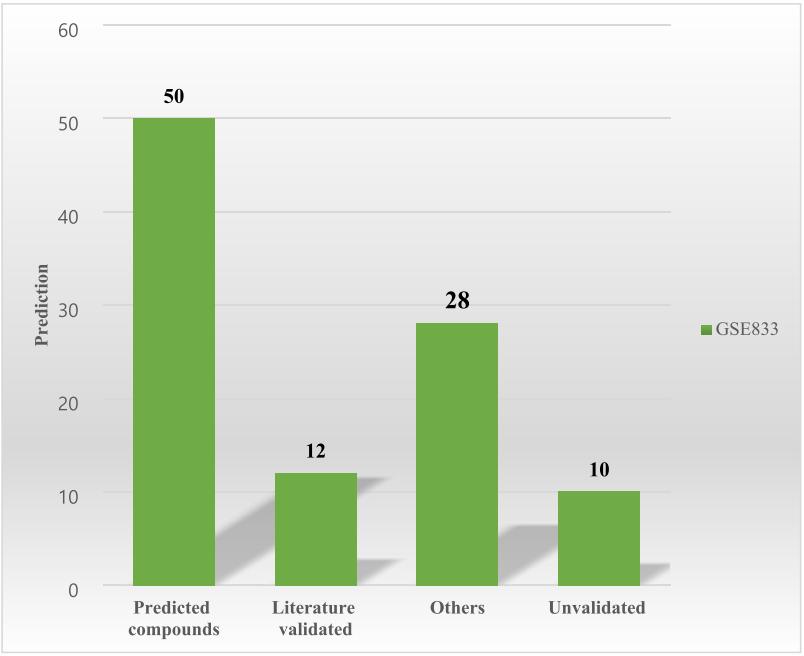

Figure 5. Line chart showing predicted drugs for sporadic ALS followed by literature-validated compounds, unvalidated candidate compounds, and other non-FDA-approved chemical compounds or inhibitors. The *y*-axis represents the number of compounds in each category, while the *x*-axis represents the respective categories. The predicted compounds refer to those identified through computational drug repurposing studies, the literature-validated compounds are those that have been previously reported to have therapeutic potential for sporadic ALS, and the unvalidated candidate compounds are those that have not been tested in preclinical or clinical studies for ALS treatment. The other compounds include chemical compounds and inhibitors that have not been approved by the FDA for any therapeutic indications.

approved drugs, and screened out 40 drug candidates, as shown in Figure 5 and Table 1. The remaining 10 drug candidates were selected for molecular docking studies. Moreover, after a thorough literature review of the gene list, we selected 10 critical genes for molecular docking studies, refer to Table 2. Moreover, we selected 10 critical genes (Paxillin, CD40, p53, P-Selectin, ubiquitin, G6PD, PPAR-γ, NOS3, and HSP90AA1) for molecular docking studies after a thorough literature review of the gene list. The first gene ofinterest is Paxillin, which has been implicated in maintaining cytoarchitectural integrity and is significantly present in the Paxillin signaling pathway. Paxillin plays a critical role in cell-extracellular matrix interactions and the proper assembly and recruitment of proteins to these sites. Disruption of Paxillin signaling can have profound consequences on cellular cytoarchitecture, response to extracellular stimuli, and activation of key signaling cascades. Targeting Paxillin as a drug repurposing strategy in sporadic ALS could help restore proper cellular function and mitigate disease pathology.[50,51] Another gene of interest is CD40, which has been associated with inflammatory markers and immune responses in sporadic ALS. ALS patients exhibit a dense infiltration of macrophages, expressing inflammatory markers such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase. CD40 receptor-positive macrophages and CD40 ligand-positive T lymphocytes are associated with damage to the tight junction protein ZO-1 in blood vessels. The involvement of CD40 in the observed inflammation suggests its potential as a drug repurposing target for sporadic ALS.[52] The transcription factor p53 has also emerged as a potential target for addressing spinal cord cell loss in ALS. Studies have shown an elevated expression of p53 in ALS mouse models and human spinal cord samples. Inhibition of p53 has been demonstrated to reduce the oxidative-stress-induced toxicity in mouse spinal cord cultures. Targeting p53 may hold promise for mitigating motor neuron death in sporadic ALS and related disorders.[53]

