Original Investigation | Özgün Araştırma

Gazi Med J

DOI: http://dx.doi.org/10.12996/gmj.2025.4444

Comprehensive Prediction of FBN1 Targeting miRNAs: A Systems Biology Approach for Marfan Syndrome

FBN1 Hedefleyen miRNA'ların Kapsamlı Tahmini: Marfan Sendromu için Sistem Biyolojisi Yaklaşımı

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ABSTRACT

Objective: Marfan syndrome (MFS) is a genetic connective tissue disorder primarily caused by mutations in the *FBN1* gene. Emerging evidence highlights the regulatory role of microRNAs (miRNAs) in modulating gene expression in MFS, but a systematic investigation into miRNAs targeting FBN1 is lacking. This study aimed to comprehensively identify miRNAs interacting with the FBN1 transcript to reveal potential molecular regulators and therapeutic targets.

Methods: Human miRNA sequences were retrieved from miRBase (Release 22.1), and the canonical FBN1 transcript (RefSeq: NM_000138.5) was used for target prediction. Computational interaction analysis was conducted using the psRNATarget server with stringent parameters to detect potential miRNA binding sites. Expression profiles and disease associations of the top candidate miRNAs were further investigated through database integration and literature review.

Results: Out of 2656 human mature miRNAs analyzed, 251 were predicted to bind FBN1, with the hsa-miR-181 family exhibiting the highest number of predicted interactions. Evidence from the literature highlighted dysregulation of hsa-miR-181 expression in MFS patients, suggesting a functional role in disease pathophysiology.

Conclusion: This study identifies key members of the hsa-miR-181 family as post-transcriptional regulators of FBN1, offering new insights into miRNA-driven mechanisms in MFS. These findings support the potential of RNA-based diagnostics and therapeutic strategies targeting miRNA-FBN1 interactions.

Keywords: Marfan syndrome, FBN1, MicroRNAs, hsa-miR-181, Bioinformatics, Post-transcriptional regulation

ÖZ

Amaç: Marfan sendromu (MFS), genellikle *FBN1* genindeki mutasyonlardan kaynaklanan genetik bir bağ dokusu bozukluğudur. Son yıllarda, gen ekspresyonunu post-transkripsiyonel düzeyde düzenleyen mikroRNA'ların (miRNA'lar) bu hastalıktaki rolü ön plana çıkmaktadır. Bu çalışmanın amacı, FBN1 transkriptini hedef alan miRNA'ları sistematik olarak tanımlamak ve potansiyel düzenleyici etkileşimleri ortaya çıkarmaktır.

Yöntemler: İnsan miRNA dizileri miRBase (Sürüm 22.1) veritabanından alınmış ve *FBN1* geninin referans transkripti (RefSeq: NM_000138.5) Ulusal Biyoteknoloji Bilgi Merkezi üzerinden elde edilmiştir. psRNATarget sunucusu kullanılarak FBN1 ile etkileşime giren miRNA'lar tahmin edilmiştir. En güçlü etkileşime sahip aday miRNA'ların ekspresyon düzeyleri ve hastalıklarla ilişkileri veritabanları ve literatür taramaları ile değerlendirilmiştir.

Bulgular: Toplamda 2656 insan miRNA'sı analiz edilmiş ve bunlardan 251'inin FBN1 transkripti ile potansiyel bağlanma bölgeleri olduğu öngörülmüştür. Özellikle hsa-miR-181 ailesi çok sayıda bağlanma bölgesiyle dikkat çekmiştir. Literatür bulguları, bu miRNA ailesinin MFS hastalarında aşağı düzenlendiğini göstermektedir.

Sonuç: Bu çalışma, hsa-miR-181 ailesinin FBN1 üzerindeki düzenleyici etkisini ortaya koyarak MFS'ndaki potansiyel moleküler mekanizmalara ışık tutmaktadır. Elde edilen bulgular, RNA temelli tedavi yaklaşımları için yeni hedeflerin geliştirilmesinde kullanılabilir.

