Iranian Journal of Pharmaceutical Sciences
2021: 17 (1): 1-18
www.ijps.ir

Original Article

Systematic Mining of Gene Co-Expression Network Suggesting a New Drug Repositioning for the Effective Treatment of Duchenne Muscular Dystrophy

Afshin Derakhshani[a], Maryam Moosavi[b], Saeed Nasseri[b], Ebrahim Miri-Moghaddam[c], Hossein Safarpour[b*], Behzad Baradaran[a,d]

[a]Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, [b]Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran, [c]Molecular Medicine Department, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran, [d]Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

Abstract

Duchenne Muscular Dystrophy (DMD) is one of the most common inherited disorders worldwide. As there is currently no absolute treatment, the present systems biology study aimed to propose a new drug repositioning for DMD therapy. A microarray dataset of 16 DMD and 6 control samples were analyzed and 208 differentially expressed genes were screened. Weighted gene co-expression network analysis (WGCNA) algorithm, was applied to obtain co-expressed gene networks for the establishment of transcriptional modules related to clinical and demographic data of DMD patients. Results indicated that a maximum of 11 co-expression modules is present in datasets with a varying number of genes. Turquoise module with 3334 genes was strongly correlated with collagen fibril organization as a positive regulator in DMD pathogenesis (r=0.98, *p*-value=2/00E-15) through which other DMD related hub-genes were identified as *COL1A1, FZD10, COL1A2, CRISPLD1, FMO1, COL5A1, COL3A1, COL5A2, TP53I3, PLAGL1, RIPK2, SBF1, MLXIP, CFAP46,* and *TYRP1*. Drug repositioning of the turquoise module identified some candidate drugs which are not presently approved for the treatment of DMD. The targets in the turquoise module indicated that some drugs might greatly affect DMD disease. Furthermore, drug repositioning introduced Zoledronic acid as a potent antagonist for *COL1A1*.

Keywords: Systems biology, Duchenne Muscle Dystrophy, WGCNA, Co-expression network, Drug Repositioning, Data analysis, *COL1A1*.

Corresponding Authors: Hossein Safarpour, Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran.
Tel: +98-5632381258
Email: H.safarpour@bums.ac.ir
Cite this article as: Derakhshani A, Moosavi M, Nasseri S, Miri-Moghaddam E, Safarpour H, Baradaran B, Systematic Mining of Gene Co-Expression Network Suggesting a New Drug Repositioning for the Effective Treatment of Duchenne Muscular Dystrophy, 2021, 17 (1): 1-18.

1. Introduction

Duchenne muscular dystrophy (DMD) is the most fatal muscular genetic disorder worldwide with a prevalence of 15.9 among 100,000 infant males [1, 2]. This neuromuscular disorder has an X-linked recessive pattern of inheritance with mutations in DMD gene encoding dystrophin as the largest structural protein [3]. DMD encompasses 2.2 Mb of the genome including79 exons. Currently, 4000 mutations have been identified in this gene [4]. Dystrophin protein is a crucial factor in integration of the extracellular matrix (ECM) into the cytoskeleton through the cell membrane as a part of the dystrophin-related glycoprotein complex [5]. The deficiency of dystrophin in the skeletal muscles results in sarcolemma fragility, intracellular signaling damage, myocyte necrosis, inflammatory infiltration and ultimately muscle replacement with fibrotic and fatty tissue [6]. Improper attachment of myofibrils to the basal lamina and their eventual separation during contraction leading to sarcolemma instability and injury are the results of lack of dystrophin in DMD patients [7].

Presently, there is no efficient treatment for DMD disease. Glucocorticoid treatment is the current standard of cure which delays the loss of ambulation by 3-4 years [8, 9] but shows no long-term treatment benefit and is often associated with debilitating side effects [10-12]. The urgency to seek therapy for DMD has resulted in parallel efforts to develop exon skipping [13, 14], termination codon read through [15], dystrophin gene replacement or editing therapies [16, 17] and non-dystrophin strategies [18-20] such as utrophin modulation [21, 22]. However, despite the recent accelerated approval of Exondys 51 (eteplirsen) in the US, disappointing clinical trials results [23] and failure of the Food and Drug Administration (FDA) approval for Ataluren and Kyndrisa drugs rekindle discussions about clinical trial designs and endpoints [24]. In spite of great knowledge of underlying DMD devastating mutations, detailed mechanisms of pathogenesis should be clarified for the development of more effective therapies. "Network Medicine" has recently been attributed to provide a platform for systematically study the molecular complexity of a particular disease as a new approach [25]. In line with the current hypothesis that genes with comparable patterns of expression may have functional similarities or share common pathways, the weighted gene co-expression network analysis (WGCNA) has shed light on exploration procedures of gene expression at the systems level for different diseases [26-29]. This bioinformatic tool constructs a network of gene co-expression according to their

expression patterns and assigns co-expressed genes into the same module. Genes in the same module could have similar functionality or be regulated through common regulatory pathways [30].

Systematic identification of gene clusters involved in DMD progression remains poorly understood. In this study, we used WGCNA to identify modules of DMD-related co-expressed gene networks. Integration of co-expression clusters with differential expression analysis and subsequent enrichment performed to find DMD-specific gene modules. This systems biology study represents a pivotal role for WGCNA algorithm in the elucidation of underlying molecular mechanisms as well as therapeutic targets regarding DMD disorder.

2. Materials and Methods

2.1. Data Collection and Preprocessing

The microarray data were obtained from the NCBI Gene Expression Omnibus (GEO) database (accession number GSE38417) [31]. This dataset operates according to GPL570 platforms and contains 22 samples including 16 DMD patients and 6 controls. The raw data were processed and quantile-normalized with the affy package of R 3.4.1 in Bioconductor. The annotation file published by Affymetrix (Affymetrix Human Genome U133 plus 2.0 Array) was applied to assign probes for appropriate genes. Unconverted data were excluded. Subsequently, the average expression data of identifiers were calculated for each sample. Gene symbols were filtered across all samples using their variance. Finally, the top 4000 genes with the highest variances were selected for the subsequent analyses.

2.2. Identification of Differentially Expressed Genes (Degs)

To identify condition-specific DEGs, GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) was used to filter DEG data of DMD and normal groups. Genes were considered as differentially expressed when they met the following criteria: Adjust p-value < 0.05, and $|\log_2 FC| \geq 1.5$.