Investigating ALS-choroid plexus (ALS-cp), researchers found elevated levels of inflammatory markers, including IL-6, IL-8, Thrombomodulin, and P-Selectin. P-Selectin, in particular, may play a significant role in sporadic ALS. Targeting P-Selectin could be relevant for drug repurposing in sporadic ALS due to its involvement in the observed inflammatory response in ALS-choroid plexus tissues.[54] The study of ubiquitin in ALS has revealed its presence in

Table 1. List of Literature-Validated Drug that Has Previously Been Used in ALS

SI NO	literature-validated drugs	mode of action	reference
1	RHO-kinase-inhibitor-III[rockout]	Rho-associated kinase inhibitor	38
2	Forskolin	adenylyl cyclase activator	39
3	Ataluren	CFTR channel agonist, Dystrophin stimulant	40
4	Picrotoxin	GABA receptor antagonist	41
5	Bicuculline	GABA receptor antagonist	42
6	deferiprone	chelating agent	43
7	tubacin	HDAC inhibitor	44
8	SB-216763	glycogen synthase kinase inhibitor	45
9	GSK-3-inhibitor-IX	glycogen synthase kinase inhibitor, Lipoxygenase inhibitor	46
10	PD-168077	bile acid	47
11	mepyramine	histamine receptor antagonist	48
12	mitomycin-c	DNA alkylating agent, DNA inhibitor, DNA synthesis inhibitor	49

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Table 2. Top Drugs and Key Genes for Molecular Docking in Sporadic ALS

Sl NO	key drugs selected for molecular docking	mode of action	original therapeutic use	potential new therapeutic use	key gene targets selected for molecular docking	active site residues/ coordinates	PDB ID
1	Acadesine	AMPK activator	cardiac reperfusion injury, cardiovascular disorders, and coronary artery disease	sporadic ALS	SELP	GLU80, ASP78, ASN82, LYS84, ASN83, ASN105, TYR48, SER97	1G1S
2	Cefaclor	bacterial cell wall synthesis inhibitor	pneumonia and ear, lung, skin, throat, and urinary tract infections	sporadic ALS	PXN	$X = -19.264$, $Y = -13.034$, $Z = 18.342$	2VZG
3	Diphenidol	acetylcholine receptor agonist	peripheral (labyrinthine) vertigo and associated nausea and vomiting of several diseases	sporadic ALS	NOS3	X center = 11.714, Y center = 12.839, Z center = 58.014	1M9M
4	Flubendazole	tubulin inhibitor	not available	sporadic ALS	CD40	TYR145, TYR146, ILE127, GLY144, HIS125, ALA124, ALA123, GLN121, LEU259, LEU261, TYR170, TYR172, ALA173, HIS224, LEU225, GLY226	1ALY
5	Fluticasone	glucocorticoid receptor agonist	asthma, inflammatory, and pruritic dermatoses	sporadic ALS	UBB	MET1, LYS6, LYS11, LYS27, LYS29, LYS33, LYS48, LYS63	5TXK
6	Lestaurtinib	FLT3 inhibitor, Growth factor receptor inhibitor, JAK inhibitor	pancreatic cancer, prostate cancer, and leukemia	sporadic ALS	PLEK	X center = −4.092, Y center = −11.059, Z center = −14.944	2I5F
7	Nadolol	adrenergic receptor antagonist	angina pectoris and hypertension	sporadic ALS	G6PD	THR236, ASN363, ARG487, ASP493, TYR503, GLU364, GLU368, ARG393, ARG370, TYR401, ASP421, TRP509, LYS403, TYR507, GLU419	6E08
8	Phenamil	TRPV antagonist	irreversible inhibitor of sodium channels in the toad urinary bladder	sporadic ALS	PPARG	ARG288, SER289, LEU330, ILE341, TYR327, CYS285, HIS323, LEU476, GLN286, LYS367, TYR473, MET364, HIS 449, PHE360, PHE363, LEU356, LEU453, PHE282, ILE281	3ET3
9	Temozolomide	DNA alkylating agent	glioblastoma and refractory anaplastic astrocytoma	sporadic ALS	TP53	CYS 229, LEU 145, THR 230, PRO 223, VAL 147, PRO 222, PRO 151, GLU 221, CYS 220, THR 155, GLY 154	2OCJ
10	Tolterodine	acetylcholine receptor antagonist	overactive bladder	sporadic ALS	HSP90AA1	VAL 150, MET 98, VAL 186, ASP 93, SER 52, ALA 55, TYR 139, ALA 111, VAL 136, PHE 138, LEU 107	3BMY

