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Integrative Network Biology Analysis of GSE6011 Uncovers Molecular Signatures in Duchenne Muscular Dystrophy

Shashikala[1], Vibha Rani[1], Shazia Haider[2,*]

Abstract

Duchenne muscular dystrophy (DMD) is a rare, severe neuromuscular disorder demonstrated by progressive skeletal muscle deterioration and premature mortality. Despite advances in supportive care, no definitive cure exists, highlighting the need to explore novel molecular targets. The current study aimed to uncover key dysregulated genes and molecular pathways in DMD through a dataset-specific network biology approach. Publicly available microarray data (GSE6011) from DMD quadriceps muscle biopsies of 22 patients and 14 age-matched healthy controls were analyzed using GEO2R to identify differentially expressed genes (DEGs) using an adjusted p-value < 0.05 and an absolute $|\log_2 FC| > 0.5$. The Protein–protein interaction (PPI) network was constructed via STRING and analyzed in Cytoscape, with hub genes identified using the Maximal Clique Centrality method in cytoHubba. Functional and pathway enrichment analyses were explored using Enrichr. A total of 159 DEGs were identified, including 19 genes that were found to be overregulated and 140 were downregulated. The PPI network revealed highly connected hub genes. Downregulated hubs included COL1A1, COL3A1, COL1A2, POSTN, LUM, COL6A3, SPARC, FBN1, COL5A2, and COL5A1, enriched in extracellular matrix organization, collagen fibril assembly, and fibrosis-related pathways. Upregulated hubs LPIN1, LPL, and UCP3 were linked to fatty acid metabolism, mitochondrial function, and oxidative stress regulation. These expression patterns suggest late-stage ECM remodeling with a concurrent metabolic shift in dystrophic muscle. Furthermore, molecular docking was performed to evaluate the binding interactions of FDA-approved DMD drugs (Amondys 45, Emflaza, and Vyondys 53) with the upregulated hub protein LPL, revealing favorable binding affinities and potential therapeutic relevance. This network biology and docking analysis highlights ECM remodeling and metabolic regulators as key components of DMD pathology, offering potential biomarkers and therapeutic targets.

Keywords: Network biology, Duchenne muscular dystrophy (DMD), differentially expressed genes (DEGs), neuromuscular disorder, protein–protein interaction (PPI), molecular docking

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INTRODUCTION

Duchenne Muscular dystrophy (DMD) is a rare, X-linked recessive neuromuscular disorder that primarily affects males, with a prevalence of approximately 1 in 3,500–5,000 live male births[1, 2]. The condition manifests with progressive skeletal muscle degeneration, typically presenting clinically between the ages of 2 and 3 years. Early signs include delayed gross motor development, difficulty running, climbing stairs, and jumping, as well as frequent falls and the development of a waddling gait. Affected children often exhibit a positive Gower's sign, using their hands and arms

to "walk" up their own body when rising from the floor [1]. Most patients lose ambulation by early adolescence (around 12–13 years of age), followed by severe cardiac complications such as dilated cardiomyopathy, arrhythmias, and respiratory disorders [3]. The DMD gene produces dystrophin, a pivotal cytoskeletal protein that facilitates the linkage between the intracellular actin filaments of muscle fibers and the extracellular matrix (ECM) via the dystrophin–glycoprotein complex at the sarcolemma. This structural connection is essential for maintaining the integrity of the muscle cell membrane by stabilizing it during repetitive cycles of contraction and relaxation, thereby minimizing susceptibility to mechanical stress and membrane damage. Mutations in the DMD gene result in the absence or severe reduction of functional dystrophin, which compromises membrane stability and integrity, making muscle fibers more susceptible to injury [4]. This leads to repeated cycles of muscle fiber damage and repair, ultimately causing progressive muscle degeneration and weakness [5]. Without intervention, DMD progresses to severe disability and premature mortality, most often due to respiratory failure or cardiac complications [6].

Currently, there is no definitive cure for DMD; existing therapeutic strategies are primarily aimed at mitigating complications and slowing the progression of the disease. Therapeutic strategies aimed at restoring dystrophin production include exon skipping, gene therapy, stop codon readthrough [7], and microdystrophin delivery via adeno-associated virus [8, 9]. Pharmacological interventions, including corticosteroids and other US FDA-approved therapies for DMD, remain central to disease management [10, 11]. While current treatments may slow disease progression and enhance quality of life, they do not offer a cure, thereby underscoring the urgent need to identify additional molecular targets for therapeutic intervention.

Network biology provides a powerful framework for uncovering such targets by analyzing complex biological interaction networks to identify critical nodes genes, or proteins central to disease mechanisms. In a protein–protein interaction (PPI) network, nodes represent proteins and edges correspond to direct physical interactions between them, collectively referred to as the interactome [12]. Constructing and analyzing a disease-specific PPI network for DMD can reveal dysregulated genes and hub proteins that may serve as biomarkers or therapeutic targets, while also illuminating key molecular pathways involved in disease progression.

Molecular docking is a computational method used to estimate the binding affinity between ligands and receptor proteins. While it holds promise in nutraceutical research, it has become a powerful and widely applied tool in drug discovery and development [13].