2.3. Construction of Co-Expression Modules for DMD Samples

Gene co-expression network of patients and control groups reconstructed using WGCNA [32]. Briefly, the matrix of the gene expression profile was converted into the matrix of pairwise gene similarity according to the Pearson test followed by conversion into the matrix of adjacency. According to already represented scale-free gene co-expression topological algorithm, when the β value is considered as 7, the adjacency matrix met the scale-free topology criteria. For the next step, topological overlap matrix (TOM) and dissimilarity TOM (dissTOM) were created using TOM similarity and dissimilarity modules based on the correlation of the pairwise gene-co-expression. Finally, the clusters of highly interconnected genes were created with a minimum module size of 30 genes and a cut height of 0.1.

2.4. Construction of Module-Trait Relationships for DMD Samples

In order to identify modules that are strongly related to the DMD clinical phenotype and calculate this correlation, module eigengene (ME) was recruited to assign expression profiles of each module. The correlation of individual genes with the DMD was measured using the gene significance (GS). Module membership (MM) was considered as the correlation of the ME and the profile of gene expression for each module. The strong correlation between GS and MM represented with the closely correlated substantial (central) elements in the modules with DMD phenotype [33]. At last, DMD related genes with both GS and MM≥0.9 were selected as hub-genes compared to the control samples.

2.5. Functional Enrichment Analysis of Significant Modules

Functional enrichment analysis was performed using the DAVID (Database for Annotation Visualization and Integrated Discovery) (https://david.ncifcrf.gov/tools.jsp), Gene Ontology (http://geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg/) databases. Enriched ontological terms and pathways with the threshold of Benjamin-adjusted *p*-value<0.05 were selected. Functional networks were constructed by GeneMANIA (https://genemania.org/) and Cytoscape v 3.0 software [34]. Venn diagram was created using the "Venny" v 2.1 software (http://bioinfogp.cnb.csic.es/tools/venny/).

2.6. Identification of Candidate Regulatory Drugs

The well-firmly established Drug-Gene Interaction Database (DGIDB) (http://www.dgidb.org/) was used to predict the functional and drug-able hub-genes with the list of commercially available or clinical trial drugs [35].

3. Results and Discussion

3.1. Results

3.1.1. Identification of WGCNA Modules

We performed quantile normalization to reduce the effects of technical noises. The plot of quantiles of expression levels across arrays is shown in Fig.1A. Based on the variance of expression values, a total of 4000 genes were included in WGCNA. No outliers were observed in 22 samples by sample clustering; thus, all samples were included in the analysis (Figure 1B). For the next step, β threshold power considered as 4 and a weighted co-expression network was reconstructed for DMD patients and normal samples (Figure 1C). Hierarchical clustering dendrogram represented with 11 modules as illustrated in long branches (Figure 1D). The number of genes on each module varied between 35 and 3334 (purple and turquoise colors, respectively) (Table 1).

3.1.2. Module-Trait Association Analysis

Module-module correlation and association of the modules with DMD disease were

evaluated using eigengenes calculation. The turquoise module was positively correlated with DMD disease (r= 0.98, *p*-value=2/00E-15) (Table 1). Furthermore, the eigengene network and heatmap indicated a relationship between modules and the DMD especially for the turquoise module (Figure 2).

3.1.3. Identification of DEGs

Overall, 208 genes were selected as DEGs (Figure 3) with the threshold of Adjust *p*-value < 0.05, and $|\log_2 FC| \geq 1.5$, including 147 up-regulated and 61 down-regulated genes. KEGG analysis of DEGs indicated that these genes are involved in the extracellular matrix organization, extracellular structure organization, and positive regulation of collagen fibril organization and with a high significance in ECM-receptor interaction functional pathways (Figure 4).

3.1.4. Hub-genes Detection and Enrichment Analysis

The correlation between features (MM and GS) of the turquoise module led to the identification of the hub-genes that are highly associated with DMD pathogenesis (Figure 5). These genes were included as: *COL1A1, FZD10, COL1A2, CRISPLD1, FMO1, COL5A1, COL3A1, COL5A2, TP53I3, PLAGL1, RIPK2, SBF1, MLXIP, CFAP46* and *TYRP1* (Figure 3). Using GeneMANIA and Cytoscape software, the co-expression network of the aforementioned module has been reconstructed (Figure 4). The most considerable pathways which are related to the turquoise module's genes were presented using the ClueGO tool. Cell-cell adhesion is an important biological function of the turquoise module (Figure 4).

3.1.5. Drug-Target Network Construction

We have assessed the turquoise module for drug development purposes. The turquoise module included well-known neuromuscular targets, promoting its significance for neuromuscular disease research. This module was also checked for the presence of targets that are not presently approved for DMD treatment. These targets included as *COL1A2* (collagenase clostridium histolyticum, ocriplasmin), *COL5A1* (collagenase clostridium histolyticum, ocriplasmin), *COL3A1* (ocriplasmin, collagenase clostridium histolyticum), *COL1A1* (zoledronic acid, collagenase clostridium histolyticum, somatropin, ocriplasmin) and *COL5A2* (collagenase clostridium histolyticum, ocriplasmin) (Figure 6).

3.2. Discussion

While there is currently no ultimate treatment for DMD as the most prevalent type of muscular dystrophy in children, some potential treatments have been introduced in the form of dystrophin substitute therapies as compensatory protein upregulation, anti-inflammatory agents, and other cellular targets. One of the current molecular therapies regarding DMD treatment is the use of oligonucleotide-mediated exon skipping by special antisense oligonucleotides (AONs), to mask the supposed splicing sites of exons. This technology is attributed to the restoration

of the correct reading frame [36]. However, these therapeutic techniques are challenging as convenient delivery of such large molecules to their intracellular targets is a matter of debate [37]. Adverse effects associated with antisense therapy may include degenerative accumulation- driven changes in the proximal tubules of the kidney, antagonism of innate immune system receptors or complement activation [38]. Recent data have shown that administration of Eteplirsen and Drisapersen drugs may constantly induce the production of functional dystrophin in DMD patients through oligonucleotide-mediated exon skipping. However Drisapersen was not getting FDA approval due to the part of safety concerns as reactions at the injection site and renal failure in DMD patients. [13, 14, 39]. Similarly, the clinical efficacy of Eteplirsen was not experientially approved as FDA assessment questioned the extent of the restoration of dystrophin expression and the validity of dystrophin levels as a possible primary consequence measure [40].