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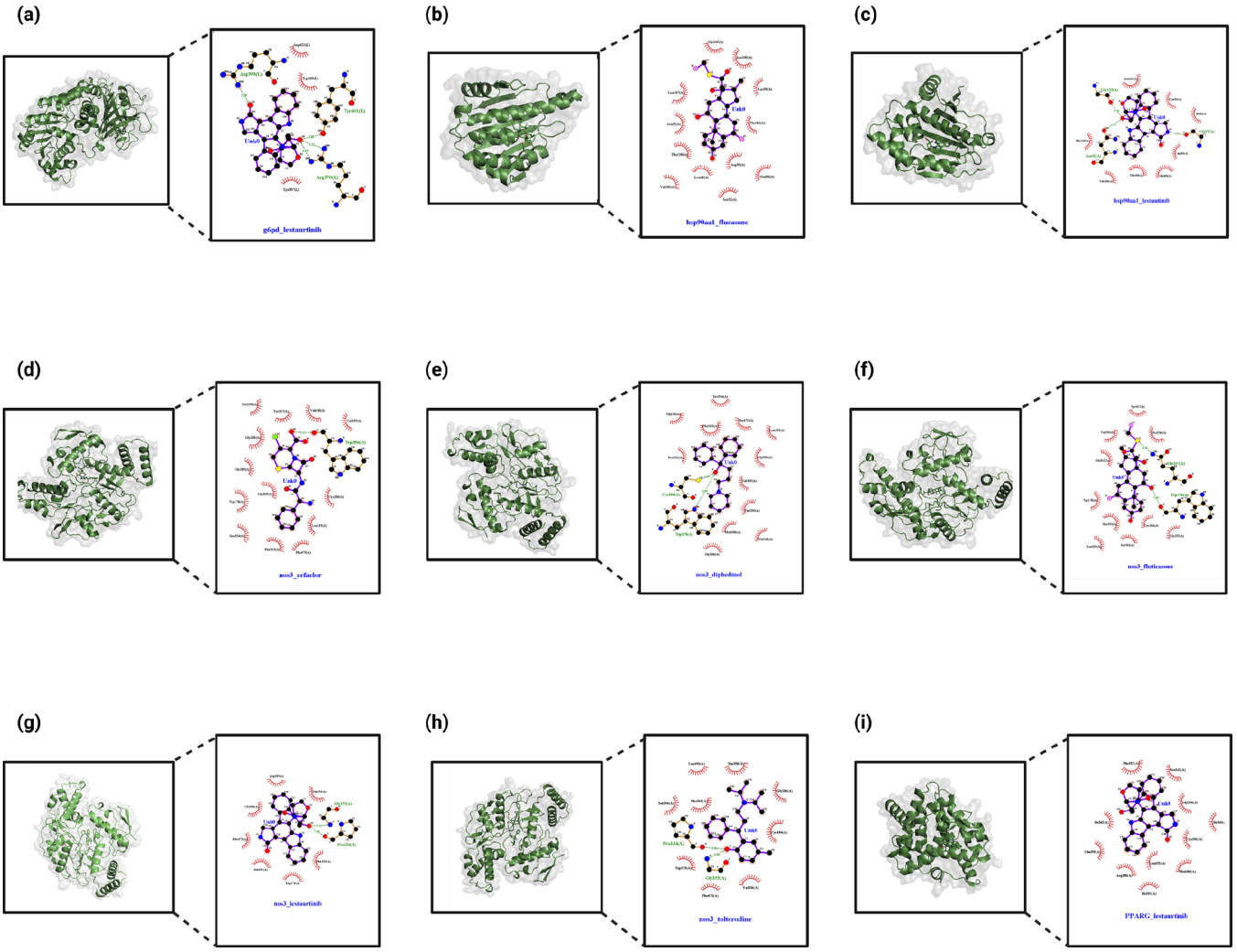

