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NETWORK-DRIVEN BIOINFORMATICS ANALYSIS OF GENE INTERACTIONS AND PATHWAYS INVOLVED IN DUCHENNE MUSCULAR DYSTROPHY

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Abstract:

Duchenne Muscular Dystrophy (DMD) is a severe X-linked neuromuscular disorder caused by mutations in the DMD gene, leading to progressive muscle degeneration. In this study, a network-based bioinformatics approach was employed to analyze disease-associated genes and molecular pathways involved in DMD using web-based bioinformatics tools. Gene–disease association analysis was performed using DisGeNET, followed by protein–protein interaction (PPI) analysis using STRING. Functional gene association and network expansion were carried out using GeneMANIA. Gene set enrichment and hub gene identification were conducted using Enrichr, and pathway mapping was performed using the Reactome Pathway Browser. The integrated analysis revealed key genes involved in muscle structure, extracellular matrix organization, immune response, and inflammatory signaling. The findings highlight the complex molecular mechanisms underlying DMD and demonstrate the utility of integrative bioinformatics approaches in understanding disease pathology and identifying potential therapeutic targets.

Keywords: *Duchenne Muscular Dystrophy, Bioinformatics, Network Analysis, Gene–Disease Association, Protein–Protein Interaction.*

1. Introduction

Duchenne Muscular Dystrophy (DMD) is a severe, inherited neuromuscular disorder characterized by progressive muscle degeneration, leading to loss of mobility, respiratory failure, and cardiac complications. It is one of the most common and clinically severe forms of muscular dystrophy, with an estimated global incidence of approximately 1 in 3,500–5,000 live male births (1, 2). The disease follows an X-linked recessive pattern of inheritance and predominantly affects males, while females are usually asymptomatic carriers. Clinical

manifestations typically appear in early childhood, often between 2 and 5 years of age, and include delayed motor milestones, difficulty in running or climbing stairs, frequent falls, and progressive proximal muscle weakness (3). As the disease progresses, muscle weakness worsens, leading to loss of independent ambulation during early adolescence. Over time, respiratory muscles and cardiac muscles become severely affected, resulting in respiratory insufficiency and dilated cardiomyopathy, which are the major causes of mortality in DMD patients (2). Despite advances in supportive care, DMD remains a life-limiting disorder, emphasizing the urgent need for a deeper molecular understanding of disease mechanisms and improved therapeutic strategies.

At the genetic level, Duchenne Muscular Dystrophy is caused by mutations in the DMD gene located on the short arm of the X chromosome (Xp21). The DMD gene is one of the largest known human genes, spanning approximately 2.4 megabases and consisting of 79 exons. It encodes dystrophin, a large cytoskeletal protein that plays a crucial role in maintaining the structural integrity of muscle fibers (4,5). Dystrophin forms an essential component of the dystrophin–glycoprotein complex (DGC), which connects the intracellular actin cytoskeleton to the extracellular matrix via transmembrane proteins. This complex protects muscle fibers from mechanical stress generated during muscle contraction and relaxation cycles.

In the absence of functional dystrophin, muscle fibers become highly vulnerable to mechanical injury. Loss of dystrophin leads to sarcolemmal instability, abnormal calcium influx, activation of proteases, and increased susceptibility to oxidative stress. These molecular disturbances trigger repeated cycles of muscle fiber degeneration and regeneration (6). Initially, muscle regeneration is supported by satellite cells; however, continuous damage eventually overwhelms regenerative capacity, resulting in progressive muscle wasting and replacement of functional muscle tissue with fibrotic and adipose tissue.

Importantly, dystrophin deficiency alone does not fully explain the complexity and variability of DMD pathology. Numerous secondary pathological processes contribute to disease progression, including chronic inflammation, immune system activation, extracellular matrix remodeling, mitochondrial dysfunction, and altered intracellular signaling pathways (7;8). Studies have demonstrated elevated expression of inflammatory cytokines, immune cell infiltration, and fibrosis-associated genes in dystrophic muscle, indicating that DMD is not solely a structural muscle disorder but also an inflammatory and metabolic disease.

Furthermore, the clinical severity of Duchenne Muscular Dystrophy varies considerably among patients, even among individuals carrying similar DMD mutations. This variability has been attributed to the influence of modifier genes and regulatory networks that affect muscle regeneration, inflammation, and fibrosis (9). Identifying such modifier genes is critical for understanding disease heterogeneity and for developing personalized therapeutic approaches.

Advances in high-throughput technologies, such as microarray analysis, RNA sequencing, and proteomics, have generated vast amounts of biological data related to Duchenne Muscular Dystrophy. Publicly available databases now provide access to gene expression profiles, protein–protein interactions, regulatory networks, and disease-associated pathways. However, extracting meaningful biological insights from these large datasets requires the application of bioinformatics and systems biology approaches (10).

Bioinformatics tools enable comprehensive analysis of gene–disease associations, identification of differentially expressed genes, construction of protein–protein interaction networks, functional enrichment analysis, and pathway mapping. Such integrative approaches are particularly valuable for complex genetic disorders like DMD,

where multiple molecular processes interact to drive disease progression (11). Network-based analysis allows the identification of key hub genes and critical biological pathways that may serve as potential biomarkers or therapeutic targets.

In recent years, bioinformatics-driven studies have significantly contributed to the understanding of Duchenne Muscular Dystrophy by revealing dysregulated signaling pathways related to inflammation, extracellular matrix organization, calcium homeostasis, and muscle regeneration (12;13). These findings highlight the importance of adopting a systems-level perspective rather than focusing solely on the primary gene defect.