In this study, we analyzed the publicly available microarray dataset GSE6011 from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) [14], which contains gene expression profiles from quadriceps muscle biopsies of DMD patients and age-matched healthy controls [15]. This dataset was selected as representing an early stage of DMD, prior to the initiation of corticosteroid treatment, thereby enabling a clearer assessment of disease-associated transcriptional alterations. Using GEO2R, differentially expressed genes (DEGs) were identified by comparing DMD samples with healthy controls. The DEGs were categorized into upregulated and downregulated groups and further analyzed using network biology approaches to identify hub genes and key molecular pathways. To explore therapeutic potential, molecular docking of the upregulated hub gene LPL (Uniprot: P06858; AF-P06858-F1) with FDA-approved drugs was performed to investigate potential ligand–target interactions. Among metabolic regulators, LPL (lipoprotein lipase) plays a key role in triglyceride hydrolysis and fatty acid uptake in skeletal muscle, and increased plasma lipid levels have been shown to exacerbate muscle pathology in the mdx mouse model of DMD, highlighting the potential contribution of LPL-mediated lipid dysregulation to disease progression [16].

Previous studies have analyzed the GSE6011 dataset alongside other GEO datasets for drug repurposing [17], whereas other studies have employed weighted gene co-expression network analysis

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(WGCNA) to identify hub genes in DMD [18]. In contrast, the present study exclusively utilizes the GSE6011 dataset, employing a comprehensive network biology framework, followed by gene ontology (GO) and pathway enrichment analyses of the identified hub genes, followed by molecular docking. This singular, network-driven approach allows for an in-depth characterization of the molecular interactions and biological processes (BP) underlying DMD, providing novel insights into potential biomarkers and therapeutic targets. To our knowledge, this is the first study to apply a singular, network-driven analysis on the GSE6011 dataset, integrating both network biology and docking approaches to provide novel insights into potential biomarkers and therapeutic targets for DMD.

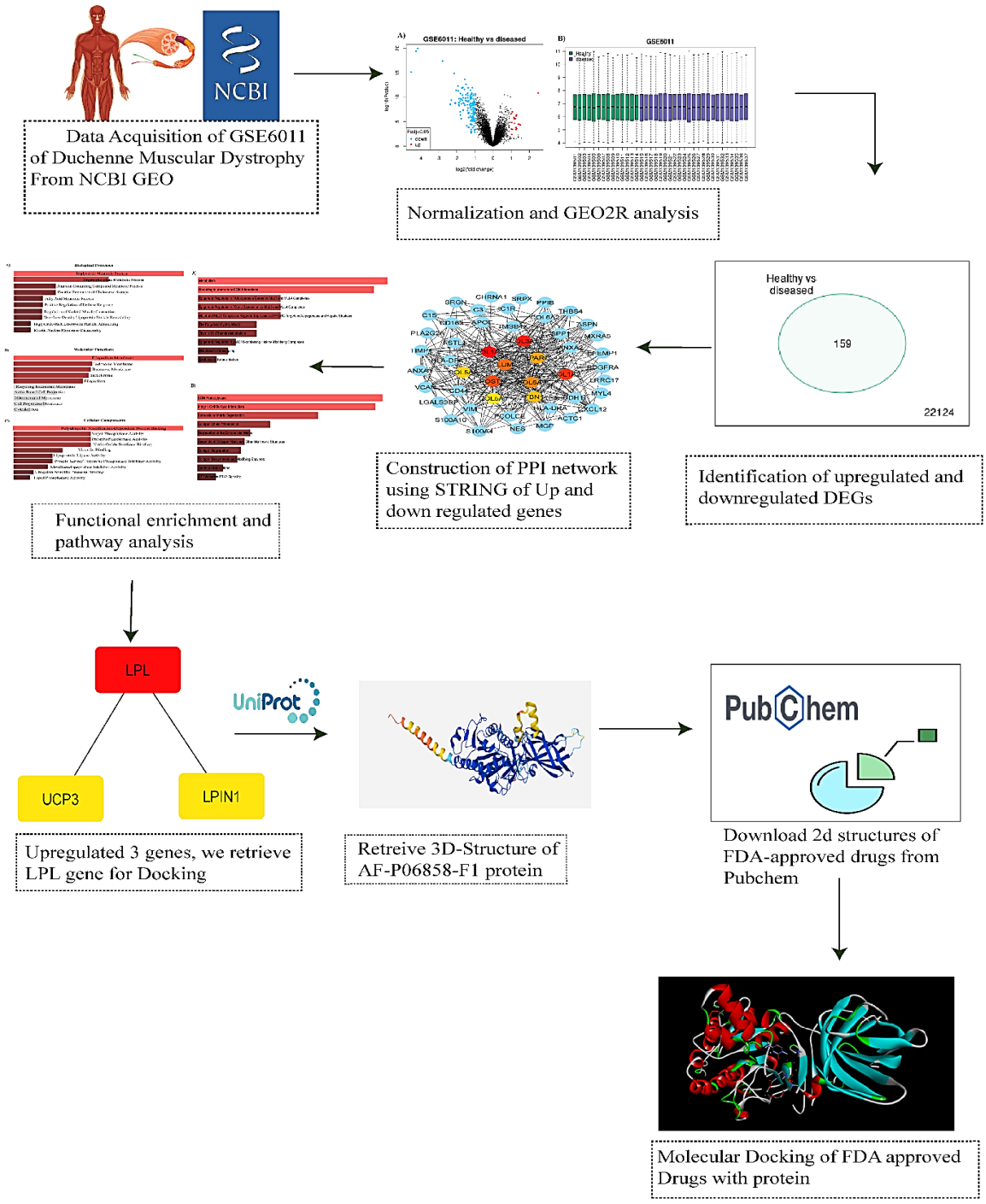

Figure 1. Workflow for identifying hub gene biomarkers in DMD using GSE6011, from dataset retrieval to functional enrichment analysis and molecular docking studies.

