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Review of FBN1 Gene Role in Marfan Syndrome Presentations Insilico Analysis

Mohanad Abdelrahim[1,2,*], Khalid EL.Khalid[3], Mohammed Elamin Faris[3], Mohamed A.Hassan[2,4], Kamal Elsiddig[5], Ahmed Siddig Muhammed[6], Mohammed Nimir[7], Mahil Abdalla[2], Asgad Suliman[2]

[1]Departement of human anatomy faculty of Medicine University of Khartoum - Sudan
[2]Daoud research group
[3]MBBS university of Khartoum
[4]Division of Molecular Genetics, university of Tubingen,- Germany
[5]Deprtement of Surgery University of Khartoum - Sudan
[6]Medical Student University of Khartoum - Sudan
[7]Institute of endemic diseases - Khartoum - Sudan
*Corresponding author: mohanadkhalid91@gmail.com

Abstract **Overview:** This is translational bioinformatics was focused on analysis of single nucleotide polymorphism of FBN1 gene and reviewing of the previous citations of the damaging SNPs. **Introduction:** Marfan syndrome is a common autosomal dominant hereditary connective tissue disorder with variable presentations, mutations in FBN1 gene were found to be responsible for Marfan syndrome and other related connective tissue disorders, SNPs contributes to gene mutations and expression variations justifying phenotypic variations among patients and hence such SNPs would be potential target for identification and analysis which may help in early diagnosis of such life threatening disorder. **Methods**: computational methods were used on this work focusing on analysis of SNPs in the coding regions of FBN1 gene found as non-synonymous variants (ns-SNP) and those in the 3'un-translated regions (3'UTR) affecting miRNA binding using computational methods including SIFT and polyphen for analysis of (nsSNPs), prediction of not previously described SNPs was done using project hope software, while (3'UTR) SNPs was analyzed using PolymiRTS tool functions interactions of FBN1 gene with functionally similar gene were predicted using Genemania software. **Results**: Out of 1134 ns-SNPs analyzed 38 SNPs were found to damaging while analysis of 175 SNP in 3'UTR prove that 24 SNPs are disturbing to their target sites and 46 SNPs are creating to new target sites.On reviewing of previous citation 31 of the predicted damaging nSNPs were reported as mutations with specific Marfan syndrome presentation while 6 nsSNP were not previously reported with high damaging probability.

Keywords: *marfan syndrome, FBN1 gene, ns-SNPs, 3'UTR SNPs, sift, polyphene, PolymiRTS. project hope, Genemania*

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1. Introduction

The Marfan syndrome is an autosomal dominant disorder of the connective tissue shows striking pleiotropism and clinical variability. The cardinal features occur in 3 systems--skeletal, ocular, and cardiovascular which is the most serious signs and symptoms associated with Marfansyndrome particularly aortic dilatation, dissection and rupture and involvement of the aortic and mitral valves, lead to a greatly reduced life expectancy [1,2].

1.1. FBN1 Gene

This gene encodes a member of the fibrillin family. The encoded protein is a large, extracellular matrix glycoprotein that serve as a structural component of 10-12 nm calcium-binding micro fibrils. These micro fibrils provide force bearing structural support in elastic and non-elastic connective tissue throughout the body. Mutations in this gene are associated with Marfan syndrome, isolated ectopialentis, autosomal dominant Weill-Marchesani syndrome, MASS syndrome, and Shprintzen-Goldberg craniosynostosis syndrome. [Provided by RefSeq, Jul 2008].

Marfan syndrome is caused by mutations in the FBN1 gene on chromosome 15 [1], which encodes fibrillin-1, a glycoprotein component of the extracellular matrix.

The fibrillin geneis located on chromosome 15, is relatively large, and the coding sequence is divided into 65 exons. Described 3 alternatively spliced exons at the 5-prime end, which they termed exon B, exon A, and exon C [3,4]. A CpG island was identified that spans the first 2 alternatively spliced exons.Estimated the size of the FBN1 gene to be 200 kb [5].