In this study, a comprehensive bioinformatics approach was employed to analyze Duchenne Muscular Dystrophy using publicly available databases and analytical tools. Gene–disease association analysis, protein–protein interaction networks, functional gene association networks, enrichment analysis, and pathway mapping were used to investigate the molecular landscape of DMD. The methodology and interpretations are strictly based on the outputs obtained from these tools, ensuring that the analysis accurately reflects the performed computational work. This study aims to demonstrate the role of integrative bioinformatics approaches in understanding the complex molecular mechanisms of Duchenne Muscular Dystrophy and to highlight their potential contribution to future therapeutic research.

2. Materials and Methodology

2.1 Study design

The present study follows a network-based bioinformatics workflow to analyze Duchenne Muscular Dystrophy-associated genes. Publicly available, web-based bioinformatics tools were used to identify gene-disease associations, construct interaction networks, perform functional enrichment analysis, and interpret biological pathways. The methodology was designed to reflect the outputs generated from each tool, ensuring reproducibility and biological relevance.

2.2 Databases and tools use

A range of well-established and publicly available bioinformatics databases were utilized to ensure comprehensive and reliable data collection.

a. DisGeNET was used to identify genes associated with Duchenne Muscular Dystrophy. Gene-disease association analysis was performed using DisGeNET to identify genes strongly linked to Duchenne Muscular Dystrophy. Genes with high association scores were selected for downstream network and functional analyses. DisGeNET is a comprehensive platform that integrates gene-disease association data from curated databases, genome-wide association studies, animal models, and scientific literature. It provides association scores that reflect the strength of evidence supporting each gene-disease relationship (11).

b. STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) was employed to construct and analyze protein-protein interaction networks. STRING integrates known and predicted protein interactions derived from experimental data, computational prediction methods, and text mining. The identified genes were analyzed using STRING to construct a protein-protein interaction network. Network properties such as number of nodes, edges, and PPI enrichment p-value were examined to assess the biological significance of the interactions. The PPI enrichment p-value generated by STRING indicates whether the observed interactions are biologically meaningful rather than random (14).

c. GeneMANIA was used for functional gene association analysis and network expansion. This tool predicts gene function based on multiple evidence types, including physical interactions, co-expression, shared pathways, co-localization, and shared protein domains. GeneMANIA also adds functionally related genes to enhance network robustness.GeneMANIA was used to analyze functional relationships among the selected genes and to identify additional genes functionally related to the core disease-associated gene set (15).

d. Enrichr was utilized for gene set enrichment analysis and hub gene identification. Functional libraries such as KEGG Human and Reactome Pathways were used to identify significantly enriched biological processes and pathways. Hub genes were identified based on functional recurrence across multiple enriched terms. Enrichr was used to perform gene set enrichment analysis across multiple functional libraries. Hub genes were identified based on their recurrence across enriched biological terms rather than solely on network topology (16).

e. Reactome Pathway Browser was applied to map disease-associated genes onto curated biological pathways. Reactome provides detailed pathway annotations along with statistical significance measures such as p-values and false discovery rate (FDR), enabling biological interpretation of enriched pathways. Reactome Pathway Browser was used to identify significantly enriched biological pathways and to interpret the molecular mechanisms involved in DMD pathogenesis (17).

3. Results

3.1 Gene–Disease Association Analysis (DisGeNET)

Gene–disease association analysis performed using the DisGeNET database identified Duchenne Muscular Dystrophy as being most strongly associated with the DMD (dystrophin) gene, which showed the highest association score among all retrieved genes. This result strongly validates the established role of dystrophin loss as the primary molecular cause of Duchenne Muscular Dystrophy. The high association score reflects extensive experimental validation, clinical reports, and curated literature evidence supporting the involvement of the DMD gene in disease pathogenesis (11).

In addition to the DMD gene, several other genes such as UTRN, ITGA7, DAG1, LAMA2, and SGCA were identified as significantly associated with the disease. These genes encode proteins involved in muscle fiber stability, sarcolemma integrity, and linkage between the cytoskeleton and extracellular matrix. Their identification suggests that Duchenne Muscular Dystrophy is influenced by a group of disease-associated and modifier genes rather than a single gene defect. Similar observations have been reported in bioinformatics studies highlighting the role of modifier genes in influencing disease severity and progression in DMD patients (12).

Overall, the DisGeNET results confirm that gene–disease association databases are effective in identifying both primary causative genes and secondary contributors involved in complex genetic disorders such as Duchenne Muscular Dystrophy.