Figure 6. Molecular docking results of potential predicted drugs for sporadic ALS against four protein targets, namely, NOS3, HSP90AA1, G6PD, and PPARG. The *y*-axis represents the binding affinity score, which ranges from −11.6 to 4 kcal/mol, indicating significant variation in the binding affinity between the ligands and protein targets. The *x*-axis displays the names of the top-scoring drugs for each protein target, along with their respective docking scores. (a) Lestaurtinib (−9.1 kcal/mol)—G6PD: this drug had the highest docking score for G6PD, indicating a solid binding affinity for the G6PD protein target. (b) Fluticasone (−8.7 kcal/mol)—HSP90AA1: this drug had the second-highest docking score for HSP90AA1, suggesting it may be an effective drug candidate for targeting this protein. (c) Lestaurtinib (−9.6 kcal/mol)—HSP90AA1: this drug had the highest docking score for HSP90AA1, indicating that it may be a particularly promising candidate for targeting this protein. (d) Cefaclor (−9.3 kcal/mol)—NOS3: this drug had the third-highest docking score for NOS3, suggesting that it may have some efficacy in targeting this protein. (e) Diphenidol (−9.2 kcal/mol)—NOS3: this drug had the fourth-highest docking score for NOS3, indicating that it may have some potential for targeting this protein. (f) Fluticasone (9.4 kcal/mol)—NOS3: this drug had the second-highest docking score for NOS3, indicating that it may be a more effective candidate than Cefaclor or Diphenidol for targeting this protein. (g) Lestaurtinib (−11.6 kcal/mol)—NOS3: this drug had the highest docking score for NOS3, suggesting that it may be the most effective candidate for targeting this protein. (h) Tolterodine (−9.1 kcal/mol)—NOS3: this drug had the fifth-highest docking score for NOS3, indicating that it may have some potential for targeting this protein but may not be as effective as the top four candidates. (i) Tolterodine—PPARG: this drug had the highest docking score for PPARG, indicating that it may be a particularly promising candidate for targeting this protein.

ubiquitin-positive inclusions, indicating a disruption of ubiquitin homeostasis. ALS-associated proteins, such as TDP-43, FUS, and SOD1, are found within motor neurons, suggesting an increased susceptibility to aggregation and disruption of ubiquitin homeostasis. Targeting ubiquitin as a drug repurposing strategy in sporadic ALS could help restore ubiquitin balance and mitigate the pathological consequences of protein aggregation in the disease.[55,56] The deregulated function of glucose-6-phosphate dehydrogenase (G6PD) has been implicated in ALS. ALS patients exhibit low levels and activities of G6PD, which may contribute to increased levels of lipid peroxidation. Targeting G6PD could potentially address the enzymatic imbalance and mitigate the oxidative stress observed in sporadic ALS, offering a novel approach for therapeutic intervention.[57,58] Neuroinflammation plays a significant role in ALS progression, and the PPAR-γ signaling pathway has been implicated in ALS pathogenesis. Modulating the activation or inactivation of PPAR-γ presents a promising strategy for controlling neuroinflammation in ALS. Synthetic PPAR-γ agonists, already approved as antidiabetic drugs, have the potential to serve as therapeutics in sporadic ALS by regulating genes associated with inflammation, oxidative stress, and apoptosis. Oxidative stress and damage induced by free radicals are key contributors to sporadic ALS progression.[59,60]