1.2. Diagnosis of Marfan Syndrome

The diagnosis of Marfan syndrome relies on a set of defined clinical criteria (Revised Ghent Nosology) developed to facilitate accurate recognition of the syndrome and improve patient management and counseling

The 2010 Revised Ghent Nosology for Marfan syndrome relies on seven rules as indicated below:

1.3. In the Absence of Family History

Aortic Root Dilatation Z score ≥ 2 AND EctopiaLentis = Marfan syndrome - The presence of aortic root dilatation (Z-score ≥ 2 when standardized to age and body size) or dissection and ectopialentis allows the unequivocal diagnosis of Marfan syndrome, regardless of the presence or absence of systemic features except where these are indicative of Shprintzen Goldberg syndrome, Loeys-Dietz syndrome, or vascular Ehlers Danlos syndrome.

Aortic Root Dilatation Z score ≥ 2 AND FBN1 = Marfan syndrome - The presence of aortic root dilatation (Z ≥ 2) or dissection and the identification of a bona fide FBN1 mutation are sufficient to establish the diagnosis, even when ectopialentis is absent.

Aortic Root Dilatation Z score ≥ 2 AND Systemic Score ≥ 7pts = Marfan syndrome - Where aortic root dilatation (Z ≥ 2) or dissection is present, but ectopialentis is absent and the FBN1 status is either unknown or negative, a Marfan syndrome diagnosis is confirmed by the presence of sufficient systemic findings (≥ 7 points, according to a scoring system) confirms the diagnosis. However, features suggestive of Shprintzen Goldberg syndrome, Loeys-Dietz syndrome, or vascular Ehlers Danlos syndrome must be excluded and appropriate alternative genetic testing (TGFBR1/2, collagen biochemistry, COL3A1, and other relevant genetic testing when indicated and available upon the discovery of other genes) should be performed.

Ectopialentis AND FBN1 with known Aortic Root Dilatation = Marfan syndrome - In the presence of ectopialentis, but absence of aortic root dilatation/ dissection, the identification of an FBN1 mutation previously associated with aortic disease is required before making the diagnosis of Marfan syndrome.

1.4. In the Presence of Family History:

Ectopialentis AND Family History of Marfan syndrome (as defined above) = Marfan syndrome - The presence of ectopialentis and a family history of Marfan syndrome (as defined in 1-4 above) is sufficient for a diagnosis of Marfan syndrome.

A systemic score ≥ 7 points AND Family History of Marfan syndrome (as defined above) = Marfan syndrome - A systemic score of greater than or equal to 7 points and a family history of Marfan syndrome (as defined in 1-4 above) is sufficient for a diagnosis of Marfan syndrome. However, features suggestive of Shprintzen Goldberg syndrome, Loeys-Dietz syndrome, or vascular Ehlers Danlos syndrome must be excluded and appropriate alternative genetic testing (TGFBR1/2, collagen biochemistry, COL3A1, and other relevant genetic testing when indicated and available upon the discovery of other genes) should be performed.

Aortic Root Dilatation Z score ≥ 2 above 20 yrs. old, ≥ 3 below 20 yrs. old) + Family History of Marfan syndrome (as defined above) = Marfan syndrome - The presence of aortic root dilatation (Z ≥ 2 above 20 yrs. old, ≥ 3 below 20 yrs. old) and a family history of Marfan syndrome (as defined in 1-4 above) is sufficient for a diagnosis of Marfan syndrome. However, features suggestive of Shprintzen Goldberg syndrome, Loeys-Dietz syndrome, or vascular Ehlers Danlos syndrome must be excluded and appropriate alternative genetic testing (TGFBR1/2, collagen biochemistry, COL3A1, and other relevant genetic testing when indicated and available upon the discovery of other genes) should be performed [6].

SNPs stand for single Nucleotide Polymorphism are DNA sequence variations that occur when a single nucleotide (A,T, C or G) in the genome is altered they are found throughout the genome in exons, introns, introgenicregions, promoters, enhancers 16 SNP in a promoter can influence gene expression [7] and thus more likely to contain an allele being more functionally or physiological relevant than other types of polymorphism.

The identification of SNPs responsible for specific phenotypes seems to be a problem, since requiring multiple testing of hundreds or thousands of SNPs in candidate genes [8]. Recently researchers found new functional polymorphisms called MiRSNPs/polymorphism located at micro RNA binding sites of functional gene that can influence gene expression by interfering with microRNA function of those SNPs within microRNAs (miRNAs) [9].

In this study the decision of choosing the right set of SNPs from the NCBI Human reference genome (www.ncbi.nlm.nih.gov), to be screened was a critical one. One possible way to overcome this problem was to prioritize SNPs according to their structural and functional significance using different bioinformatics prediction tools.