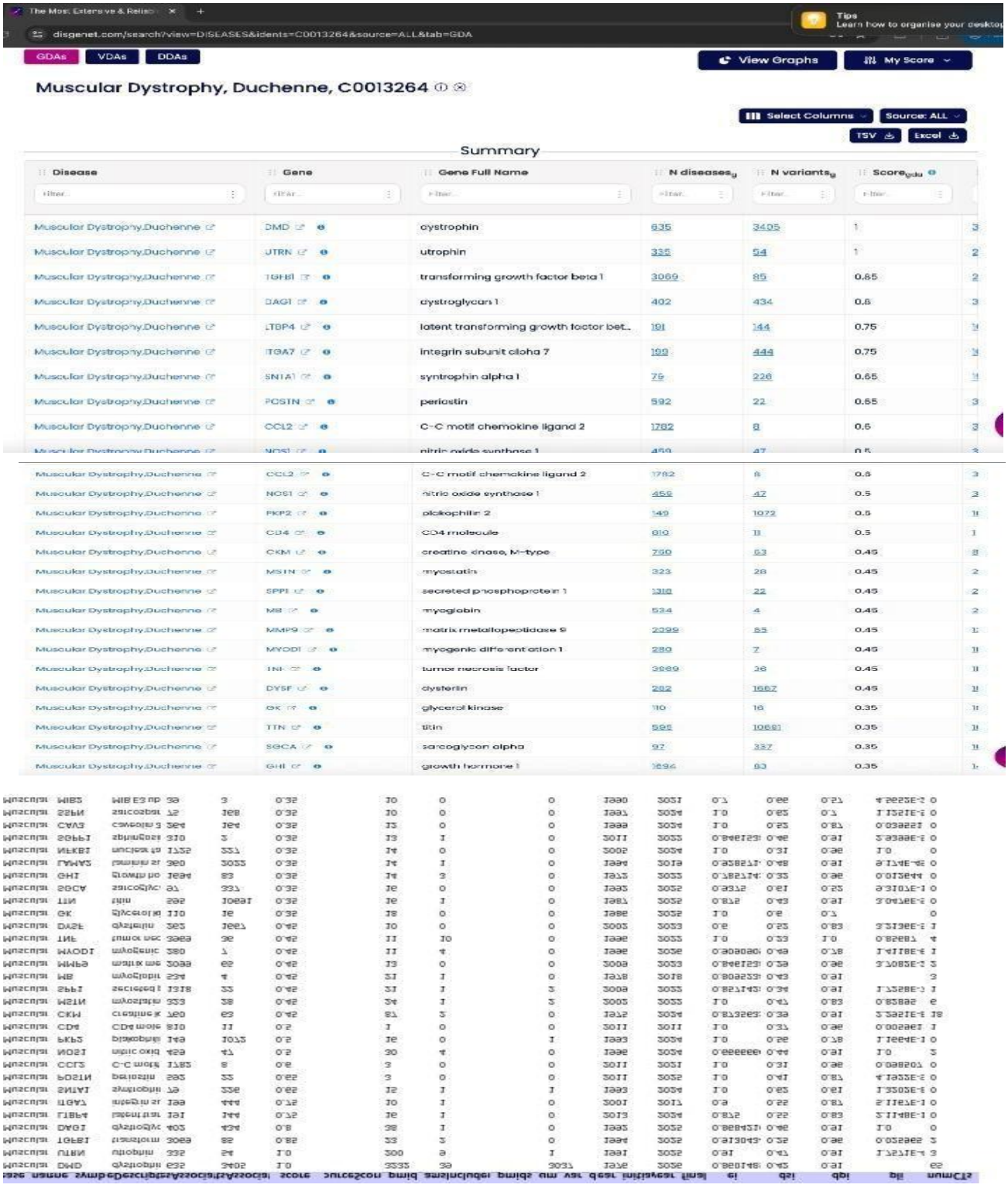


Figure 1: Gene–disease association results for Duchenne Muscular Dystrophy (DMD) obtained from DisGeNET database

3.2 Protein–Protein Interaction (PPI) Network Analysis (STRING)

Protein–protein interaction analysis was carried out using the STRING database to explore functional interactions among DMD-associated genes. The resulting network consisted of 30 nodes, representing interacting proteins, and displayed a dense interaction pattern, indicating extensive functional connectivity. The protein–protein interaction enrichment p-value was reported as $< 1 \times 10^{-16}$, demonstrating that the observed interactions are significantly enriched and not due to random chance.

Gene Ontology enrichment analysis of the interaction network revealed strong enrichment of muscle-related biological processes, including muscle contraction, muscle system process, and muscle structure development. Cellular component analysis highlighted key structures such as the dystrophin-associated glycoprotein complex and the sarcolemma, which are directly affected in Duchenne Muscular Dystrophy. These findings are consistent with the known pathological mechanism where dystrophin loss compromises muscle fiber stability (14).

Additional enrichment of cytoskeletal organization, ion transport regulation, inflammatory response, and extracellular matrix remodeling pathways suggests the involvement of secondary mechanisms contributing to disease progression. The presence of inflammation-related processes indicates ongoing muscle damage and repair cycles, which are hallmarks of dystrophic muscle tissue. Similar protein interaction with patterns have been reported in network-based analyses of neuromuscular disorders, reinforcing the relevance of the STRING-based results (18).