MATERIALS & METHODS

The analytical workflow followed in this study is outlined in Figure 1. Initially, the GSE6011 microarray dataset was retrieved from the GEO database. Differential expression analysis was performed to identify significantly upregulated and downregulated genes between DMD and control samples. The resulting DEGs were used to construct a PPI network, which was analyzed to determine key hub genes based on network topological parameters. Subsequently, GO and pathway enrichment analyses were conducted to investigate the biological functions and signaling pathways associated with these hub genes. Each step of the analysis is described in detail in the following subsections.

Retrieval of Dataset and Identification of DEGs

The normalized expression matrix from the GSE6011 dataset was utilized, containing transcriptomic profiles derived from quadriceps muscle biopsies of DMD patients and age-matched healthy individuals [15]. This dataset was specifically chosen to capture gene expression changes during the early phase of the disease, prior to the administration of corticosteroid therapy. It was accessed from the GEO database, a publicly available functional genomics repository maintained by the NCBI [14].

The dataset is based on microarray gene expression profiling using Affymetrix Human Genome U133A Arrays (platform ID: GPL96), comprising 22 DMD samples and 14 controls. Differential expression analysis was performed using the GEO2R web tool (http://www.ncbi.nlm.nih.gov/geo/geo2r/). To account for multiple testing, the Benjamini–Hochberg method was applied to adjust *p*-values and control the false discovery rate. For each gene, the $\log_2$ fold change ($\log_2$FC) was computed; genes with $\log_2FC > +0.5$ were defined as upregulated, whereas those with $\log_2FC < -0.5$ were classified as downregulated. Genes meeting the threshold of an adjusted *p*-value < 0.05 were considered significantly differentially expressed.

Construction of PPI Network and Hub Genes Identification

To explore the functional interactions among the DEGs, a PPI was constructed. The list of DEGs was submitted to the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database [19], which provides known and predicted interactions based on experimental data, computational prediction, and public text collections. The interaction confidence score was set to ≥ 0.4 (medium confidence) to ensure biologically meaningful associations.

Hub genes, key nodes with significant topological and biological importance, were identified using the cytoHubba plugin in Cytoscape [20]. CytoHubba offers multiple topological algorithms for node ranking, including Degree, Betweenness, Closeness, and Maximal Clique Centrality (MCC). The MCC algorithm, known for its high precision in predicting essential proteins within PPI networks, was employed to rank and extract the top 10 hub genes. Additionally, the Centrality function, which integrates results from 12 ranking methods, was used to enhance the robustness of hub gene identification.

GO and Pathway Enrichment Analysis

GO enrichment analysis was conducted to assess the functional roles of the identified DEGs as well as the upregulated and downregulated hub genes derived from the PPI network. GO terms were categorized into three main domains: BP, molecular functions (MF), and cellular components (CC).

To further explore the signaling pathways associated with these genes, pathway enrichment analysis was performed using the Enrichr web tool, incorporating the Reactome pathway database [21]. This analysis enabled the identification of significantly enriched GO terms and pathways, providing functional insights into the roles of the selected genes and their potential involvement in the molecular mechanisms underlying DMD.

Docking Studies of FDA-Approved Drugs with Protein

Molecular docking was performed to evaluate the binding interactions of FDA-approved drugs for DMD, including Amondys 45, Emflaza, and Vyondys 53, with the target protein LPL (Uniprot: P06858;

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AF-P06858-F1), which exhibited the highest degree among the other upregulated genes. The 3D structures of these drugs were retrieved from PubChem [22] and prepared by converting to 3D format, followed by energy minimization using Open Babel [23] in PyRx (v0.8) [24] and energy-minimized to optimize geometries before docking. Protein and ligand structures were prepared by adding polar hydrogens and assigning Gasteiger charges. Docking was carried out using AutoDock Vina (v1.2.5) within PyRx, with a grid box defined to encompass all predicted binding pockets. For each ligand, the conformation with the lowest binding free energy was selected for further analysis of molecular interactions using Discovery Studio Visualizer and PyMOL.

RESULTS

Screening of DEGs

The analysis began with the normalization of gene expression data across all samples, enabling accurate comparison between DMD patients and matched controls (Figure 2A). This preprocessing step minimized technical biases and enhanced the reliability of downstream differential expression analysis. Following normalization, we identified a distinct set of genes exhibiting significant expression changes between the two groups. In total, 19 genes showed significant upregulation**,** whereas 140 genes were found to be downregulated, based on predefined thresholds for $\log_2$ fold change and adjusted *p*-value (Supplementary File 1). The overall distribution and magnitude of these changes are presented in the volcano plot (Figure 2B), clearly distinguishing upregulated from downregulated genes within the dataset.