In the current study, we conducted an assessment called drug repositioning for DMD neuromuscular disease. Drug repositioning means whether a drug can be used for the treatment of disease besides its current approved applications for a known disorder. This situation is faster per se to get a new application approval for a known drug and less risky in clinical trials compared to the development of a new drug [41].

In line with this hypothesis, we have intentionally evaluated DMD as a candidate disease for systems biology. To the best of our knowledge, systematic identification of gene clusters involved in DMD progression is not yet reported. To address the co-expression network of genes involved in DMD, we have reconstructed the DMD dataset using WGCNA package. Module-module correlation and association of the modules with DMD disease suggested that the turquoise module should be strongly contributed to the disease pathogenesis. Accordingly, this module was chosen for further evaluation. It has a correlation value of 0.98 and a *p*-value of 2/00E-15. With a LogFC=5.4. *COL1A1* was the most up-regulated gene among the hub-genes detected from the turquoise module (Figure 3). Several pieces of evidence indicate that collagen I plays a crucial role in DMD pathogenesis. Fibrosis in muscular dystrophy is widely recognized by aggregation of collagen type I and type III [42]. The fibrillary ECM consists of collagen I (COL1) and collagen III (*COL3A1*) and is adjacent to the layers of ECM in the muscle and the tendon outside the musculotendinous junction [43]. Our results are consistent with other findings, as one report has indicated the critical role of the *COL1A1* gene in DMD-patients compared to the control group. [44]. Indeed, another survey has shown that type I and III collagens have been regulated in the endomysium and perimysium of normal muscles and congenital myopathies [45]. Moreover, in a study conducted by Porter and coworkers, the main components of the collagen disease load index (DLI), in particular, *COL1A1*, *COL1A2*, and *COL3A1* have a higher expression in mdx skeletal muscle [46]. In the current study, we

used the DGIDB to predict drug targets for the hub-genes in the turquoise module. The results indicated that Zoledronic acid has a potential interaction with the *COL1A1* gene. Zoledronic acid is used in postmenopausal osteoporosis women to reduce fractures and also in breast and prostate cancer therapy and other malignancies [47]. This drug can shift the macrophage balance from M2 to M1, which inhibits tumor progression [48]. The M1 macrophages induce inflammation, while M2 macrophages reduce inflammatory conditions and accelerate wound healing and fibrosis [49]. In response to fibrinogen stimulation and the production of pro-inflammatory cytokines, TGF-β is produced and initiated alternative macrophage activation and directly induces collagen synthesis [50]. Using zoledronic acid in combination with steroids can enhance vertebral morphology and ameliorates DMD symptoms by retaining the mobility status in the affected children without serious adverse effects [41].

Given the present results, we have practically introduced the application of drug repositioning for a known human disorder using bioinformatics and database tools. Based on our findings, Zoledronic acid can be used as a potential therapeutic agent against DMD and may reduce muscle fibrosis. Practical explorations are required to establish the idea of therapeutic intervention of Zoledronic acid in DMD patients as well as its safety, tolerability, and efficacy in a pre-clinical trial setting.

4. Conclusion

In summary, the result of WGCNA study on a microarray DMD dataset showed a significantly correlated module containing 15 hub-genes which can be used as candidate genes in DMD pathogenicity for further evaluations. Also, drug-target network analysis has demonstrated four FDA-approved drugs as potential candidates for the treatment of DMD patients.

Acknowledgments

This research was supported by Tabriz and Birjand University of Medical Sciences.

References

[1] Filareto A, Maguire-Nguyen K, Gan Q, Aldanondo G, Machado L, Chamberlain JS, Rando TA. Monitoring disease activity noninvasively in the mdx model of Duchenne muscular dystrophy. *PNAS*. (2018) 115(30): 7741-7746.

[2] Birnkrant DJ, Bushby K, Bann CM, Apkon SD, Blackwell A, Brumbaugh D, Case LE, Clemens PR, Hadjiyannakis S, Pandya S, Street N, Tomezsko J, Wagner KR, Ward LM, Weber DR. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. *Lancet Neurol*. (2018) 17(3): 251-267.

[3] Lionarons JM, Hellebrekers DMJ, Klinkenberg S, Faber CG, Vles JSH, Hendriksen JGM. Methylphenidate use in males with Duchenne muscular dystrophy and a comorbid attention-deficit hyperactivity disorder. *Eur. J. Paediatr. Neurol*. (2019) 23(1):152- 157.

[4] Amoasii L, Long C. Single-cut genome editing restores dystrophin expression in a new mouse model of muscular dystrophy. *Sci. Transl. Med.* (2017) 9(418):eaan8081.

[5] Akpulat U, Wang H, Becker K, Contreras A, Partridge TA, Novak JS, Cirak S. Shorter Phosphorodiamidate Morpholino Splice-Switching Oligonucleotides May Increase Exon-Skipping Efficacy in DMD. *Mol. Ther. Nucleic Acids*. (2018) 13:534-542.

[6] Min YL, Bassel-Duby R, Olson EN. CRISPR Correction of Duchenne Muscular Dystrophy. *Annu Rev. Med*. (2019) 70:239-255.

[7] Rancourt A, Dufresne SS, St-Pierre G, Levesque JC, Nakamura H, Kikuchi Y, Satoh MS, Frenette J, Sato S. Galectin-3 and N-acetylglucosamine promote myogenesis and improve skeletal muscle function in the mdx model of Duchenne muscular dystrophy. *Faseb j*. (2018) fj201701151RRR.

[8] McAdam LC, Mayo AL, Alman BA, Biggar WD. The Canadian experience with long-term deflazacort treatment in Duchenne muscular dystrophy. *Acta Myol*. (2012) 31(1):16-20.

[9] Henricson EK, Abresch RT, Cnaan A, Hu F, Duong T, Arrieta A, Han J, Escolar DM, Florence JM, Clemens PR, Hoffman EP, McDonald CM. The cooperative international neuromuscular research group Duchenne natural history study: glucocorticoid treatment preserves clinically meaningful functional milestones and reduces rate of disease progression as measured by manual muscle testing and other commonly used clinical trial outcome measures. *Muscle Nerve*. (2013) 48(1): 55-67.