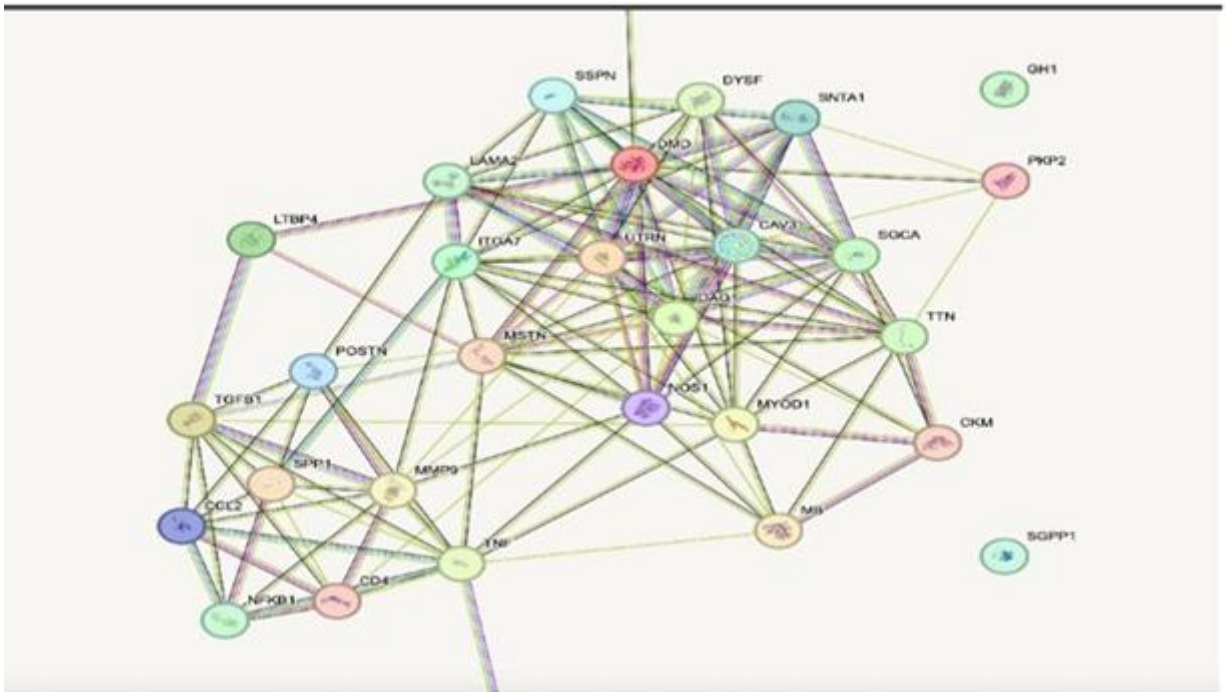
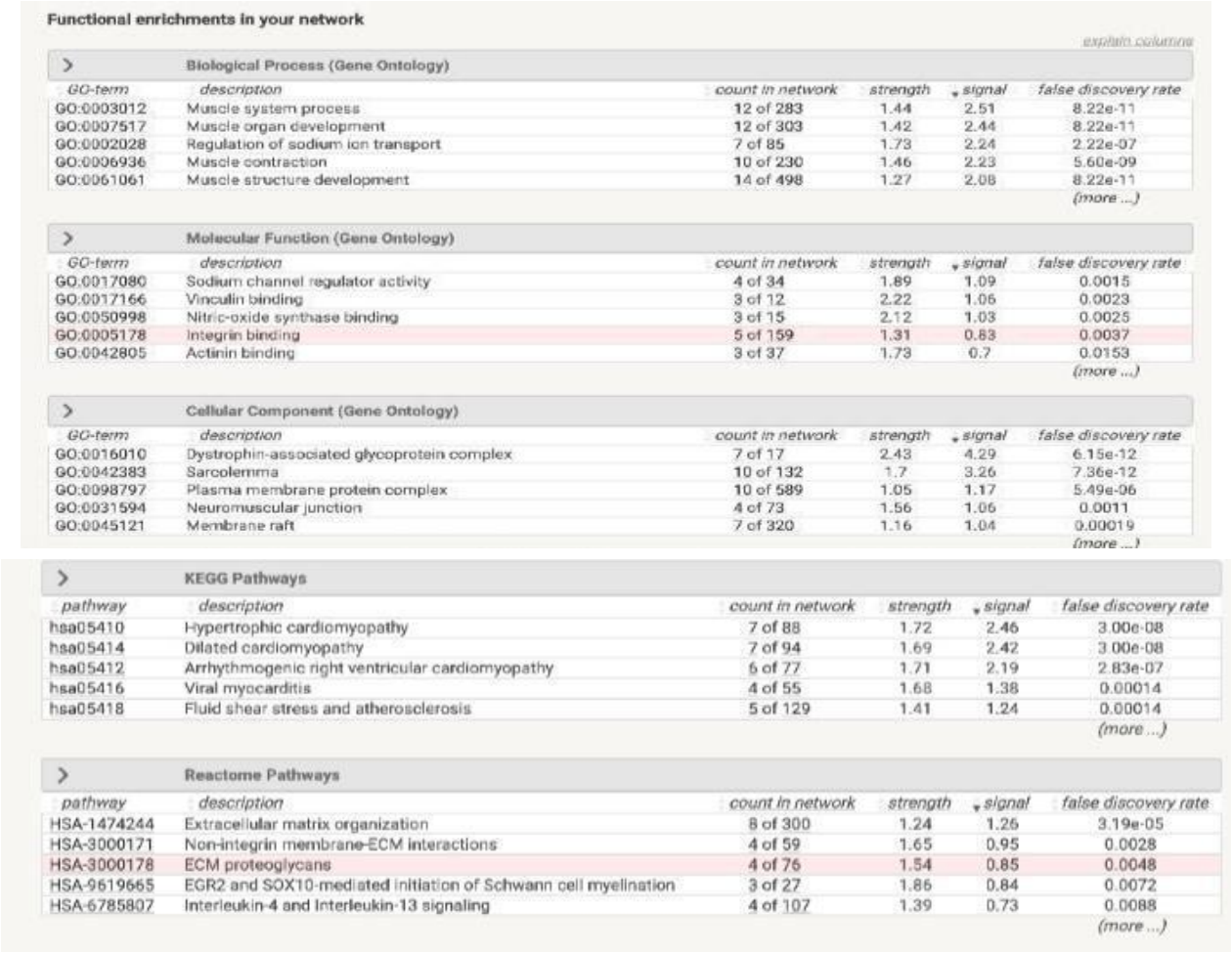


Figure 2: STRING protein–protein interaction network connectivity among muscular dystrophy genes



Functional enrichments in your network

explain columns

Biological Process (Gene Ontology)

GO-term	description	count in network	strength	signal	false discovery rate
GO:0003012	Muscle system process	12 of 283	1.44	2.51	8.22e-11
GO:0007517	Muscle organ development	12 of 303	1.42	2.44	8.22e-11
GO:0002028	Regulation of sodium ion transport	7 of 85	1.73	2.24	2.22e-07
GO:0006936	Muscle contraction	10 of 230	1.46	2.23	5.60e-09
GO:0061061	Muscle structure development	14 of 498	1.27	2.08	8.22e-11

(more ...)