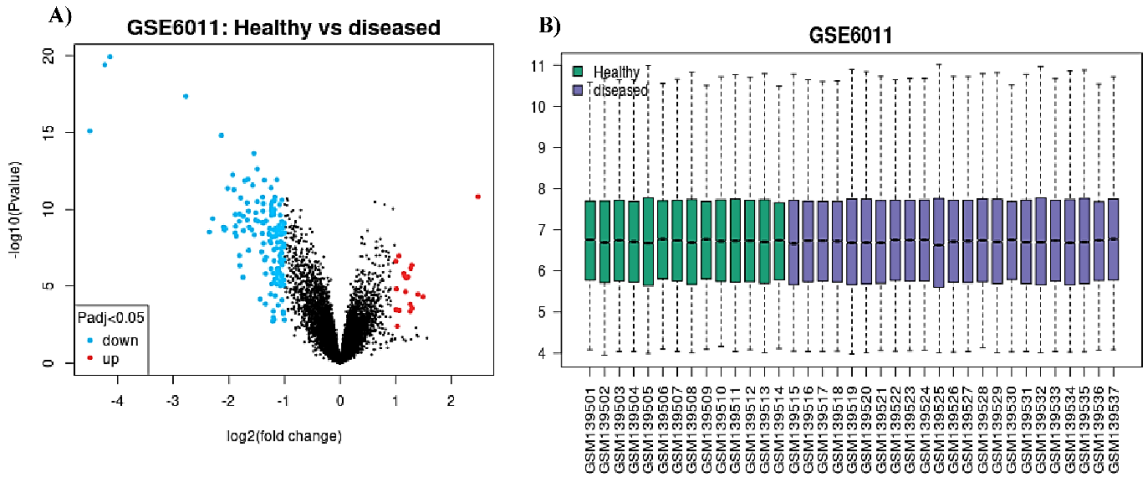

Figure 2. Differential gene expression analysis of the GSE6011 dataset. (A) Normalized expression profiles across all samples. (B) Volcano plot showing significantly upregulated and downregulated genes.

PPI Network Construction and Hub Gene Identification

A PPI network was constructed using the STRING database, consisting of 102 nodes and 580 edges, representing the interactions among the identified DEGs. The resulting network displayed a scale-free topology, a characteristic feature of biological networks, in which a limited number of nodes (hub genes) exhibit high connectivity, while the majority possess relatively few interactions [25]. The MCC algorithm identified hub nodes with high connectivity, with the top 10. Notably, the downregulated hub proteins, including COL1A1, COL3A1, COL1A2, POSTN, LUM, COL6A3, SPARC, FBN1, COL5A2, and COL5A1 exhibited significantly higher connectivity compared to non-hub proteins, suggesting their potential biological significance in the DMD. Similarly, the upregulated hub genes LPIN1, LPL, and UCP3 also demonstrated strong interaction strength within the network (Figure 3, Supplementary File 2).

Gene Enrichment and Pathway Analysis

To assess the biological relevance and functional pathways associated with the identified hub genes, GO and pathway enrichment analyses were performed.

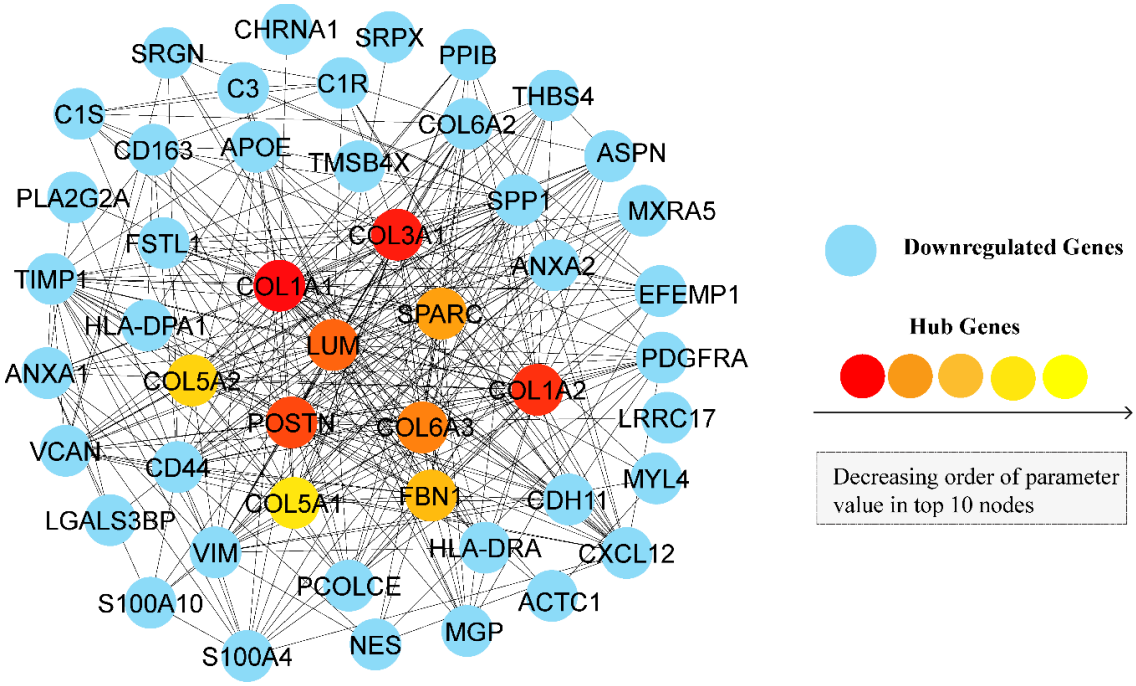

Figure 3. Downregulated protein–protein interaction (PPI) network of DEGs from the GSE6011 dataset. Inner nodes represent hub genes identified by cytoHubba, with node colors indicating gene rank.