Anahtar Sözcükler: Marfan sendromu, FBN1, MikroRNA, hsa-miR-181, biyoinformatik, gen ekspresyon düzenlemesi

Cite this article as: Orhan ME, Demirci YM, Saçar Demirci MD. Comprehensive prediction of FBN1 targeting miRNAs: a systems biology approach for marfan syndrome. Gazi Med J. [Epub Ahead of Print]

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Received/Geliş Tarihi: 29.04.2025
Accepted/Kabul Tarihi: 11.08.2025
Epub: 22.09.2025

INTRODUCTION

Marfan syndrome (MFS) is a systemic, autosomal dominant connective tissue disorder with manifestations affecting the skeletal system, eyes, cardiovascular system, and lungs. Clinical hallmarks include aortic aneurysm and dissection, ectopia lentis, scoliosis, and tall stature. The disorder arises primarily from mutations in the *FBN1* gene, which encodes fibrillin-1, a structural glycoprotein essential for the formation of elastic fibers in connective tissue (1). Pathogenic variants in FBN1 result in abnormal microfibril formation and dysregulated transforming growth factor-beta (TGF-β) signaling, contributing to tissue fragility and disease progression (2). In modern practice, healthcare providers use the revised Ghent criteria from 2010 to diagnose related conditions. When there is no positive family history, a diagnosis requires either (i) an aortic-root Z-score of 2 or higher in combination with ectopia lentis, or (ii) an aortic-root Z-score of 2 or higher along with a pathogenic variant in the *FBN1* gene. If a first-degree relative is affected, the criteria are less strict; a diagnosis can be made with either one major sign (such as aortic dilatation or ectopia lentis) or a total score of 7 or higher based on skeletal, craniofacial, and pulmonary features. These guidelines emphasize the importance of FBN1 genetics while also taking measurable physical signs into account, highlighting the need to explore factors that regulate FBN1 after it is transcribed (3).

While genetic testing can confirm the diagnosis of MFS, significant variability in phenotype even among individuals with the same FBN1 mutation suggests the presence of additional regulatory factors (4). In recent years, microRNAs (miRNAs)-short, non-coding RNA molecules that regulate gene expression post-transcriptionally-have emerged as key players in disease modulation (5). MiRNAs influence diverse biological processes such as extracellular matrix remodeling, apoptosis, and inflammation-hallmarks of cardiovascular pathology in MFS (6,7). Despite advances in understanding FBN1-related pathology, systematic investigations into miRNA-mediated regulation of FBN1 remain scarce (7,8). Previous studies have identified miRNA dysregulation in MFS patients, such as downregulation of hsa-miR-181 in serum samples (6) which may affect aortic wall integrity through modulation of smooth muscle cell behavior (9). Moreover, miRNAs like hsa-miR-143 and hsa-miR-145 have been implicated in the phenotypic plasticity of vascular cells (10), potentially contributing to the progression of aneurysms.

This study aimed to bridge this knowledge gap by applying a bioinformatics pipeline to systematically predict and prioritize miRNAs that target the FBN1 transcript. By integrating interaction prediction, expression profiling, and disease association data, we highlight key miRNA regulators with potential therapeutic relevance in MFS.

MATERIALS AND METHODS

Figure 1 illustrates a schematic representation of the study workflow employed in this research.

Retrieval of miRNA and mRNA Sequences

Human mature miRNA sequences were downloaded from miRBase (Release 22.1), and the canonical transcript of the human *FBN1* gene (RefSeq: NM_000138.5) was retrieved from the National Center for Biotechnology Information database.

Prediction of miRNA–FBN1 Interactions

Target prediction was conducted using the psRNATarget web server. The following parameters were used: Maximum expectation = 5.0; HSP length = 19; Seed region = nucleotides 2-13; Mismatches allowed = 2; Penalties: pairing between Guanine and Uracil = 0.5, mismatch = 1.0, opening gap = 2.0, extending gap = 0.5; Bulges in target allowed; Seed weight = 1.5.

Prioritization of Candidate miRNAs

Predicted miRNAs with high binding frequency and complementarity were further analyzed for their expression and disease associations using HMDD v4.0 and literature search.

Statistical Analysis

Descriptive statistics summarized the binding distributions. All computational analysis was performed using R free and open-source software package (version 4.2.0).

RESULTS

Broad Prediction of miRNAs Targeting FBN1

Out of 2656 human mature miRNAs analyzed, 251 were predicted to have binding sites on the *FBN1* transcript. The number of binding sites per miRNA varied, with certain families showing enriched targeting.