2. Material and Methods

Computational methods were used in this study with proitrizing SNPs in the coding region (exonal SNPs) that are non-synonymous (nsSNP) and SNPs at un-translated region at 3'ends (3'UTR) to predict the effect on miRNA binding on these regions that may greatly associated with Marfan syndrome [10]. The SNPs and the related ensembles protein (ESNP) were obtained from the SNPs database (dbSNPs) for computational analysis from http://www.ncbi.nlm.nih.gov/snp/ and Uniprot database http://www.uniprot.org for related protein sequences.

SIFT is an online computational tool to detect a harmful non-synonymous single-base nucleotide polymorphism (nsSNP); the genetic mutation that causes a single amino acid substitution (AAS) in a protein sequence subsequently altering the carrier's phenotype and health status. The software traces AAS and Sorting Intolerant From Tolerant (SIFT) and predicts whether these substitutions affect protein function by using sequence homology, SIFT predicts the effects of all possible substitutions at each position in the protein sequence [11]. Furthermore, the algorithm performs a comprehensive search in protein repositories to find the tolerance of each candidate compared to the conserved counterparts [12] Non-synonymous reference SNPs identity (rsSNPs ID) were

downloaded from online dbSNPs of NCBI, and then submitted to SIFT. Results are expressed as damaging (Intolerated) or benign (Tolerated) depending on cutoff value 0.05; as values below or equal to 0.0 _ 0.04 predicted to be damaging or intolerant while (0.05_1) is benign or tolerated, then the damaging SNPs were re-analyzed by Polyphen software which predicts the effect of mutations on both structural and functional sides. SIFT is available as online tool at http://sift.jcvi.org.

2.1. Prediction of Functional Modification Using Polyphen-2 (Polymorphism Phenotyping v2):

It is a software tool to predict possible impact of an amino acid substitution on both structure and function of a human protein by analysis of multiple sequence alignment and protein 3D structure, in addition it calculates position-specific independent count scores (PSIC) for each of two variants, and then calculates the PSIC scores difference between two variants. The higher a PSIC score difference, the higher the functional impact a particular amino acid substitution is likely to have. Prediction outcomes could be classified as probably damaging, possibly damaging or benign according to the value of PSIC as it ranges from (0_1); values closer to zero considered benign while values closer to 1 considered probably damaging and also it can be indicated by a vertical black marker inside a color gradient bar, where green is benign and red is damaging [13] nsSNPs that predicted to be intolerant by Sift has been submitted to Polyphen as protein sequence in FASTA format that obtained from UniproktB/Expasy after submitting the relevant ensemble protein (ESNP) there, then we entered position of mutation, native amino acid and the new substituent for both structural and functional predictions.

PolyPhen version 2.2.2 is available at http://genetics.bwh.harvard.edu/pph2/index.shtml

2.2. b- Project Hope (version 0.4.1):

Project hope is a new online web-server to search protein 3D structures by collecting structural information from a series of sources, including calculations on the 3D protein structure, sequence annotations in UniproktB and predictions from DAS-servers. HOPE works online where one can submit a sequence and mutation only for those that predicted to be damaging by both SIFT and Polyphen (Double Positive) servers. Protein sequences were submitted to project hope server in order to analyze the structural and conformational variations that have resulted from single amino acid substitution corresponding to single nucleotide substitution. Project Hope is available at: http://www.cmbi.ru.nl/hope [14].

2.3. PolymiRTS Data Base (version 3.0) for Polymorphism in microRNA Target Site

PolymiRTS database was designed specifically for the analysis of non-coding SNPs namely 3'UTR, it aims to identify single-nucleotide polymorphisms (SNPs) that affect miRNA targets in human and mouse. We used this computational server in order to determine 3'UTR SNPs in *APC g*ene that may alter miRNA binding on target sites resulting in diverse functional consequences. All SNPs located in that region were selected and submitted to PolymiRTS (v3.0), available at: http://compbio.uthsc.edu/miRSNP/.

The previous citation sof each indvidual SNP predicted by SIFT and polyphene were revised with the help of uniprot website.