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NOS3, which is the endothelial enzyme responsible for producing nitric oxide (NO), acts as an antioxidant agent. Targeting NOS3 could enhance the antioxidant response and mitigate oxidative damage in ALS patients, promoting NO release and its antioxidant properties. The heat-shock protein HSP90AA1 has been identified as playing a role in ALS progression and in the survival of motoneurons. Activation of the heat-shock response through compounds such as Arimoclomol has shown promising results in delaying disease progression in ALS mouse models. Pharmacologically targeting HSP90AA1 and promoting the survival of motoneurons could serve as a therapeutic strategy for sporadic ALS and other neurodegenerative disorders.[61] Overall, these 10 critical genes, including Paxillin, CD40, p53, P-Selectin, ubiquitin, G6PD, PPAR-γ, NOS3, and HSP90AA1, have been implicated in various aspects of sporadic ALS pathogenesis, such as cellular architecture, inflammation, apoptosis, oxidative stress, and protein aggregation. Targeting these genes through drug repurposing strategies may offer potential therapeutic interventions for sporadic ALS, with the aim to restore cellular function, mitigate disease progression, and enhance patient outcomes.

Further, we performed molecular docking to evaluate the binding affinity between the selected drug candidates and target proteins associated with sporadic ALS. By employing molecular docking analysis, we aim to assess the potential binding strength and affinity of the drug candidates toward their target proteins. This information is crucial in predicting the effectiveness of the drug candidates in modulating the biological activity of the target proteins and ultimately influencing the disease mechanisms in sporadic ALS. The outcomes of these molecular docking studies will facilitate the identification of promising drug candidates for further preclinical and clinical investigations.

3.5. Molecular Docking Results. In this study, we employed molecular docking analysis using AutoDock Vina to investigate the binding affinity of predicted ligands for sporadic ALS against selected host protein targets. Ligand structures were obtained from PubChem in the 3D SDF format and converted to the PDB format using PyMol software. The prepared ligands and protein targets were subjected to docking, and the resulting binding affinity scores were analyzed. The analysis revealed a widerange of binding affinity scores, ranging from −11.6 to 4 kcal/mol, indicating significant variability in the binding affinity between ligands and protein targets. Notably, Lestaurtinib exhibited the highest binding affinity for NOS3 (Figure 6g) with a score of −11.6 kcal/mol, followed by Fluticasone (Figure 6f) (9.4 kcal/mol), Cefaclor (Figure 6d) (−9.3 kcal/mol), Diphenidol (Figure 6e) (−9.2 kcal/mol), and Tolterodine (Figure 6h) (−9.1 kcal/mol). For HSP90AA1, Lestaurtinib (Figure 6c) (−9.6 kcal/mol) and Fluticasone (Figure 6b) (−8.7 kcal/mol) demonstrated the highest scores. Regarding G6PD, Lestaurtinib (Figure 6a) (−9.1 kcal/mol) showed the most favorable binding affinity, while Tolterodine (Figure 6i) (−9.7 kcal/mol) exhibited the highest score for PPARG. Remarkably, Lestaurtinib exhibited strongbinding affinity toward multiple protein targets, suggesting its potential as a broad-spectrum therapeutic agent for sporadic ALS. Furthermore, NOS3 was found to interact with all the short-listed drugs, indicating its possible involvement in the underlying mechanisms of these drugs' therapeutic potential in sporadic ALS. Based on the docking analysis, Cefaclor, Diphenidol, Flubendazole, Fluticasone, Lestaurtinib, Nadolol, Phenamil, Temozolomide, and Tolterodine are proposed as potential drug candidates for repurposing in the treatment of sporadic ALS, as depicted in Figure 4, Figure 6, and Supplementary Table 5. In conclusion, our study demonstrates the utility of molecular docking analysis in predicting the binding affinity of selected drug candidates to protein targets implicated in sporadic ALS. The findings highlight Lestaurtinib as a promising candidate due to its strong binding affinity to multiple protein targets. The potential involvement of NOS3 in the therapeutic mechanisms of these drug candidates warrants further investigation. These results lay the groundwork for repurposing existing drugs for the treatment of sporadic ALS, offering potential avenues for the development of novel therapies for this debilitating disease.