Upregulated hub genes were significantly enriched in BP related to fatty acid metabolism, monocarboxylic acid metabolism, carboxylic acid metabolism, triglyceride metabolism, oxoacid metabolism, and organic acid metabolism. Pathway enrichment analysis indicated their involvement in several key signaling pathways, including glycolipid metabolism, SREBP (sterol regulatory element-binding proteins) signaling, epigenetic regulation of gene expression by MLL3 and MLL4 complexes, and adipogenesis (Figure 4A, Supplementary File 3).

In contrast, downregulated hub genes were enriched in BP terms associated with collagen fibril organization, ECM organization, skin and skeletal system development, supramolecular fiber organization, eye morphogenesis, wound healing, heart development, and the TGF-β receptor signaling pathway. MF enrichment indicated associations with platelet-derived growth factor binding, protease binding, metal and calcium ion binding, hormone activity, and receptor–ligand interactions. These genes were primarily localized to CC such as the collagen-containing ECM, microfibrils, intracellular organelle lumen, platelet alpha granule membranes, extracellular vesicles, Golgi lumen, and secretory vesicles (Figure 4(B), Supplementary File 4).

Further pathway analysis revealed that downregulated hub genes participated in multiple ECM-related pathways, including ECM proteoglycans, integrin cell surface interactions, collagen chain trimerization, collagen biosynthesis and degradation, and collagen formation. Additional pathways included RNA polymerase II transcription, cytokine signaling, interleukin signaling, post-translational protein modification, protein metabolism, gene expression, hemostasis, and platelet activation responses, including response to elevated cytosolic Ca^{2+} (Figure 5).

Docking Studies of FDA-Approved Drugs with LPL

Expression profiling identified LPL as a significantly upregulated hub gene in DMD muscle, indicating altered lipid metabolism as part of the disease pathology. To explore its potential as a therapeutic target, molecular docking was performed using the AlphaFold-predicted structure of LPL (AF-P06858-F1). The FDA-approved drugs Amondys 45, Emflaza, and Vyondys 53 were docked, and their binding affinities were evaluated using AutoDock Vina in PyRx.

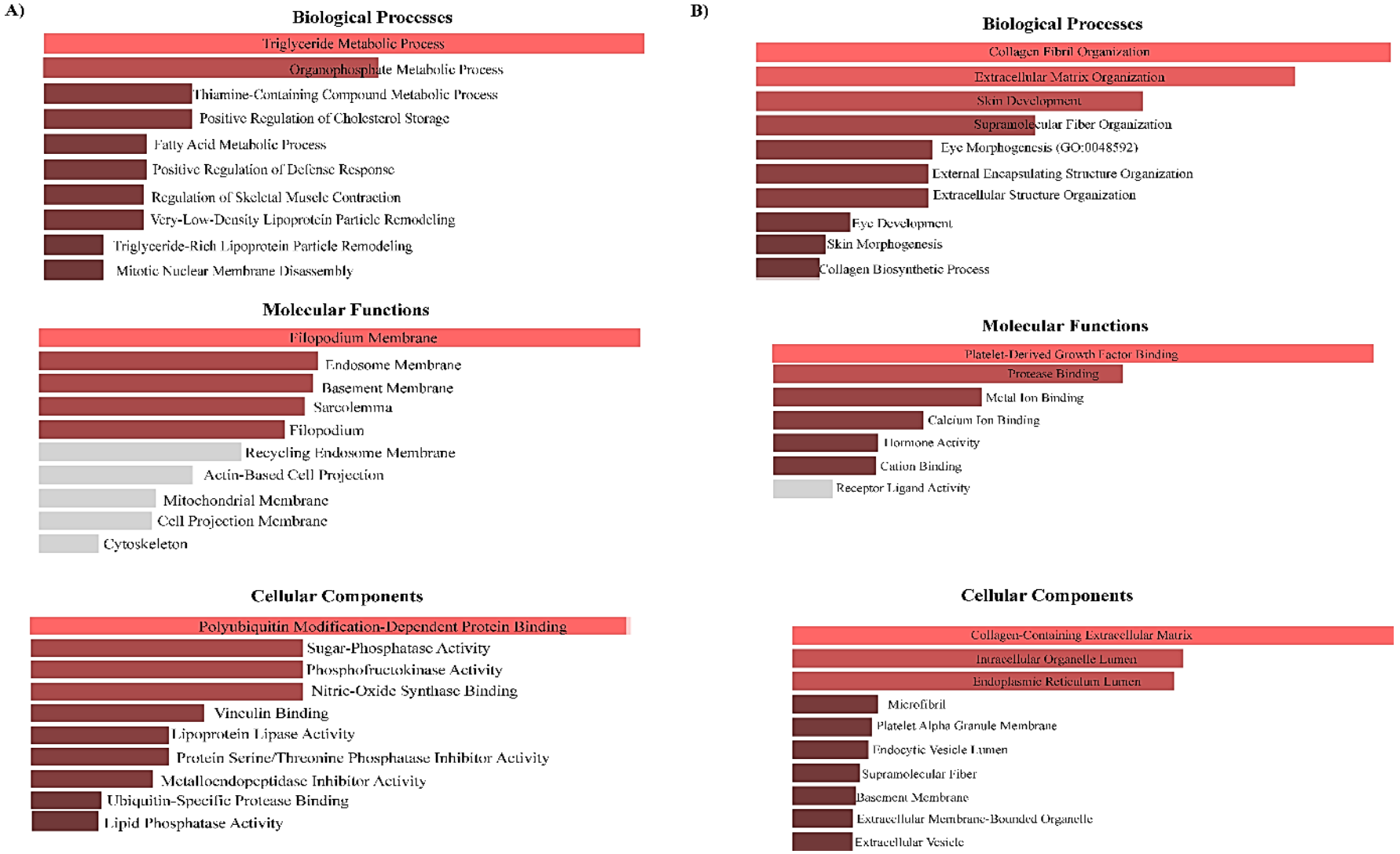

Figure 4. (A) Gene enrichment analysis of upregulated and (B) downregulated hub genes. GO terms include biological processes (SRING), molecular functions (MF), and cellular components (CC). Pathways were identified from the Reactome database.