3. Results and Discussion

FBN1 gene was investigated with NCBi and it was found to contains total of 1134 SNPs 642 were nsSNPs and 161 SNPs on the 3UTR region

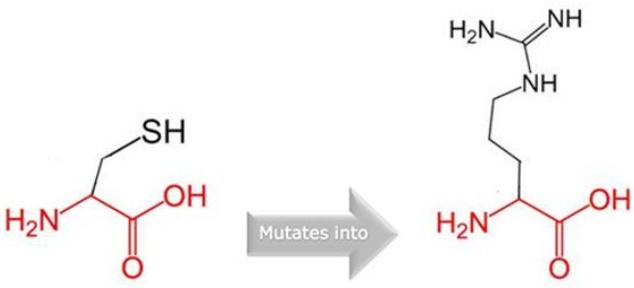
3.1. Predictions by SIFT and Polyphen

Predictions of deleterious nsSNPs was performed by SIFT and Polyphensoftwares; out of 642snSNPs only 38 (5.9%) were predicted to be damaging by both servers. First, we submitted batch nsSNPs (rs SNPs) to Sift server; then the resultant damaging nsSNPs were submitted to Polyphen as query sequences in FASTA Format, it traced 37 probably damaging nsSNPsand only one nsSNP was scored as possibly damaging. While reviewing of the predicted nsSNPs there are 6 nsSNPs were not cited and all of the rest were cited with one of the manifestations of Marfan Syndrome Results are shown in the table below.

3.2. Project Hope Predictions of No Cited Mutations

1. rs140592 C996R: [27]

The figure below shows the schematic structures of the original (left) and the mutant (right) amino acid.(mutation of a cysteine into a arginine at position 996) The backbone, which is the same for each amino acid, is colored red. The side chain, unique for each amino acid, is colored black.



Each amino acid has its own specific size, charge, and hydrophobicity-value. The original wild-type residue and newly introduced mutant residue often differ in these properties.

The mutant residue is bigger than the wild-type residue.

The wild-type residue was neutral, the mutant residue is positively charged.

The wild-type residue is more hydrophobic than the mutant residue.

2. rs140647 N1282S [27]

Mutation of an asparagine into a serine at position 1282. The figure below shows the schematic structures of the original (left) and the mutant (right) amino acid. The backbone, which is the same for each amino acid, is colored red. The side chain, unique for each amino acid, is colored black.

Table 1. 6 nsSNPs were not cited and all of the rest were cited with one of the manifestations of Marfansyndrome

number	SNP ID	Amino acid change	Polyphene results	Age	Gender	Family Hx.	Clinical features	references
1	rs25403	R62C	probably damaging	31	M	+ve	EL, RD, [15]	Katzke S
2	rs140592	C996R	probably damaging					No pup.
3	rs140597	D1113G	probably damaging	4	F	-ve	MVP, SS [16]	Rommel K
4	rs140599	C1153Y	probably damaging	<1	F	-ve	AD, AR, MVP, TVR, EL [17]	Arbustini E
5	rs140627	C1672F	probably damaging					No pup.
6	rs140647	N1282S	possibly damaging					No pup.
7	rs363804	C2038Y [16]	probably damaging	42 11 13	F F F	-ve +ve +ve	MVP, EL, SS, Sk AD, SS AD, MVP,SS [16]	Rommel K
8	rs363815	C2111R	possibly damaging	-	3 patients	+ve	OS, SS, CVS [18]	Hayward C
9	rs363821	C2170F	probably damaging					No pup.
10	rs363853	C177R	probably damaging	4	F	-ve	EL [17]	Arbustini E
11	rs363855	G363S	probably damaging					No pup.
12	rs111401431	R1530C[19]	probably damaging	32	-	-ve	OS, mild AD, EL [19]	Loeys B
13	rs111588631	C2318R[20]	probably damaging					Beatens M
14	rs111929350	C1835Y[19]	probably damaging	29	-	+ve		Loeys B
15	rs112375043	C1431W[20]	probably damaging					Beatens M
16	rs112660651	C89F[19]	probably damaging	28	-	-ve	SS, CVS [19]	Loeys B
17	rs112728248	C1876Y	probably damaging	27	M	+ve	AD, MVP, RD [17]	Arbustini E
18	rs112836174	C2251R	probably damaging					No pup.
19	rs113544411	C2535W	probably damaging	11	F	+ve	AD, MVP, SS [17]	Arbustini E
20	rs113722038	Y2793H[20]	probably damaging					Beatens M
21	rs137854457	C2307S[21]	probably damaging					Dietz H.C.
22	rs137854458	C1249S[21]	probably damaging					Dietz H.C.
23	rs137854459	C1663R[17]	probably damaging	59	M	+ve	AD, AoDi, MVP, MVR, EL, SS	Arbustini E
24	rs137854460	C2221S[21]	probably damaging					Dietz H.C.
25	rs137854461	N2144S[16]	probably damaging	40	M	-ve	AD, AI, SS	Rommel K
26	rs137854462	N548I[21]	probably damaging					Dietz H.C.
27	rs137854463	D723A[15]	probably damaging	8	F		EL, AD, MVP	Katzke S
28	rs137854464	E2447K[22]	probably damaging					Kainulainen K.
29	rs137854465	C1074R[18]	probably damaging	4	-	_ve	CVS, SS.	Hayward C
30	rs137854469	C1223Y[23]	probably damaging	66	F	-ve	EL, SS,	Hewett D.R.
31	rs137854470	C1117Y[24]	probably damaging					Tynan K
32	rs137854471	C1242Y[22]	probably damaging					Kainulainen K.
33	rs137854473	N1131Y	probably damaging					No pup.
34	rs137854474	C1265R[25]	probably damaging					Montgomery R.A.
35	rs137854477	G985E[19]	probably damaging	18	-	+ve	SS, CVS	Loeys B
36	rs137854478	E1073K[24]	probably damaging	Neonate	-	-	SS, AD, MVP	Nijbroek G.
37	rs137854480	R240C[19]	probably damaging	18	-	-ve	SS, OS, CVS	Loeys B
38	rs137854482	C1129Y[26]	probably damaging	No. Of pt. 27	-	-	All of them had SS, OS, CVS	El-Aleem A.A.