Molecular Function (Gene Ontology)

GO-term	description	count in network	strength	signal	false discovery rate
GO:0017080	Sodium channel regulator activity	4 of 34	1.89	1.09	0.0015
GO:0017166	Vinculin binding	3 of 12	2.22	1.06	0.0023
GO:0050998	Nitric-oxide synthase binding	3 of 15	2.12	1.03	0.0025
GO:0005178	Integrin binding	5 of 159	1.31	0.83	0.0037
GO:0042805	Actinin binding	3 of 37	1.73	0.7	0.0153

(more ...)

Cellular Component (Gene Ontology)

GO-term	description	count in network	strength	signal	false discovery rate
GO:0016010	Dystrophin-associated glycoprotein complex	7 of 17	2.43	4.29	6.15e-12
GO:0042383	Sarcolemma	10 of 132	1.7	3.26	7.36e-12
GO:0098797	Plasma membrane protein complex	10 of 589	1.05	1.17	5.49e-06
GO:0031594	Neuromuscular junction	4 of 73	1.56	1.06	0.0011
GO:0045121	Membrane raft	7 of 320	1.16	1.04	0.00019

(more ...)

KEGG Pathways

pathway	description	count in network	strength	signal	false discovery rate
hsa05410	Hypertrophic cardiomyopathy	7 of 88	1.72	2.46	3.00e-08
hsa05414	Dilated cardiomyopathy	7 of 94	1.69	2.42	3.00e-08
hsa05412	Arrhythmogenic right ventricular cardiomyopathy	6 of 77	1.71	2.19	2.83e-07
hsa05416	Viral myocarditis	4 of 55	1.68	1.38	0.00014
hsa05418	Fluid shear stress and atherosclerosis	5 of 129	1.41	1.24	0.00014

(more ...)

Reactome Pathways

pathway	description	count in network	strength	signal	false discovery rate
HSA-1474244	Extracellular matrix organization	8 of 300	1.24	1.26	3.19e-05
HSA-3000171	Non-integrin membrane-ECM interactions	4 of 59	1.65	0.95	0.0028
HSA-3000178	ECM proteoglycans	4 of 76	1.54	0.85	0.0048
HSA-9619665	EGR2 and SOX10-mediated initiation of Schwann cell myelination	3 of 27	1.86	0.84	0.0072
HSA-6785807	Interleukin-4 and Interleukin-13 signaling	4 of 107	1.39	0.73	0.0088

(more ...)

Figure 3: STRING-based Protein–Protein Interaction (PPI) network and functional/pathway enrichment analysis of the selected genes

Table 1: Summary of Enriched Biological Themes and Representative GO Terms from STRING-Based Network Analysis:

Biological Theme	Representative GO terms
Muscle structure & development	Muscle contraction, muscle system process
Sarcolemma integrity	Dystrophinassociated glycoprotein complex, sarcolemma
Cytoskeleton & adhesion	Actin binding, integrin binding
Ion transport & signaling	Sodium ion transport regulation
Inflammation & ECM remodeling	Inflammatory response, ECM organization

3.3 Functional Gene Association Network (GeneMANIA)

Functional gene association analysis using GeneMANIA revealed a highly interconnected network with a dense central core of hub genes. The network was supported by multiple types of evidence, including physical interactions, co-expression, shared pathways, co-localization, shared protein domains, and predicted functional associations. The high contribution of physical interactions and co-expression suggests strong functional coordination among disease-associated genes.

Several key genes, including LAMA2, DAG1, DMD, ITGA7, SGCA, TGFB1, TNF, NFKB1, MMP9, CCL2, and SPP1, were positioned centrally within the network, indicating their importance in maintaining network stability. These genes are involved in extracellular matrix organization, immune signaling, inflammatory regulation, and muscle fiber maintenance. The central placement of inflammatory mediators such as TNF and NFKB1 highlights the contribution of immune-mediated mechanisms to Duchenne Muscular Dystrophy pathology.

Additionally, GeneMANIA predicted and incorporated functionally related genes, further expanding the network and supporting the existence of shared biological pathways and co-regulatory mechanisms. Such dense and multi-layered networks have been reported in previous transcriptomic and systems-biology studies of Duchenne Muscular Dystrophy (19).

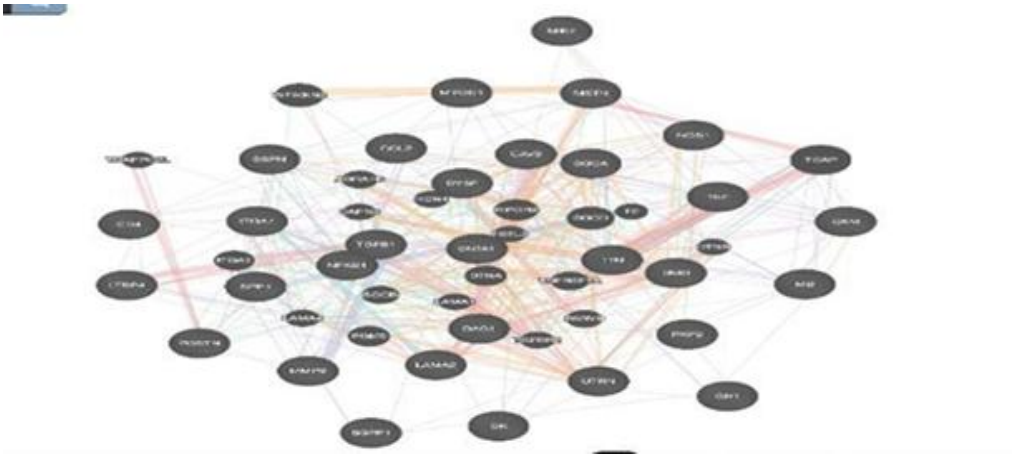


Figure 4: Integrated GeneMANIA interaction network illustrating the complex connectivity among Duchenne Muscular Dystrophy (DMD) genes and their predicted partners