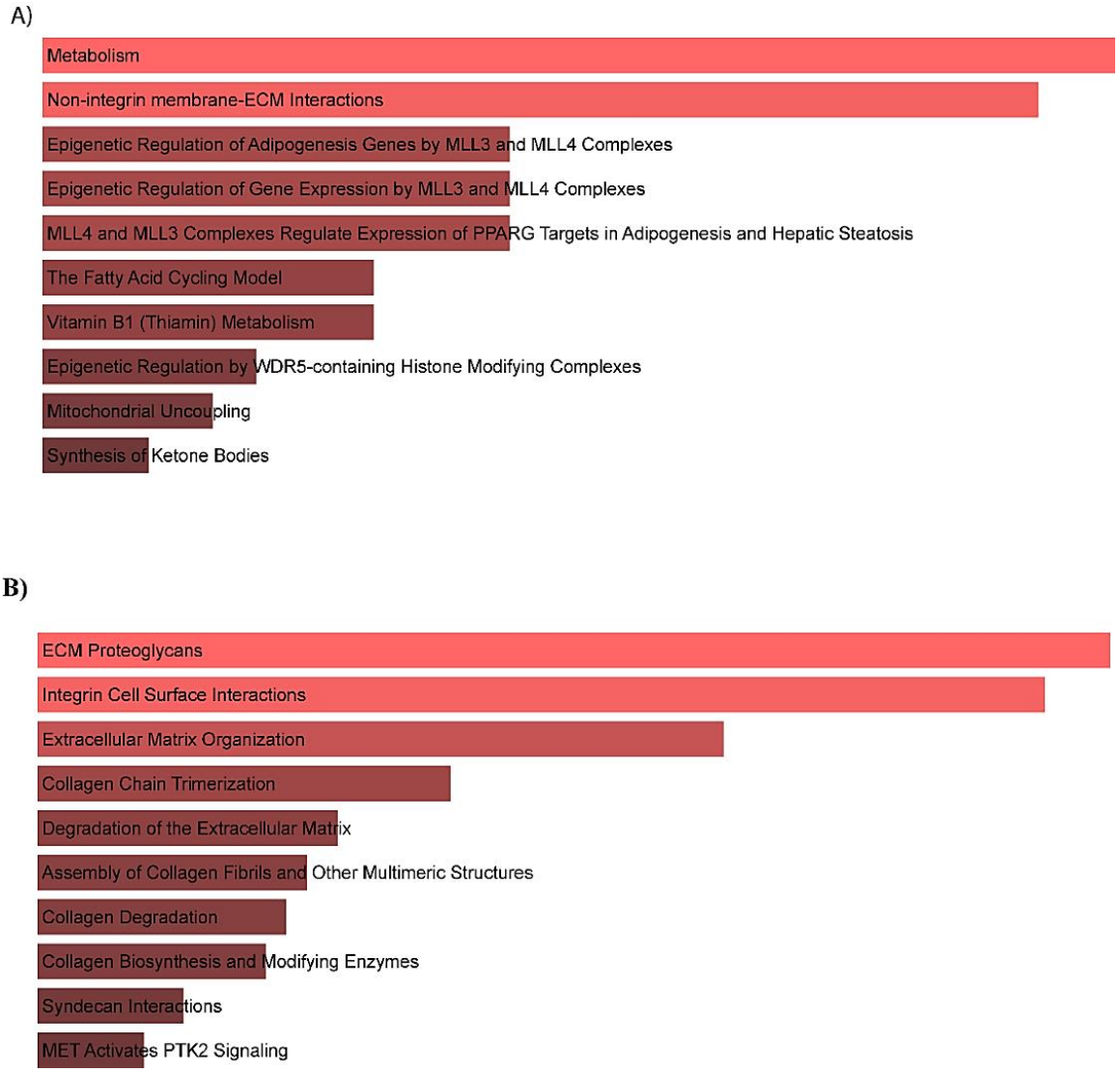

Figure 5. Pathway enrichment analysis of upregulated and downregulated hub genes.

All three ligands demonstrated favorable binding to LPL, with Amondys 45 showing the strongest binding affinity (–7.2 kcal/mol), followed by Emflaza (–5.7 kcal/mol) and Vyondys 53 (–5.3 kcal/mol). Multiple docking poses were generated for each ligand, with the lowest-energy conformations selected for analysis. Visualization in PyMOL and Discovery Studio confirmed that the ligands occupied predicted binding cavities within LPL and were stabilized primarily through hydrogen bonds, hydrophobic contacts, and van der Waals interactions. Amondys 45 formed more extensive hydrogen-bond interactions compared to Emflaza and Vyondys 53, consistent with its stronger binding affinity. The receptor is represented in cartoon format, where α-helices are depicted in red, β-sheets in cyan, and random coils in green (Figure 6). The ligand is displayed in stick representation within the binding pocket. The docking results revealed that the ligand was stably accommodated inside the active site cavity of the protein, forming close contact with surrounding amino acid residues.