EL: EctopiaLentis, **RD**: Retinal Detachment, **MVP**: Mitral Valve Prolapse, **MVR**: Mitral Valve Regurgitation, **SS**: Skeletal System envolvement, **AD**: Aortic root Dilatation, **AR**: Aortic Regurgitation, **AoDi**: Aortic Dissection, **TVR**: Tricuspid Valve Regurgitation, **Sk**: Skin involvement, **OS**: Ocular System involvement, **RD**: Retinal Detachment, **AI**: Aortic Insufficiency, **CVS**: Cardiovascular system involvement.

Each amino acid has its own specific size, charge, and hydrophobicity-value. The original wild-type residue and newly introduced mutant residue often differ in these properties.

The mutant residue is smaller than the wild-type residue.

The mutant residue is more hydrophobic than the wild-type residue.

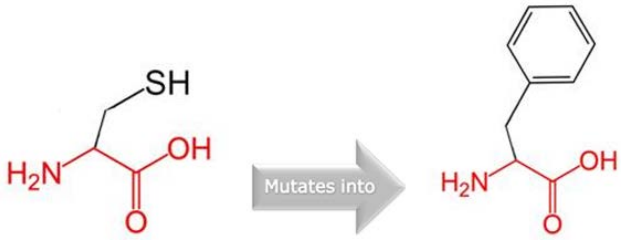
The report will evaluate the effect of the mutation on the following features: Contacts made by the mutated residue, structural domains in which the residue is located, modifications on this residue and known variants for this residue. A feature will only be shown when information is

available. A short conclusion based on just the amino acid properties is shown always. In case a 3D-structure/model is available you will also find images and animations in the report.

3. rs363821 (C2170F) [27]

Mutation of a cysteine into a phenylalanine at position 2170.

The figure below shows the schematic structures of the original (left) and the mutant (right) amino acid. The backbone, which is the same for each amino acid, is colored red. The side chain, unique for each amino acid, is colored black.



Each amino acid has its own specific size, charge, and hydrophobicity-value. The original wild-type residue and newly introduced mutant residue often differ in these properties.

The mutant residue is bigger than the wild-type residue.

The mutated residue is not in contact with a metal, however, one of the neighboring residues does make a metal-contact that might be affected by the mutation in its vicinity.

In the 3D-structure can be seen that this residue is involved in a cysteine bridge, which is important for stability of the protein. Only cysteine can make these type of interactions, the mutation causes loss of this interaction and will have a severe effect on the 3D-structure of the protein.

Together with loss of the cysteine bond, the differences between the old and new residue can cause destabilization of the structure.

The wild-type and mutant amino acids differ in size.

The mutant residue is bigger than the wild-type residue.

The residue is located on the surface of the protein, mutation of this residue can disturb interactions with other molecules or other parts of the protein.