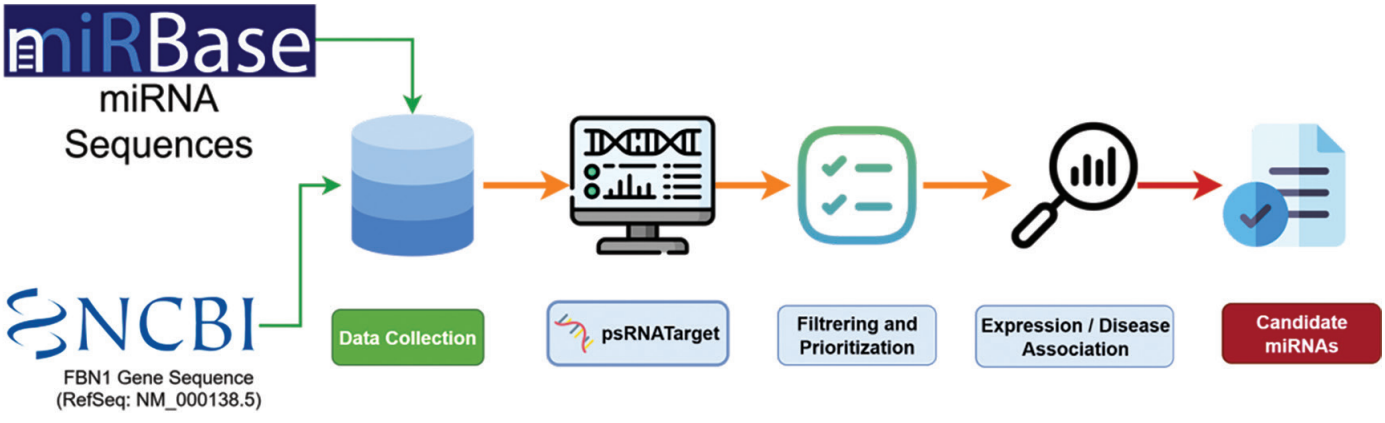

Figure 1. The schema of the study workflow

NCBI: National Center for Biotechnology Information

The hsa-miR-181 family emerged as particularly prominent, with multiple members exhibiting 10 or more binding interactions with *FBN1* (Table 1 and Figure 2).

High-Confidence miRNA–mRNA Interactions

Table 1 summarizes representative alignments of hsa-miR-181d-5p, hsa-miR-181b-5p, and hsa-miR-181a-5p with distinct regions on the FBN1 mRNA. These alignments were characterized by high complementarity in the seed regions and occurred in both coding and 3' untranslated regions of the transcript.

Expression Profiles and Disease Associations

Cross-referencing with HMDD v4.0 showed that the hsa-miR-181 family is associated with over 400 disease states, with cardiovascular and connective tissue conditions frequently appearing. Notably, hsa-miR-181d was found to be downregulated in MFS patient plasma samples (6), suggesting its potential involvement in disease modulation. This aligns with prior findings on miRNA dysregulation in aneurysmal diseases (7-9).

DISCUSSION

This study provides a systems-level exploration of miRNAs that may regulate *FBN1*, the primary gene mutated in MFS. Our analysis revealed that members of the hsa-miR-181 family exhibit extensive potential for *FBN1* regulation through multiple high-affinity binding sites, supported by experimental and computational data.

Previous investigations into MFS-related miRNAs have predominantly been candidate-driven, concentrating on select miRNAs or restricted expression panels analyzed in patient blood, or aneurysmal tissues. To date, no study has implemented a comprehensive whole-miRNome screening approach encompassing all 2,656 mature human miRNAs against the canonical FBN1 transcript. Furthermore, there has been a lack of integration of binding-site density with cross-cohort expression data and disease association meta-analyses. Our methodology, therefore, offers the first systems-level mapping of potential miRNA regulators of FBN1, resulting in a prioritized catalogue that can inform targeted experimental validation and facilitate therapeutic development.

Functional Implications of miRNA-FBN1 Regulation

Given the central role of *FBN1* in maintaining connective tissue elasticity, its post-transcriptional regulation by miRNAs may directly influence phenotypic severity in MFS. The hsa-miR-181 family has been previously implicated in cardiovascular remodeling, vascular smooth muscle cell apoptosis, and extracellular matrix regulation-pathways relevant to MFS aortopathy (10-12).

Translational Relevance

The miR-181 family demonstrates promising translational potential in human cohorts, potentially offering additional clinical insights beyond conventional FBN1 genotyping. In a two-stage blood profiling study involving patients with MFS (discovery cohort n=7, validation cohort n=26), Abu-Halima et al. (6) identified a down-regulation of miR-181d-5p, which exhibited an inverse correlation with aortic root Z-scores and left ventricular diameter. This study asserts that these miRNAs are minimally invasive biomarkers for assessing disease severity. Further supporting these findings, a recent serum analysis involving 388 patients with thoracic aortic aneurysm (TAA) established that levels of miR-181b-5p were independently correlated with TAA presence, achieving an area under the receiver operating characteristic curve of 0.82 for differentiating TAA from both coronary artery disease and healthy controls (13). The integration of these findings with established functional connections to extracellular matrix turnover and TGF-β signaling pathways suggests that circulating miR-181 levels could serve to inform: (i) baseline risk stratification, (ii) longitudinal monitoring of aortic dilation, and (iii) the selection of patients for potential miRNA-modulating therapies.