3.6. Selected Drug Targets and Their Mechanism in Sporadic ALS. In our study, we investigated the binding affinity of Tolterodine (−9.1 kcal/mol) to PPARγ and suggested its potential therapeutic effect in reducing the progression of ALS disease progression. Building upon our findings, we can correlate our results with those of the previous study that explored the role of PPARs in ALS and lipid peroxidation, as referenced. This correlation provides a basis for further discussion and speculation on the potential implications of Tolterodine's interaction with PPARγ in the context of ALS.The previous study demonstrated that PPARγ-driven transcription selectively increased in the spinal cord of symptomatic hSOD1G93A ALS transgenic mice. This increase in PPARγ activity correlated with the upregulation of target genes involved in scavenging lipid peroxidation byproducts. The enhanced PPARγ immunoreactivity within motor neuronal nuclei indicated its role in neutralizing harmful lipoperoxidation derivatives within motor neurons. The study also highlighted that lipid peroxidation end products, elevated in the cerebrospinal fluid and spinal cord of ALS patients, can activate PPARγ.[62] Based on these findings, it can be hypothesized that Tolterodine's high binding affinity to PPARγ, as indicated in our study, may modulate PPARγ activity and its downstream effects. PPARγ activation has been associated with neuroprotective effects in various neurodegenerative diseases, including ALS. Therefore, Tolterodine's potential interaction with PPARγ may offer a novel approach for attenuating neurodegeneration in ALS by limiting the damage induced by lipid peroxidation derivatives. Additionally, we examined the binding affinity of various compounds for HSP90AA1, a heat-shock protein associated with the pathogenesis of amyotrophic lateral sclerosis. Among the ligands tested, Lestaurtinib (−9.6 kcal/mol) (Figure 6a) and Fluticasone (−8.7 kcal/mol) exhibited the highest scores, indicating strong binding to HSP90AA1 (Figure 6b). This finding suggests that these compounds have the potential to interact with HSP90AA1 and may hold therapeutic relevance for ALS (Figure 6c). Previous research has implicated heat-shock proteins, including HSP90, in the progression of ALS. In a separate investigation, elevated serum levels of HSP70 and HSP90 were observed in ALS patients compared to control individuals.[60] Importantly, these increased levels persisted throughout the disease course, indicating a potential role of HSPs in ALS progression and motor neuron degeneration.[63] Given these findings, it is reasonable to propose that compounds with strong binding affinity to HSP90AA1, such as Lestaurtinib and Fluticasone, could modulate the activity of HSP90AA1 and potentially impact sporadic ALS pathogenesis. To further understand the underlying mechanisms, additional