[10] Wong BL, Christopher C. Corticosteroids in Duchenne muscular dystrophy: a reappraisal. *J. Child Neurol.* (2002) 17(3): 183-190.

[11] Manzur AY, Kuntzer T, Pike M, Swan A. Glucocorticoid corticosteroids for Duchenne muscular dystrophy. *Cochrane Database Syst Rev*. (2008) (1): CD003725.

[12] Ricotti V, Ridout DA, Scott E, Quinlivan R, Robb SA, Manzur AY, Muntoni F. Long-term benefits and adverse effects of intermittent versus daily glucocorticoids in boys with Duchenne muscular dystrophy. *J Neurol. Neurosurg. Psychiatry*. (2013) 84(6): 698-705.

[13] Voit T, Topaloglu H, Straub V, Muntoni F, Deconinck N, Campion G, De Kimpe SJ, Eagle M, Guglieri M, Hood S, Liefaard L, Lourbakos A, Morgan A, Nakielny J, Quarcoo N, Ricotti V, Rolfe K, Servais L, Wardell C, Wilson R, Wright P, Kraus JE. Safety and efficacy of drisapersen for the treatment of Duchenne muscular dystrophy (DEMAND II): an exploratory, randomised, placebo-controlled phase 2 study. *Lancet Neurol*. (2014) 13(10): 987-96.

[14] Mendell JR, Goemans N, Lowes LP, Alfano LN, Berry K, Shao J, Kaye EM, Mercuri E. Longitudinal effect of eteplirsen versus historical control on ambulation in Duchenne muscular dystrophy. *Ann. Neurol.* (2016) 79(2): 257-71.

[15] Bushby K, Finkel R, Wong B, Barohn R, Campbell C, Comi GP, Connolly AM, Day JW, Flanigan KM, Goemans N, Jones KJ, Mercuri E, Quinlivan R, Renfroe JB, Russman B, Ryan MM, Tulinius M, Voit T, Moore SA, Lee Sweeney H, Abresch RT, Coleman KL, Eagle M, Florence J, Gappmaier E, Glanzman AM, Henricson E, Barth J, Elfring GL, Reha A, Spiegel RJ ,O'Donnell M W, Peltz SW, McDonald CM. Ataluren treatment of patients with nonsense mutation dystrophinopathy. *Muscle Nerve.* (2014) 50(4): 477-87.

[16] Wang Z, Storb R, Halbert CL, Banks GB, Butts TM, Finn EE, Allen JM, Miller AD, Chamberlain JS, Tapscott SJ. Successful regional delivery and long-term expression of a dystrophin gene in canine muscular dystrophy: a preclinical model for human therapies. *Mol. Ther*. (2012) 20(8): 1501-7.

[17] Nik-Ahd F, Bertoni C. *Ex vivo* gene editing of the dystrophin gene in muscle stem cells mediated by peptide nucleic acid single stranded oligodeoxynucleotides induces stable expression of dystrophin in a mouse model for Duchenne muscular dystrophy. *Stem Cells*. (2014) 32(7): 1817-30.

[18] Nelson MD, Rader F, Tang X, Tavyev J, Nelson SF, Miceli MC, Elashoff RM, Sweeney HL, Victor RG. PDE5 inhibition alleviates functional muscle

ischemia in boys with Duchenne muscular dystrophy. *Neurology*. (2014) 82(23): 2085-91.

[19] Mendell JR, Sahenk Z, Malik V, Gomez AM, Flanigan KM, Lowes LP, Alfano LN, Berry K, Meadows E, Lewis S, Braun L, Shontz K, Rouhana M, Clark KR, Rosales XQ, Al-Zaidy S, Govoni A, Rodino-Klapac LR, Hogan MJ, Kaspar BK. A phase 1/2a follistatin gene therapy trial for becker muscular dystrophy. *Mol. Ther.* (2015) 23(1): 192-201.

[20] Campbell C, McMillan HJ, Mah JK, Tarnopolsky M, Selby K, McClure T, Wilson DM, Sherman ML, Escolar D, Attie KM. Myostatin inhibitor ACE-031 treatment of ambulatory boys with Duchenne muscular dystrophy: Results of a randomized, placebo-controlled clinical trial. *Muscle Nerve.* (2017) 55(4): 458-464.

[21] Ricotti V, Spinty S, Roper H, Hughes I, Tejura B, Robinson N, Layton G, Davies K, Muntoni F, Tinsley J. Safety, Tolerability, and Pharmacokinetics of SMT C1100, a 2-Arylbenzoxazole Utrophin Modulator, following Single- and Multiple-Dose Administration to Pediatric Patients with Duchenne Muscular Dystrophy. *PLoS One.* (2016) 11(4): e0152840.

[22] Guiraud S, Squire SE, Edwards B, Chen H, Burns DT, Shah N, Babbs A, Davies SG, Wynne GM, Russell AJ, Elsey D, Wilson FX, Tinsley JM, Davies KE. Second-generation compound for the modulation of utrophin in the therapy of DMD. *Hum. Mol. Genet.* (2015) 24(15): 4212-24.

[23] Victor RG, Sweeney HL, Finkel R, McDonald CM, Byrne B, Eagle M, Goemans N, Vandenborne K, Dubrovsky AL, Topaloglu H, Miceli MC, Furlong P, Landry J, Elashoff R, Cox D. A phase 3 randomized placebo-controlled trial of tadalafil for Duchenne muscular dystrophy. *Neurology.* (2017) 89(17): 1811- 1820.

[24] Guiraud S, Edwards B, Squire SE, Babbs A, Shah N, Berg A, Chen H, Davies KE. Identification of serum protein biomarkers for utrophin based DMD therapy. *Sci. Rep*. (2017) 7: 43697.

[25] Chen J, Piquette- Miller M, Smith B. Network medicine: finding the links to personalized therapy. *Clin. Pharmacol. Ther*. (2013) 94(6): 613- 616.

[26] Wang X, Song P, Huang C, Yuan N, Zhao X, Xu C. Weighted gene co‐expression network analysis for identifying hub genes in association with prognosis in Wilms tumor. *Mol. Med. Rep*. (2019) 19(3): 2041-2050.