The study's findings support the feasibility of RNA-targeted therapeutics in MFS. Technologies such as antagomirs, miRNA mimics, and lipid nanoparticle-mediated delivery could be leveraged to normalize miRNA expression and rebalance *FBN1* levels. Personalized medicine approaches may incorporate miRNA expression profiling to predict disease progression or treatment response (12-17).

Study Limitation

Predictions were based on in silico data and required experimental validation through reporter assays or transcript knockdown models. To convert our ranked catalog into biologically credible candidates, future work should follow a staged validation roadmap. This process includes (i) molecular confirmation of the highest-affinity binding

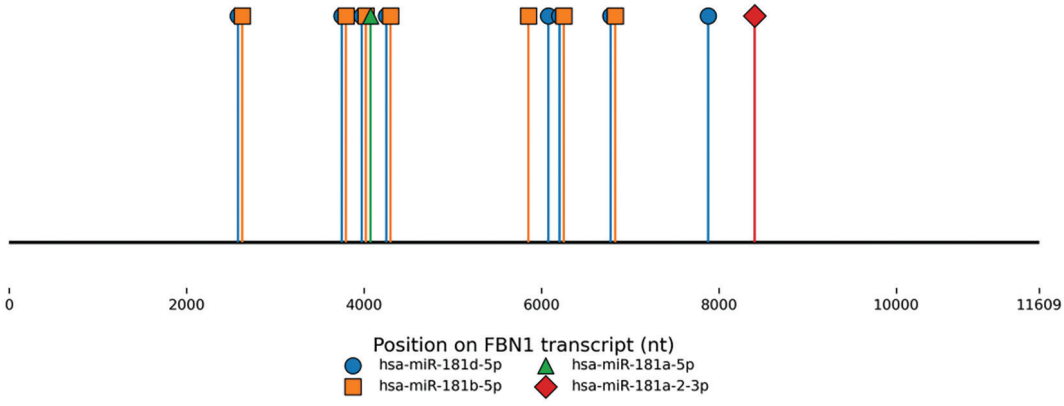

Figure 2. Lollipop plot of miR-181 binding sites on *FBN1*

Table 1. Predicted binding sites of hsa-miR-181 family members on *FBN1* transcript

MiRNA	Alignment
hsa-miR-181d-5p	miRNA 23 UGGGUGGCUGUUGUUACUUACAA 1 . | . | | | | | | | | | | | | | | | Target 3772 GUGUAUCGACAUCAAUGAAUGUG 3794
hsa-miR-181d-5p	miRNA 23 UGGGUGGCUGUUGUUACUUACAA 1 | . | | | | | | | | | | | | . | | | | . | | | Target 4021 AUGCACCGACAUCGAUGAGUGUG 4043
hsa-miR-181d-5p	miRNA 23 UGGGUGGCUGUUGUUACUUACAA 1 . | | | | | | | | | | | | | | | | | Target 4273 CUGUACAGACAUCAAUGAAUGUG 4295
hsa-miR-181d-5p	miRNA 23 UGGGUGGCUGUUGUUACUUACAA 1 | | . . | | . | | | | | | | | | | | | Target 7879 CUGCAUUGAUAACAAUGAAUGCA 7901
hsa-miR-181d-5p	miRNA 23 UGGGUGGCUGUUGUUACUUACAA 1 | . . . | | . | . | | | | | | | | | | Target 2602 CUGCGUUGAUAUUAAUGAAUGUG 2624
hsa-miR-181d-5p	miRNA 23 UGGGUGGCUGUUGUUACUUACAA 1 | . . | . | | | | | | | | | | | | | | Target 6079 CCUUUCUCACAACAAUGACUGUA 6101
hsa-miR-181d-5p	miRNA 23 UGGGUGGCUGUUGUUACUUACAA 1 | . . . | | . | . . | | | | | | | | | | Target 6805 AUGUGUAGAUACUGAUGAAUGUU 6827
hsa-miR-181d-5p	miRNA 23 UGGGUGGCUGUUGUUACUUACAA 1 . . . | | . | | | | | | | | | | | | Target 6226 CUGUGUGGAUAUCAAUGAAUGUC 6248
hsa-miR-181b-5p	miRNA 23 UGGGUGGCUGUCGUUACUUACAA 1 . | . | | | | | | | | | | | | | | | Target 3772 GUGUAUCGACAUCAAUGAAUGUG 3794
hsa-miR-181b-5p	miRNA 23 UGGGUGGCUGUCGUUACUUACAA 1 | . | | | | | | | | | | . | | | | . | | | Target 4021 AUGCACCGACAUCGAUGAGUGUG 4043
hsa-miR-181b-5p	miRNA 23 UGGGUGGCUGUCGUUACUUACAA 1 . | | | | | | | | | | | | | | | | | Target 4273 CUGUACAGACAUCAAUGAAUGUG 4295
hsa-miR-181b-5p	miRNA 23 UGGGUGGCUGUCGUUACUUACAA 1 | . . . | | . | . | | | | | | | | | | Target 2602 CUGCGUUGAUAUUAAUGAAUGUG 2624
hsa-miR-181b-5p	miRNA 23 UGGGUGGCUGUCGUUACUUACAA 1 | | . | | . | . | | | | | | | | | | Target 5854 GUGCAAUGAUCGUAAUGAAUGUC 5876
hsa-miR-181b-5p	miRNA 23 UGGGUGGCUGUCGUUACUUACAA 1 | . . . | | . | . . | | | | | | | | | | Target 6805 AUGUGUAGAUACUGAUGAAUGUU 6827
hsa-miR-181b-5p	miRNA 23 UGGGUGGCUGUCGUUACUUACAA 1 . . . | | . | | | | | | | | | | | | Target 6226 CUGUGUGGAUAUCAAUGAAUGUC 6248