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research is necessary. *In vitro* studies employing cell cultures can provide insights into the effects of Lestaurtinib and Fluticasone on HSP90AA1 expression and downstream pathways in motor neurons. Animal models, including sporadic ALS transgenic mice, can be utilized to assess the impact of these compounds on sporadic ALS-related pathologies and motor function. Furthermore, clinical studies involving sporadic ALS patients are crucial to evaluating the therapeutic potential of Lestaurtinib and Fluticasone in targeting HSP90AA1-related pathways and their effectiveness in ALS treatment. The distinct elevation of serum HSP70 and HSP90 levels in ALS patients, as observed in previous investigations, strengthens the rationale for targeting HSP90AA1 in sporadic ALS therapy. However, comprehensive investigations are warranted to validate these hypotheses and ascertain the safety and efficacy of these compounds in the context of sporadic ALS treatment. Moreover, G6PD, which exhibited the most favorable binding affinity with Lestaurtinib (−9.1 kcal/mol), can explore its potential therapeutic implications in sporadic ALS based on previous findings. Metabolic dysregulation, characterized by hypermetabolism, is a well-known feature of ALS patients.[64] However, understanding the underlying cellular-level metabolic changes associated with this clinical observation remains a challenge. In a study utilizing Drosophila as a model organism, researchers investigated how specific metabolic changes in neurons and glia contribute to disease progression. Metabolic profiling revealed that the neuronal expression of TDP-43, a protein associated with ALS pathology, led to an increase in the level of pyruvate, indicating enhanced glycolysis. Interestingly, similar metabolite changes have been observed in the plasma of ALS patients, suggesting dysregulation of glycolysis in the disease. Consistent with these findings, transcriptional profiling of ALS spinal cords and patient-derived motor neurons demonstrated significant upregulation of PFK, a key enzyme in glycolysis. Furthermore, metabolic profiling in flies suggested an increased level of glycolytic input into the pentose phosphate pathway, which plays a crucial role in counteracting oxidative stress by generating NADPH. Supporting this possibility, G6PD, the rate-limiting enzyme of the pentose phosphate pathway, was found to be upregulated in both Drosophila ALS models and human ALS ventral and spinal cords. Oxidative stress is a well-established feature of ALS, and drugs targeting this aspect, such as Radicava, have shown therapeutic benefits. The alignment of molecular and metabolic alterations observed in the fly model with patient-derived motor neurons and spinal cords highlights the relevance and predictive power of the fly model in studying ALS pathomechanisms.[65] Given the upregulation of G6PD in sporadic ALS tissues and its role in counteracting oxidative stress, targeting G6PD with Lestaurtinib (Figure 6g) represents a promising therapeutic approach for sporadic ALS. However, further investigations are warranted to validate this hypothesis, including assessing the safety and efficacy of modulating G6PD in the context of sporadic ALS treatment. Furthermore, we investigated the binding affinity of several drugs to NOS3 and explored their potential implications in the context of sporadic ALS. Among the drugs tested, Lestaurtinib demonstrated the highest binding affinity to NOS3, followed by Fluticasone (Figure 6f), Cefaclor (Figure 6d), Diphenidol (Figure 6e), and Tolterodine (Figure 6h,i), as indicated by the respective kcal/mol values. These interactions between NOS3 and the identified drugs suggest their potential involvement in the underlying mechanisms of therapeutic efficacy in sporadic ALS. Nitric oxide (NO) is a versatile molecule involved in signaling and nonspecific immune defense processes, playing a crucial role in maintaining the delicate balance between normal and pathological outcomes. NOS consists of three major forms, namely, neuronal (nNOS/NOS1), inducible (iNOS/NOS2), and endothelial NOS (eNOS/NOS3), which contribute to NO production. The specific roles of these distinct NOS types in sporadic ALS pathogenesis are not yet fully understood. However, previous studies have demonstrated elevated NO levels in ALS mice carrying the mtSOD1 (G93A) mutation with significant upregulation of iNOS gene expression in astrocytes and notable downregulation of nNOS gene expression in motor neurons. Superoxide, a free radical generated in various biological processes, can give rise to reactive oxygen species that contribute to the oxidative damage of lipids, proteins, and DNA, thereby playing a role in the development of various diseases. Notably, L-arginine supplementation has been shown to exert immunostimulatory effects in breast cancer patients and tumor-bearing mice, enhancing the host defenses. Furthermore, administration of L-arginine to ALS transgenic mice carrying the mtSOD1 (G93A) mutation resulted in a significant delay in the onset of lumbar spinal cord neuropathology and motor dysfunction, ultimately prolonging the lifespan of the mice.[66,67] Based on these findings, we propose a hypothesis suggesting that drugs with strong binding affinity to NOS3, including Lestaurtinib and the other identified drugs, may modulate NOS3 activity and downstream processes. The interactions observed between these drugs and NOS3 imply their potential to regulate NO production and maintain the delicate balance between normal and pathological outcomes. Further experimental investigations are needed to validate this hypothesis and understand the therapeutic implications of targeting NOS3 in sporadic ALS. By establishing a correlation between the binding affinity of the identified drugs for NOS3 and the role of nitric oxide, particularly NOS3, in sporadic ALS, we propose a promising approach to attenuate sporadic ALS pathogenesis. Drugs exhibiting a high affinity for NOS3, such as Lestaurtinib, have the potential to modulate NO production and its downstream effects, offering a novel avenue for reducing the level of sporadic ALS progression. However, extensive investigations, including *in vitro* studies using cell culture experiments, animal models such as sporadic ALS transgenic mice, and clinical trials involving human subjects, are necessary to validate this hypothesis and evaluate the safety and efficacy of targeting NOS3, PPARγ, HSP90AA1, and G6PD in sporadic ALS treatment.