[27] Chen W, Chen X, Wang Y, Liu T, Liang Y, Xiao Y, Chen L. Construction and Analysis of lncRNA-Mediated ceRNA Network in Cervical Squamous Cell Carcinoma by Weighted Gene Co-Expression Network Analysis. *Med. Sci. Monit*. (2019) 25: 2609- 2622.

[28] Huayan L, Runhong T. Identification of Temporal Characteristic Networks of peripheral blood changes in Alzheimer's disease Based on Weighted correlation network analysis. *Front. Aging Neurosci*. (2019) 11:83.

[29] Guo N, Zhang N, Yan L, Lian Z, Wang J, Lv F, Wang Y, Cao X. Weighted gene co‐expression network analysis in identification of key genes and networks for ischemic-reperfusion remodeling myocardium. *Mol. Med. Rep*. (2018) 18(2):1955-1962.

[30] Langfelder P, Horvath S. Eigengene networks for studying the relationships between co-expression modules. *BMC Syst. Biol*. (2007) 1:54.

[31] https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE38417.

[32] Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC bioinformatics.* (2008) 9(1): 559.

[33] Zhang B, Horvath S. A general framework for weighted gene co-expression network analysis. *Stat Appl Genet Mol Biol.* (2005) 4: Article17.

[34] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT ,Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* (2003) 13(11): 2498-504.

[35] Cao W, Wu W, Yan M, Tian F, Ma C, Zhang Q, Li X, Han P, Liu Z, Gu J, Biddle FG. Multiple region whole-exome sequencing reveals dramatically evolving intratumor genomic heterogeneity in esophageal squamous cell carcinoma. *Oncogenesis.* (2015) 4(11): e175-e175.

[36] Benchaouir R, Meregalli M, Farini A, D'Antona G, Belicchi M, Goyenvalle A, Battistelli M, Bresolin N, Bottinelli R, Garcia L, Torrente Y. Restoration of human dystrophin following transplantation of exon-skipping-engineered DMD patient stem cells into dystrophic mice. *Cell Stem Cell*. (2007) 1(6): 646-57.

[37] Arechavala-Gomeza V, Anthony K, Morgan J, Muntoni F. Antisense oligonucleotide-mediated exon skipping for Duchenne muscular dystrophy: progress and challenges. *Curr. Gene Ther*. (2012) 12(3): 152-60.

[38] Rinaldi C, Wood MJA. Wood, Antisense oligonucleotides: the next frontier for treatment of neurological disorders. *Nat. Rev. Neurol*. (2018) 14(1): 9-21.

[39] Komaki H, Nagata T. Nagata. Systemic administration of the antisense oligonucleotide NS-065/NCNP-01 for skipping of exon 53 in patients with Duchenne muscular dystrophy. *Sci. Transl. Med.* (2018) 10(437): eaan0713.

[40] U.S. Food and Drug Administration (FDA),(D.A.P.E.I., w.a.f.g.d.d.n. (eteplirsen) (FDA, and TOC.cfm.

[41] Ashburn TT, Thor KB. Drug repositioning: identifying and developing new uses for existing drugs. *Nat. Rev. Drug Discov.* (2004) 3(8): 673-83.

[42] Foidart M, Foidart JM ,Engel WK. Collagen localization in normal and fibrotic human skeletal muscle. *Arch. Neurol*. (1981) 38(3): 152-7.

[43] Smith LR, Meyer G, Lieber RL. Systems analysis of biological networks in skeletal muscle function. *Wiley Interdiscip. Rev. Syst. Biol. Med.* (2013) 5(1): 55-71.

[44] Zanotti S, Gibertini S, Curcio M, Savadori P, Pasanisi B, Morandi L, Cornelio F, Mantegazza R, Mora M. Opposing roles of miR-21 and miR-29 in the progression of fibrosis in Duchenne muscular dystrophy. *Biochim. Biophys. Acta.* (2015) 1852(7): 1451-64.

[45] Hantai D, Labat-Robert J, Grimaud JA, Fardeau M. Fibronectin, laminin, type I, III and IV collagens in Duchenne's muscular dystrophy, congenital muscular dystrophies and congenital myopathies: an immunocytochemical study. *Connect. Tissue Res.* (1985) 13(4): 273-81.

[46] Porter JD, Merriam AP, Leahy P, Gong B, Feuerman J, Cheng G, Khanna S. Temporal gene expression profiling of dystrophin-deficient (mdx) mouse diaphragm identifies conserved and muscle group-specific mechanisms in the pathogenesis of muscular dystrophy. *Hum. Mol. Genet*. (2004) 13(3): 257-269.

[47] Lyles KW, Colón-Emeric CS, Magaziner JS, Adachi JD, Pieper CF, Mautalen C, Hyldstrup L, Recknor C, Nordsletten L, Moore KA, Lavecchia C, Zhang J ,Mesenbrink P, Hodgson PK, Abrams K, Orloff JJ, Horowitz Z, Eriksen EF, Boonen S. Zoledronic Acid and Clinical Fractures and Mortality after Hip Fracture. *N. Engl. J. Med.* (2007) 357(18): 1799-1809.

[48] Veltman JD, Lambers ME, van Nimwegen M, Hendriks RW, Hoogsteden HC, Hegmans JP, Aerts JG. Zoledronic acid impairs myeloid differentiation to tumour-associated macrophages in mesothelioma. *Br. J. Cancer.* (2010) 103(5): 629-641.

[49] Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep.* (2014) 6: 13.

[50] Vidal B, Serrano AL, Tjwa M, Suelves M, Ardite E, De Mori R, Baeza-Raja B, Martínez de Lagrán M, Lafuste P, Ruiz-Bonilla V, Jardí M, Gherardi R, Christov C, Dierssen M, Carmeliet P, Degen JL, Dewerchin M, Muñoz-Cánoves P. Fibrinogen drives dystrophic muscle fibrosis via a TGFbeta/alternative macrophage activation pathway. *Genes Dev*. (2008) 22(13):1747-1752.

Tables:

Table 1. Module colors characterization. The co-expression modules identified by WGCNA.