The visualization clearly demonstrates the positioning of the ligand relative to the secondary structural elements of the receptor, suggesting that the binding pocket provides a favorable environment for ligand accommodation. These findings support the predicted docking score and confirm the potential binding affinity of the compound toward the target protein. These findings suggest that FDA-approved drugs for DMD can establish stable interactions with LPL, providing further evidence that this upregulated metabolic regulator may serve as a potential secondary drug target in DMD (Table 1).

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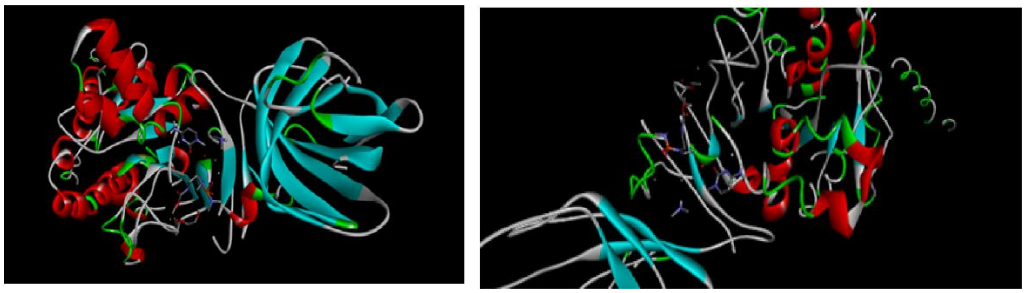

Figure 6. Interaction of the docked ligand Amondys with the target protein, showing a favorable binding affinity.

Table 1. Results of docking of FDA-approved drugs with LPL (AF-P06858-F1 model) using AutoDock Vina.

S.N.	Ligand	Best Binding Affinity (kcal/mol)	Interaction Notes
1	Amondys 45	–7.2	Multiple H-bonds + hydrophobic contacts.
2	Emflaza	–5.7	Moderate H-bonds, stable pose.
3	Vyondys 53	–5.3	Fewer H-bonds, weaker binding.

DISCUSSION

In the present study, the GSE6011 microarray dataset was analyzed to elucidate the molecular mechanisms underlying DMD. We identified DEGs and constructed a PPI network. The resulting network exhibited high connectivity, highlighting the potential central roles of several hub genes in the pathogenesis of DMD.

GO and pathway enrichment analyses revealed the significantly involved genes in biological BP, such as ECM organization, fatty acid metabolism, fibril formation, and skeletal system development pathways, that are strongly associated with disease progression. The enrichment of both ECM-related and metabolic pathways underscores the dual contribution of structural remodeling and metabolic dysregulation in DMD pathology. While earlier studies have integrated the GSE6011 dataset with other transcriptomic datasets for drug repurposing [17] and applied WGCNA to identify hub genes in DMD [18] , our study takes a distinct approach. By focusing exclusively on GSE6011 within a network biology framework, we generated more refined insights into disease-associated molecular alterations that may be diluted in meta-analyses.

It is noteworthy that in our DMD PPI network, the expressions of COL1A1, COL3A1, COL1A2, POSTN, LUM, COL6A3, SPARC, FBN1, COL5A2, and COL5A1 were downregulated while LPIN1, LPL, and UCP3 were upregulated. This suggests a potential role in these genes in DMD pathology and highlights their strong interaction strength within the network.

COL1A1, the gene encoding the alpha-1 chain of type I collagen, is a well-established marker of collagen-secreting fibroblasts and is critically involved in both muscle tissue regeneration and fibrotic processes. Normally, injury triggers transient PDGFRα activation in mesenchymal progenitors, leading to controlled COL1A1 expression and limited scar formation. In DMD, chronic muscle damage maintains PDGFRα signaling active, driving persistent COL1A1 expression, excessive collagen deposition, and fibrosis [26]. In our study, however, COL1A1 was downregulated in DMD samples, possibly reflecting differences in disease stage, sample composition, or a late-stage state where collagen synthesis slows despite existing fibrosis. This aligns with the idea that PDGFRα activation drives fibrosis early in disease, while later phases may show reduced COL1A1 transcription even in fibrotic tissue. Several genes associated with the ECM and fibrosis, including COL3A1, COL1A2, POSTN, LUM, COL6A3, SPARC, FBN1, COL5A2, and COL5A1, were found to be downregulated. These genes encode critical structural and regulatory components of fibrotic tissue. Specifically, collagen-encoding genes, such as COL1A1/1A2, COL3A1, COL5A1/5A2, and COL6A3, are responsible for

producing fibrillar and network-forming collagens, which contribute to the tensile strength and structural integrity of the ECM. These collagens are typically upregulated during active fibrotic remodeling in DMD-affected muscle, highlighting the complex regulation of ECM dynamics in disease progression [27].

POSTN (Periostin) is a matricellular protein secreted by activated fibroblasts that supports collagen crosslinking and ECM organization and is generally elevated in DMD to promote fibrosis [28]. It is downregulated in our study may reflect late-stage disease, where fibrosis is sustained by preexisting ECM rather than active transcription, or differences in sample composition. Despite this, its established pro-fibrotic role in DMD remains relevant and warrants further cell-type–specific investigation.