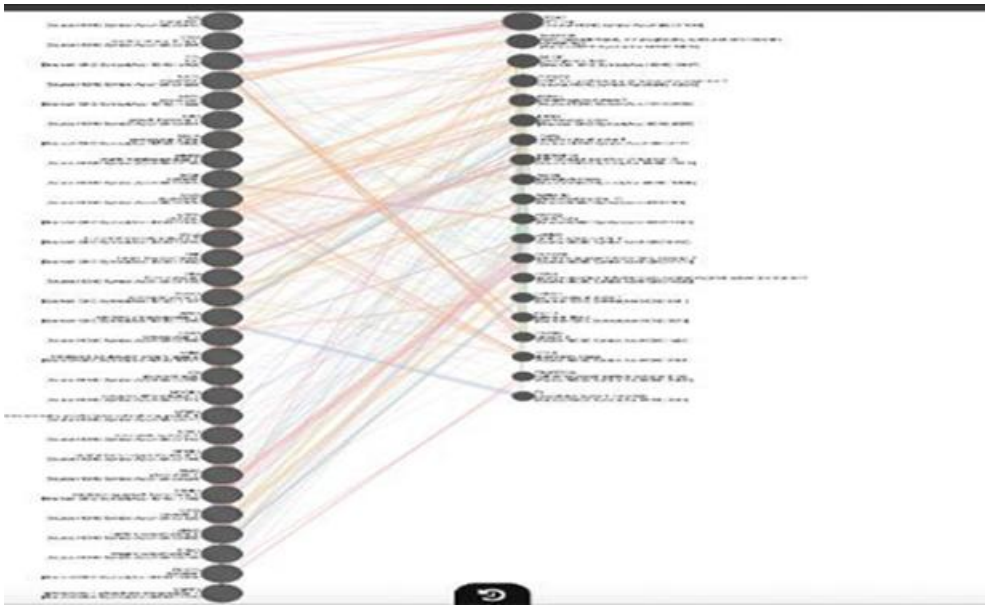


Figure 5: GeneMANIA-based gene interaction network for Duchenne Muscular Dystrophy (DMD) illustrating functional relationships among the selected genes

3.4 Functional Enrichment and Hub Gene Identification Using Enrichr

Gene set enrichment analysis performed using Enrichr identified multiple significantly enriched functional terms across Reactome and KEGG libraries. Hub genes were identified based on functional recurrence, meaning genes that appeared repeatedly across several enriched biological processes and pathways were considered functionally central. Clustergram analysis revealed that genes displaying multiple red blocks across enriched terms demonstrated high functional involvement. Based on this recurrence-based approach, the final set of hub genes included LAMA2, DAG1, DMD, ITGA7, SGCA, TGFB1, TNF, NFKB1, MMP9, SPP1, CCL2, and UTRN. These genes were associated with pathways related to extracellular matrix organization, immune signaling, inflammatory response, and muscle structure maintenance. The identification of these hub genes indicates that Duchenne Muscular Dystrophy is driven by coordinated dysregulation of structural and immune-related molecular processes. Similar enrichment-based hub gene identification strategies have been successfully used in previous bioinformatics studies of complex genetic disorders (20).

Figure 6: Enrichr output showing enriched KEGG pathways and biological processes associated with Duchenne Muscular Dystrophy (DMD) related genes

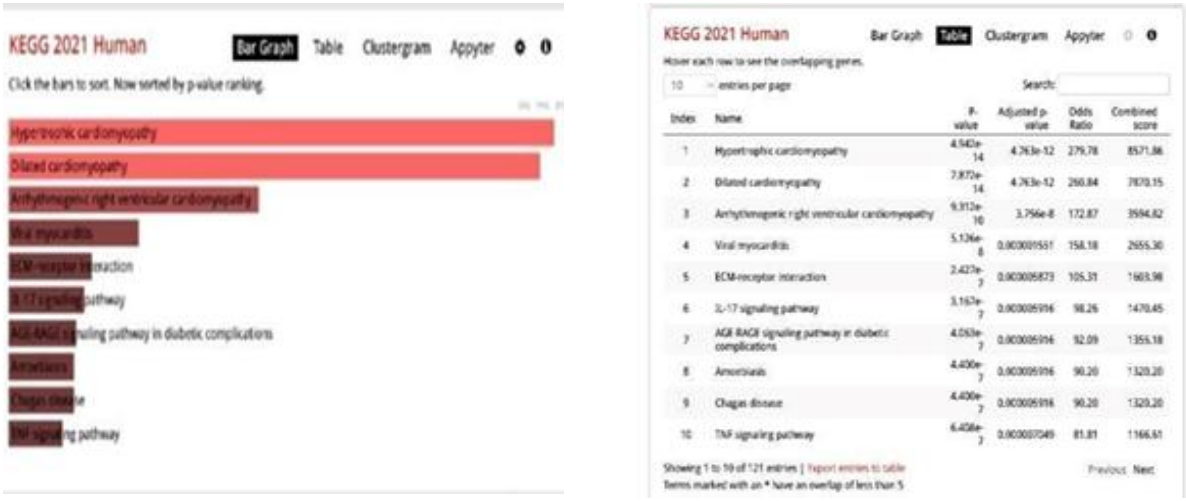


Figure 7: Heatmap Representation of KEGG Pathway Enrichment in Duchenne Muscular Dystrophy (DMD) related genes