Module color	**Correlation**	***p*-value**	**#Genes**
turquoise	0.98	2/00E-15	3334
brown	-0.49	0.02	86
blue	-0.48	0.03	89
yellow	-0.46	0.03	84
green	-0.45	0.04	81
magenta	-0.44	0.04	68
pink	-0.42	0.05	68
Black	0.33	0.1	74
purple	0.28	0.2	35
red	0.23	0.3	74
grey	0.054	0.8	7
Black	0.33	0.1	74

Figures:

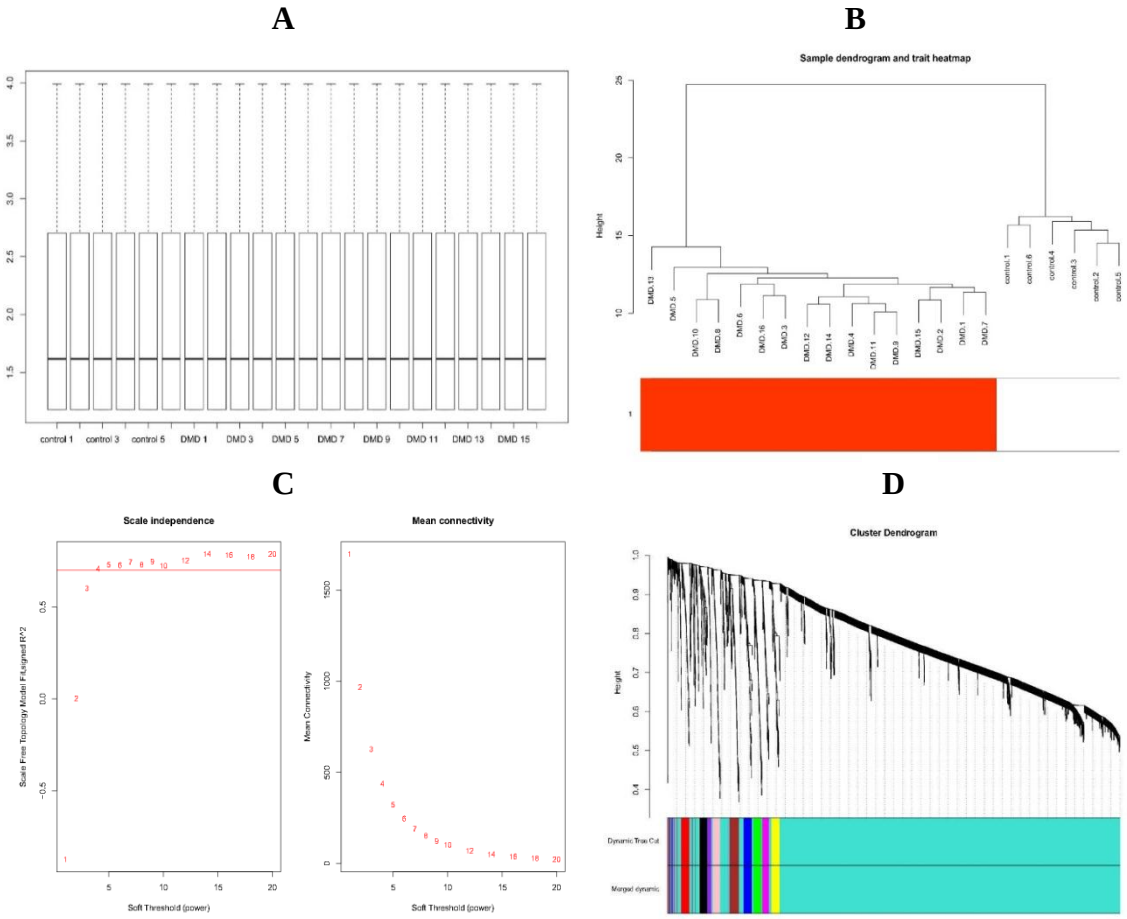


Figure 1. (**A)** Quantile-normalization of samples. Box plots of expression data after normalization. The quantile normalization algorithms were used to adjust the values of the background-subtracted mean pixel intensities of GSE38417. In contrast to the pre-normalization boxplots (top panel), the post-normalized box plots have been distributed in the same intervals with the same density center, indicating successful adjustment of data. The post-normalized data were used for further analysis. (**B)** Sample clustering to detect outliers. The color is proportional to the pathological stage (Red=DMD samples and white=normal samples). (**C)** Selection of the soft-thresholding powers. The left panel shows the scale-free fit index (y-axis) as a function of the soft-thresholding power (x-axis). The right panel displays the mean connectivity (degree, y-axis) as a function of the soft-thresholding power (x-axis). The power was set as 7 for the next analysis. (**D)** Cluster dendrogram and module assignment from WGCNA. The branches correspond to highly interconnected groups of genes. Colors in the horizontal bar represent the modules.

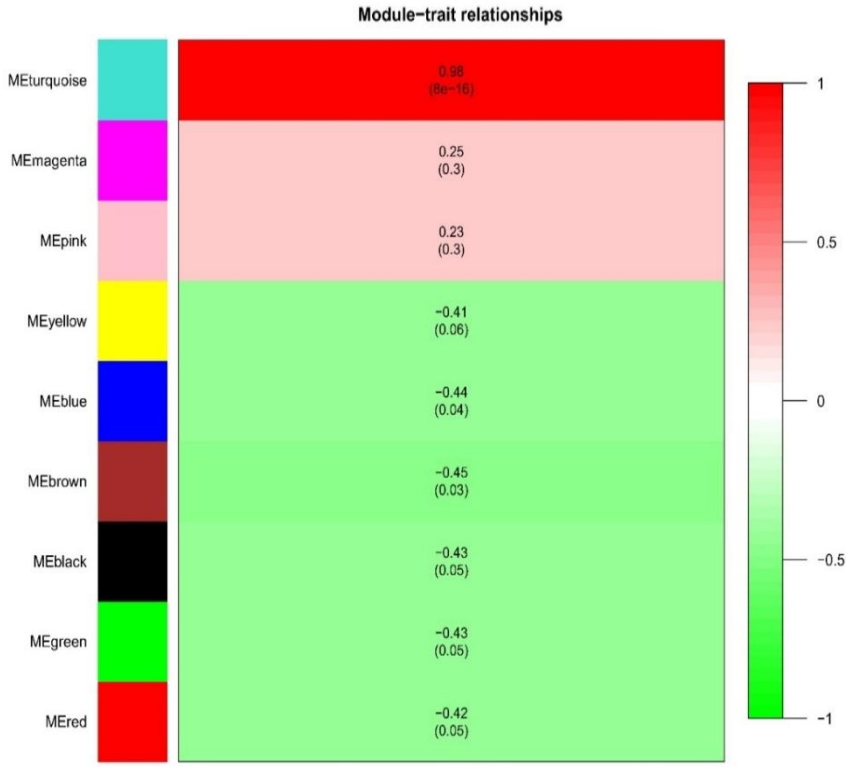
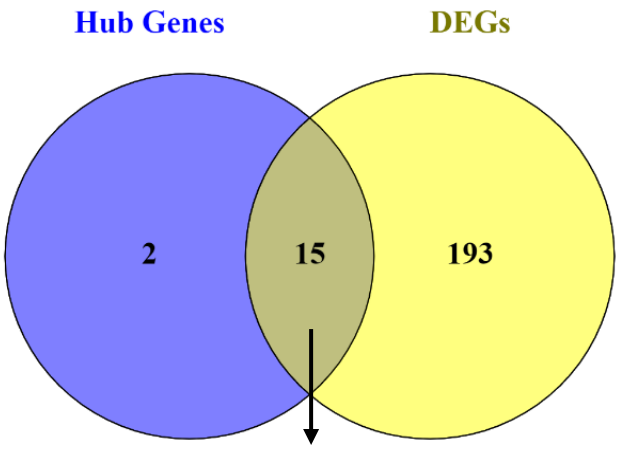