4. CONCLUSIONS

This study presents a drug repurposing pipeline for identifying potential therapeutic options for sporadic ALS. Through analysis of gene expression data, key genes and pathways involved in sporadic ALS pathogenesis were identified using bioinformatics tools. Nine potential drug candidates were short-listed for repurposing in sporadic ALS treatment, including Cefaclor, Diphenidol, Flubendazole, Fluticasone, Lestaurtinib, Nadolol, Phenamil, Temozolomide, and Tolterodine. Lestaurtinib demonstrated high binding affinity toward multiple proteins, suggesting its potential as a broad-spectrum therapeutic agent for sporadic ALS. Furthermore, our analysis revealed NOS3 as the gene that interacts with all the short-listed drugs, suggesting its possible involvement in the mechanisms underlying the therapeutic potential of these

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drugs in sporadic ALS. The study also identified key interacting genes and signaling pathways involved in sporadic ALS pathogenesis, including the ubiquitin-proteasome system, oxidative stress, and immune response pathways. This study provides a systematic framework for identifying potential drug candidates for sporadic ALS therapy, which may aid in the development of effective treatments for this devastating disease. Drug repurposing offers several advantages, including faster development timelines and lower costs, compared to traditional drug discovery approaches. While our study identified several promising drug candidates, further experimental validation is necessary to confirm their effectiveness. Future studies could explore the potential of using combinations of the identified drugs to achieve synergistic effects and improve therapeutic outcomes.

■ ASSOCIATED CONTENT

Data Availability Statement

Supplementary data will be provided upon request.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c07296.

Top 50 drug candidates with negative scores from the connectivity map for further analysis, protein−protein interaction network score from the STRING database, upregulated genes and associated pathways in sporadic ALS from DAVID, downregulated genes and associated pathways in sporadic ALS from DAVID, ligand protein binding energy score, and differentially expressed genes in ALS (PDF)

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N.S.: conceptualization, formal analysis, original draft, writing—review and editing, and images, F.A.: formal analysis, investigation, validation, A.R.C.S.: writing, formal analysis, investigation, validation, review & editing, and images, J.H.L.: conceptualization, review and editing, resources, supervision, K.H.C.: conceptualization, review and editing, resources, supervision, funding acquisition.

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Notes

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Ethics approval and consent to participate will be provided on demand.

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