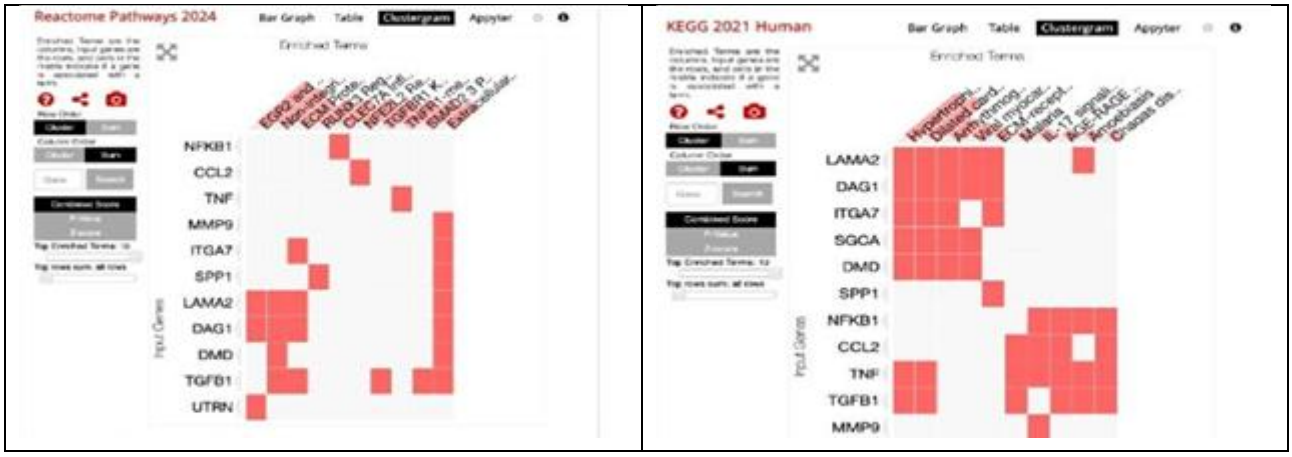


Figure 8: Functional enrichment analysis performed using Enrichr illustrating significantly enriched Reactome and KEGG pathways and their gene associations through clustogram visualization for DMD-associated genes

3.5 Pathway mapping and biological interpretation using Reactome

Reactome pathway analysis revealed significant enrichment of multiple biologically relevant pathways. Among the top enriched pathways were Interleukin-4 and Interleukin-13 signaling, extracellular matrix organization, and non-integrin membrane–ECM interactions, all of which showed highly significant p-values and false discovery rates.

The enrichment of Interleukin-4 and Interleukin-13 signaling pathways indicates strong involvement of immune and inflammatory responses in Duchenne Muscular Dystrophy. This suggests immune dysregulation plays a key role in disease progression. Extracellular matrix organization pathways highlight alterations in tissue integrity, cell adhesion, and muscle fiber support, which are critical contributors to progressive muscle weakness. Non-integrin membrane–ECM interaction pathways suggest impaired communication between muscle cells and their surrounding matrix, leading to structural and functional abnormalities.

These findings are consistent with systems-level studies describing Duchenne Muscular Dystrophy as a disorder involving interconnected structural, inflammatory, and signaling networks rather than isolated molecular defects (21,22).

Figure 9: Reactome pathway mapping and enrichment results for DMD-associated genes.

Table 2: Pathway Mapping and Biological Interpretation (Reactome)

Pathway Name	Entities Found (Hits)	Entities Total	p-value	FDR
Interleukin-4 and Interleukin13 signaling	8	201	1.48×10^{-10}	3.58×10^{-8}
Extracellular matrix organization	8	355	2.52×10^{-10}	3.58×10^{-8}
Non-integrin membrane ECM interactions	3	43	9.38×10^{-10}	8.88×10^{-8}
Formation of the dystrophinglycoprotein complex (DGC)	5	37	1.26×10^{-9}	8.86×10^{-8}
Signaling by interleukins	9	646	4.98×10^{-9}	2.84×10^{-6}

4. Discussion

The present study employs a network-based bioinformatics framework to investigate the molecular mechanisms underlying Duchenne Muscular Dystrophy (DMD), providing an integrative and systems-level view of disease pathogenesis. By combining gene–disease association analysis, protein–protein interaction (PPI) networks, functional gene association analysis, enrichment studies, and pathway mapping, this approach captures the complex interactions among genes and pathways that collectively contribute to disease onset and progression. Such integrative analyses are particularly valuable for DMD, where the primary genetic defect initiates multiple downstream molecular disturbances that drive progressive muscle degeneration.