Figure 2. Module-trait relationship. Each row corresponds to a module eigengene and each column represents one DMD status. Numbers in each cell corresponds to the corresponding correlation and *p*-values.



GSE38417	
Hub-Genes	**LogFC**
COL1A1	5.481
FZD10	4.506
COL1A2	4.289
CRISPLD1	4.238
FMO1	4.029
COL5A1	3.859
COL3A1	3.759
COL5A2	3.551
TP53I3	3.516
PLAGL1	3.493
RIPK2	2.087
SBF1	-1.782
MLXIP	-2.533
CFAP46	-3.852
TYRP1	-5.709

Figure 3. Similarity assessment between DEGs and hub genes of the turquoise module using a Venn diagram. A total of 15 hub genes which were similar in both lists were chosen and then imported to GeneMANIA to construct a co-expression network.

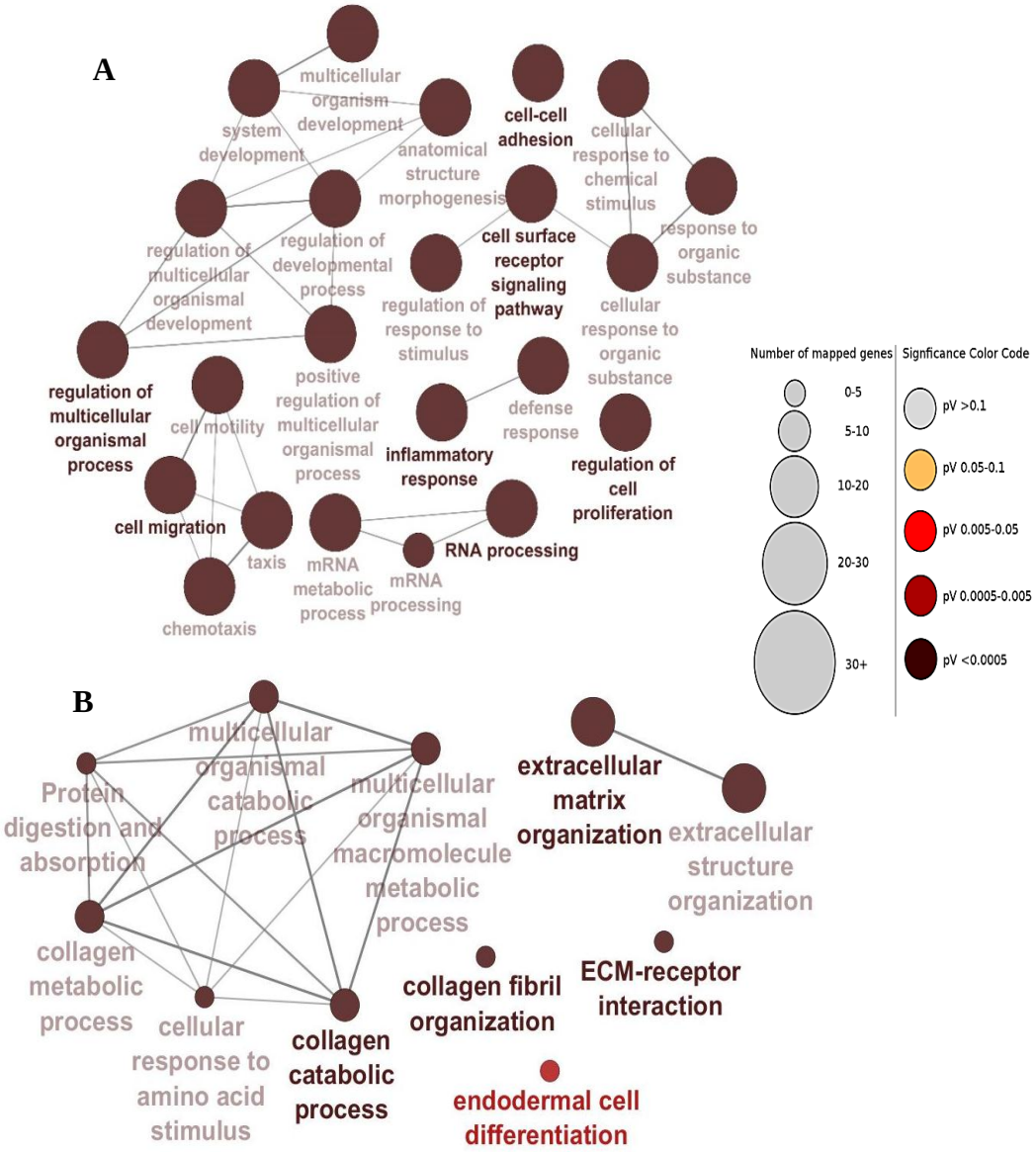


Figure 4. Processes and pathways identified within the DEGs and turquoise module. Gene ontology and pathway analysis were performed using significant genes across all datasets. Node size corresponds to the number of associated genes, and node color reflects the statistical significance. The darker the pathway node, the more statistically significant it is, with a gradient from red (*p*-value 0.05-0.005) to black (*p*-value < 0.0005).