In advanced DMD, fibrosis is often maintained by preexisting ECM rather than ongoing high transcription of fibrotic genes, consistent with late-stage remodeling rather than active fibrogenesis [26]. Alternatively, reduced expression could be due to depletion or altered transcriptional programming of fibro/adipogenic progenitors in chronic disease. LUM (lumican) regulates collagen fibril assembly and ECM organization. Deficiency in LUM has been associated with structural defects such as skin fragility and corneal opacity [29]. In DMD, LUM supports collagen network integrity during fibrosis, and its reduced expression could impair ECM stability. SPARC (Secreted Protein Acidic and Rich in Cysteine) is classified as a matricellular protein that regulates cell–matrix interactions and promotes collagen deposition, thereby contributing to tissue remodeling and fibrotic processes [30]. FBN1 (fibrillin-1) is a key structural glycoprotein that forms microfibrils in the ECM, providing elasticity and mechanical stability to connective tissues [31]. Downregulation of SPARC and FBN1 may indicate reduced active ECM remodeling at the time of sampling or late-stage fibrosis maintenance by existing ECM rather than ongoing synthesis.

In contrast, metabolic regulators, including LPIN1, LPL, and UCP3, were upregulated, suggesting an adaptive response in dystrophic muscle to meet altered energy demands. LPIN1 (lipin-1) encodes a phosphatide phosphatase involved in triglyceride and phospholipid metabolism, as well as regulation of energy homeostasis. It also influences mitochondrial function and fatty acid oxidation in skeletal muscle [32]. Dysregulation of LPIN1 has been associated with altered lipid metabolism and muscle pathology. While UCP3 (uncoupling protein-3) is a mitochondrial inner membrane transporter involved in proton leak and regulation of mitochondrial efficiency. In skeletal muscle, UCP3 modulates energy expenditure, reactive oxygen species balance, and lipid utilization [33]. Notably, LPL emerged as a central hub gene, encoding the enzyme responsible for hydrolyzing circulating triglycerides into free fatty acids for uptake and utilization by muscle and adipose tissue. In skeletal muscle, LPL activity supports lipid-based energy metabolism and can shift substrate utilization toward fatty acids during endurance or metabolic stress [32]. This is consistent with clinical evidence showing a high prevalence of plasma lipid abnormalities in DMD and BMD patients and canine models, even in unaffected carriers, supporting the concept that dyslipidemia is a primary metabolic feature of dystrophinopathies rather than a secondary consequence of muscle wasting [34].

To further investigate the therapeutic relevance of LPL, docking studies were performed with FDA-approved drugs for DMD. The study revealed favorable binding of all ligands, with Amondys 45 showing the strongest affinity (–7.2 kcal/mol), followed by Emflaza (–5.7 kcal/mol) and Vyondys 53 (–5.3 kcal/mol). Amondys 45 formed multiple hydrogen bonds and hydrophobic interactions within the predicted binding site, consistent with its superior docking score. These findings suggest that current FDA-approved drugs may exert secondary effects by interacting with metabolic regulators, such as LPL, potentially broadening their therapeutic benefits beyond dystrophin modulation. Importantly, LPL has been shown to be pharmacologically targetable, as evidenced by the development of novel small-molecule LPL activators [35], reinforcing the biological plausibility of our docking results.

Taken together, the downregulation of ECM-associated genes and upregulation of metabolic regulators highlight a dual mechanism of structural and metabolic remodeling in DMD. Among these,

LPL stands out as both a biomarker of metabolic imbalance and a potential therapeutic node. The integration of network biology and docking analyses underscores the emerging role of lipid dysregulation in DMD pathophysiology and supports further exploration of LPL as a druggable target. Nonetheless, these in silico findings require validation through molecular dynamics simulations, enzymatic assays, and in vivo studies to clarify whether drug–LPL interactions can translate into measurable therapeutic effects.

CONCLUSIONS

In this study, network biology analysis of the GSE6011 dataset revealed key molecular signatures underlying DMD. Downregulation of collagen and ECM-associated genes indicates altered fibrosis dynamics, consistent with a transition from active fibrogenesis to late-stage ECM remodeling. Concurrently, upregulation of metabolic regulators, including LPIN1, LPL, and UCP3, reflects a compensatory shift in energy metabolism and mitochondrial activity in dystrophic muscle. Notably, molecular docking demonstrated that FDA-approved DMD drugs, particularly Amondys 45, exhibit favorable binding to LPL, highlighting its potential as a druggable target and suggesting secondary therapeutic modulation of lipid metabolism. By focusing on a single, untreated early-stage dataset, this approach minimized heterogeneity and captured disease-specific molecular features. Hub genes, such as COL1A1, COL3A1, POSTN, LPIN1, LPL, and UCP3, emerge as promising biomarkers and candidate therapeutic targets. Future studies should pursue cell-type-specific transcriptomics, functional assays, and experimental validation of LPL–drug interactions to advance translational insights and therapeutic strategies for DMD.

Authors' Contribution

SK designed the study, developed the protocol, prepared the figures and tables, and wrote the first draft of the manuscript. SZ, VBR interpret the data and provided important input during the analysis. They also reviewed and edited the manuscript and checked the references for accuracy. All authors reviewed the final version of the manuscript, approved it, and agreed to take responsibility for the content as per guidelines.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this work.

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