Gene–disease association analysis using DisGeNET consistently identified the *DMD* gene as the central determinant of Duchenne Muscular Dystrophy, reaffirming its critical role in maintaining sarcolemmal integrity and muscle fiber stability. The absence of dystrophin disrupts the dystrophin–glycoprotein complex, weakening the mechanical link between the cytoskeleton and the extracellular matrix and increasing susceptibility of muscle fibers to contraction-induced damage (11). However, the identification of additional genes such as *UTRN, LAMA2, DAG1, ITGA7,* and *SGCA* highlights the influence of modifier genes in modulating disease severity and progression. These genes are involved in cell–matrix adhesion, extracellular matrix organization, and structural reinforcement of the sarcolemma, suggesting that impaired anchoring mechanisms significantly worsen muscle fiber degeneration (7). Their repeated identification across analyses supports the view that DMD pathology extends beyond dystrophin loss alone.

Protein–protein interaction analysis using STRING revealed a highly interconnected molecular network, indicating that dystrophin deficiency affects a wide array of interacting proteins and cellular processes. The enrichment of biological processes related to muscle contraction, cytoskeletal organization, ion transport, and extracellular matrix remodeling reflects the extensive impact of dystrophin loss on muscle cell homeostasis (14). Disruption of cytoskeletal organization compromises mechanical stability, while altered ion transport, particularly calcium dysregulation, contributes to muscle fiber necrosis and degeneration. In addition, increased extracellular matrix remodeling observed in the network suggests progressive fibrosis and replacement of functional muscle tissue, a key feature of advanced DMD.

A notable finding from the PPI network was the presence of immune- and inflammation-related components, emphasizing the role of chronic inflammation in disease progression. Persistent inflammatory signaling leads to

repeated cycles of muscle damage and incomplete repair, promoting infiltration of immune cells and excessive deposition of fibrotic tissue. This inflammatory environment not only accelerates muscle degeneration but also interferes with normal muscle regeneration, further contributing to disease severity. These observations support growing evidence that immune activation is an integral component of DMD pathophysiology rather than a secondary consequence of muscle injury (8).

Functional gene network analysis using GeneMANIA further strengthened these findings by demonstrating strong co-expression patterns and physical interactions among DMD-associated genes (15). Inflammatory regulators such as *TNF, NFKB1,* and *CCL2* occupied central positions within the functional network, indicating sustained activation of immune signaling pathways in dystrophic muscle. Chronic activation of these pathways is known to enhance muscle fiber damage, promote fibrosis, and impair satellite cell function, thereby limiting the muscle's regenerative capacity. The involvement of these inflammatory mediators highlights their importance in driving disease progression and suggests their potential relevance as therapeutic targets.

Enrichment analysis using Enrichr and hub gene identification provided additional support for the regulatory importance of genes involved in extracellular matrix organization, immune signaling, and muscle structural integrity (16). The recurrence of specific genes across multiple enriched biological processes underscores their functional centrality within the disease network. Hub genes identified through this analysis are likely to play key regulatory roles and may represent promising candidates for biomarker development or targeted therapeutic intervention. Reactome pathway analysis further contextualized these findings by mapping associated genes to pathways related to interleukin signaling, dystrophin–glycoprotein complex assembly, and non-integrin membrane–ECM interactions (17). These pathways collectively illustrate how structural disruption, immune activation, and extracellular matrix remodeling act in a coordinated manner to drive progressive muscle degeneration in DMD.

Taken together, the consistent identification of structural, inflammatory, and extracellular matrix–related pathways across multiple bioinformatics tools highlights the robustness of the analysis and the interconnected nature of DMD pathogenesis. Rather than functioning as isolated mechanisms, these processes appear to reinforce one another, creating a self-perpetuating cycle of muscle damage, inflammation, and fibrosis. This integrative perspective emphasizes the limitations of therapeutic strategies that focus exclusively on dystrophin restoration and underscores the importance of addressing secondary pathological processes to achieve sustained clinical benefit (23).

Overall, this network-based bioinformatics study demonstrates the value of systems-level approaches in unraveling the molecular complexity of Duchenne Muscular Dystrophy. By revealing key genes, interactions, and pathways that contribute to disease progression, the findings provide a strong foundation for future experimental validation and support the development of multi-targeted therapeutic strategies aimed at slowing disease progression and improving patient outcomes.

Conclusion

Network-based bioinformatics analysis provides a comprehensive overview of the molecular events associated with Duchenne Muscular Dystrophy. While mutations in the *DMD* gene and the resulting loss of dystrophin (24) remain the primary cause of the disease, the findings clearly demonstrate that disease progression is influenced by a wider network of interacting genes and pathways. Genes involved in muscle structural integrity, extracellular

matrix organization, and cell–matrix interactions were consistently identified across multiple analyses, indicating their important contribution to muscle fiber instability and degeneration.

The integration of gene–disease association data, protein–protein interaction networks, functional gene association analysis, and pathway enrichment approaches allowed a more holistic interpretation of DMD pathogenesis. The repeated identification of immune- and inflammation-related genes suggests that chronic inflammatory responses play a significant role in accelerating muscle damage and promoting fibrotic tissue replacement. In parallel, alterations in extracellular matrix remodeling and cytoskeletal organization further compromise muscle function and regenerative capacity (25).

Importantly, the consistency of results obtained from different bioinformatics tools strengthens the reliability of the analysis and highlights the usefulness of integrative network-based methods for studying complex genetic disorders such as DMD. Rather than acting independently, the identified genes and pathways appear to function in a coordinated manner, collectively contributing to progressive muscle degeneration.

Overall, this study emphasizes that effective therapeutic strategies for Duchenne Muscular Dystrophy should extend beyond dystrophin restoration alone. Targeting secondary pathological processes such as inflammation, fibrosis, and extracellular matrix dysregulation may be necessary to slow disease progression and improve patient outcomes. The findings of this study provide a valuable foundation for future experimental validation and may support the development of multi-targeted therapeutic approaches for the management of DMD.

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