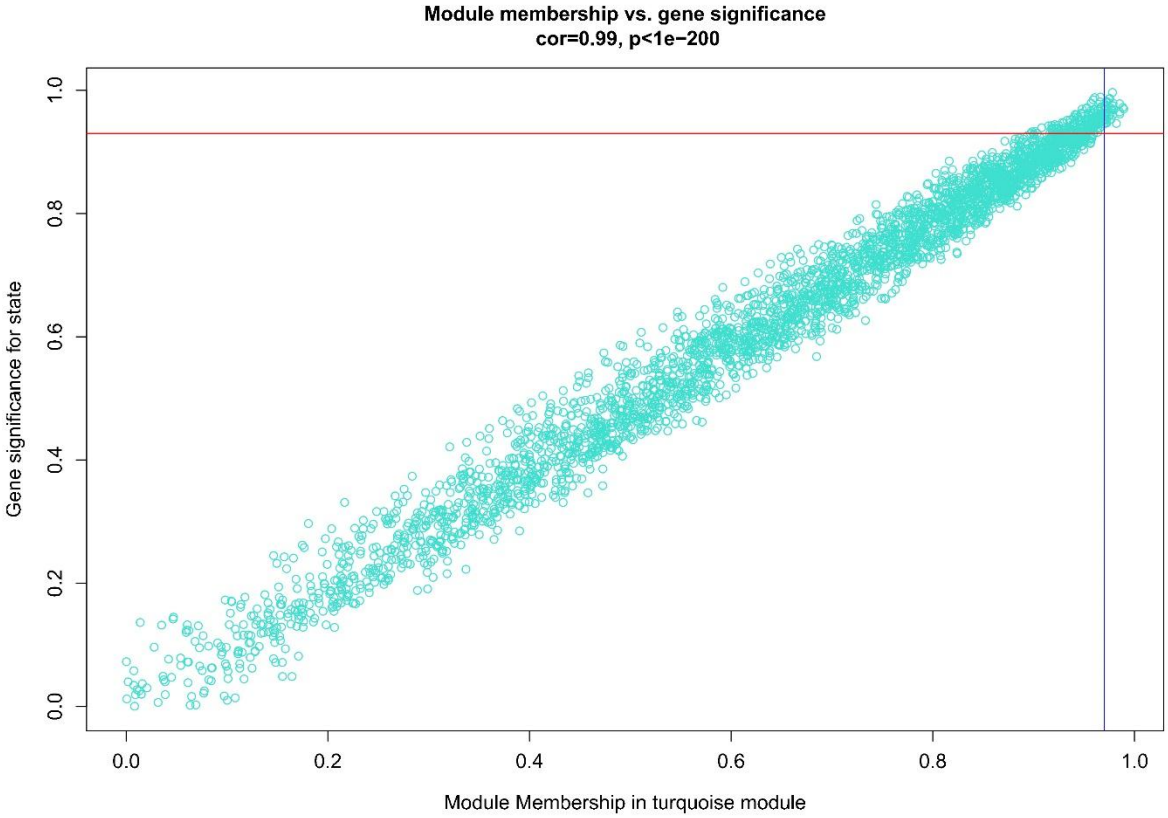


Figure 5. Module features of GS and MM (a) Modules are significantly correlated with DMD status (control vs. patient). Each point represents an individual gene within each module, which are plotted by GS on the y-axis and MM on the x-axis.

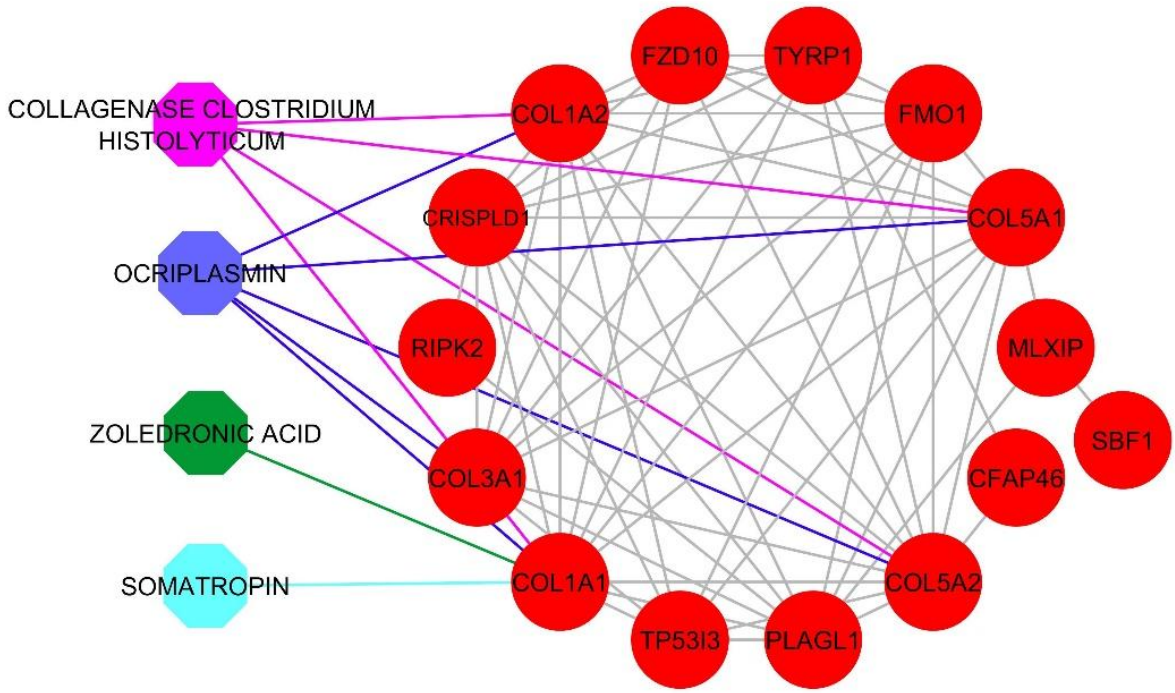


Figure 6. Drug-target network of turquoise module hub-genes. FDA approved drugs were acquired from DGIDB database for each gene.

ONLINE SUBMISSION

www.ijps.ir