Table 1. Continued

MiRNA	Alignment
hsa-miR-181a-5p	miRNA 23 UGAGUGGCUGUCGCAACUUACAA 1 |. |||||||| || |||.||| Target 4021 AUGCACCGACAUCGAUGAGUGUG 4043
hsa-miR-181a-2-3p	miRNA 22 CCAUGUCAGUUGCCAGUCACCA 1 | || || | ||||||||| Target 8404 CCCAGAGCCACCUGUCAGUGGU 8425

Base pair interactions and alignment sites on the corresponding RNA sequences are shown in the alignment column. "|" indicates matches, "." shows pairing between Guanine and Uracil

sites using dual-luciferase assays; (ii) mapping of endogenous interactions through RT-qPCR or high-throughput sequencing following Argonaute immunoprecipitation; (iii) conducting perturbation studies, such as transient delivery of miR-181 mimics or inhibitors, or Clustered Regularly Interspaced Short Palindromic Repeats editing of seed sites, in vascular smooth muscle and fibroblast models; and (iv) investigating the clinical correlation of circulating miR-181 levels with aortic-root growth in longitudinal cohorts of MFS patients. Although these experimental steps extend beyond the scope of our current computational study, they are crucial next steps for transforming *in silico* predictions into mechanistically and clinically actionable insights.

CONCLUSION

This study offers a comprehensive computational analysis of miRNAs that potentially regulate *FBN1*, the principal gene implicated in MFS. By applying a stringent target prediction approach, we identified members of the hsa-miR-181 family as top candidate regulators with multiple high-confidence binding sites on the *FBN1* transcript. The literature supports the downregulation of these miRNAs in MFS patients, implicating them in the disease's molecular etiology.

These findings reinforce the concept that miRNA-based regulation may significantly influence connective tissue homeostasis and suggest that therapeutic modulation of miRNA expression could serve as a strategy to restore FBN1 function. Furthermore, the study advocates for integrating miRNA profiling into personalized medicine frameworks, enabling tailored risk assessment and treatment planning in genetically complex disorders like MFS.

Future directions include experimental validation of predicted interactions using luciferase assays, as well as *in vivo* studies to assess the phenotypic consequences of miRNA modulation. These steps are critical to translating the current findings into clinical applications.

Ethics

Ethics Committee Approval: This study did not require ethical approval because it is entirely based on the analysis of publicly available datasets. No experiments involving humans, animals, or the use of personally identifiable information were performed.

Informed Consent: Not applicable, as no individual persons' data are included in this manuscript.

Footnotes

Authorship Contributions

Concept: M.E.O., Y.M.D., M.D.S.D., Design: M.D.S.D., Data Collection or Processing: M.E.O., Y.M.D., M.D.S.D., Analysis or Interpretation: M.E.O., Y.M.D., M.D.S.D., Literature Search: M.E.O., Y.M.D., M.D.S.D., Writing: M.E.O., Y.M.D., M.D.S.D.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This work has been supported by the Abdullah Gül University Support Foundation.

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