4. rs363855 (G363S) [27]

Mutation of a glycine into a serine at position 363.

The figure below shows the schematic structures of the original (left) and the mutant (right) amino acid. The backbone, which is the same for each amino acid, is colored red. The side chain, unique for each amino acid, is colored black.

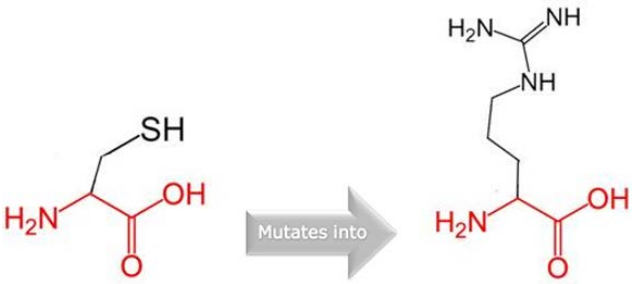
Each amino acid has its own specific size, charge, and hydrophobicity-value. The original wild-type residue and newly introduced mutant residue often differ in these properties.

The mutant residue is bigger than the wild-type residue.

5. rs112836174 (C2251R) [27]

Mutation of a cysteine into a arginine at position 2251.

The figure below shows the schematic structures of the original (left) and the mutant (right) amino acid. The backbone, which is the same for each amino acid, is colored red. The side chain, unique for each amino acid, is colored black.



Each amino acid has its own specific size, charge, and hydrophobicity-value. The original wild-type residue and newly introduced mutant residue often differ in these properties.

The mutant residue is bigger than the wild-type residue.

The wild-type residue was neutral, the mutant residue is positively charged.

The wild-type residue is more hydrophobic than the mutant residue.

6. rs140627 (C1672F) [27]

The mutation of a cysteine into a phenylalanine at position 1672.

The figure below shows the schematic structures of the original (left) and the mutant (right) amino acid. The backbone, which is the same for each amino acid, is colored red. The side chain, unique for each amino acid, is colored black.

Each amino acid has its own specific size, charge, and hydrophobicity-value. The original wild-type residue and newly introduced mutant residue often differ in these properties.

The mutant residue is bigger than the wild-type residue, The mutated residue is located in a domain that is important for binding of other molecules. Mutation of the residue might disturb this function

3.3. Analysis of 3UTR Region

Analysis of SNPs at the 3UTR prove that 24 SNPs are dturbing to their target sites Table 2, and 46 SNPs are creating to new target sits Table 3.

Table 2. 24 SNPs are disturbing to their target sites

Target sites disrupted by SNPs and INDELs in miRNA seeds								
Location	**miR ID**	**dbSNP ID**	**miR Seed**	**Allele**	**Wobble base pair**	**miRSite**	**Conservation**	**context+ score change**
48701662	hsa-miR-7152-5p	rs35654725	U[-/C]CCUGU	-/C	0	CAGGAAA	2	0.041
48701227	hsa-miR-6814-5p	rs185472832	CCCAA[G/A]G	G/A	1	CCUUGGG	3	-0.181
48701668	hsa-miR-938	rs12416605	[G/A]CCCUUA	G/A	1	UAAGGGCA	3	-0.107
48701004	hsa-miR-6509-3p	rs145322812	UC[C/T]ACUG	C/T	0	CAGUGGA	2	-0.164
48701459	hsa-miR-1302	rs74647838	UGGGA[C/T]A	C/T	0	GUCCCAA	2	-0.106
48701404	hsa-miR-6754-5p	rs113246861	CAG[G/A]GAG	G/A	1	CUCCCUG	2	-0.054
48702226	hsa-miR-4749-3p	rs148982635	[G/A]CCCCUC	G/A	1	GAGGGGC	8	-0.176
48702226	hsa-miR-4749-3p	rs200056596	GC[C/T]CCUC	C/T	0	GAGGGGC	8	-0.176
48701406	hsa-miR-6726-5p	rs199607738	[G/A]GGAGCU	G/A	1	AGCUCCC	4	-0.131
48700978	hsa-miR-1269b	rs7210937	UGGA[C/G]UG	C/G	0	AGUCCAA	2	-0.138
48703176	hsa-miR-432-3p	rs201401049	UGGAU[G/C]G	G/C	0	CCAUCCA	12	No Change
48702443	hsa-miR-4322	rs114399468	UGUGG[G/A]C	G/A	1	GCCCACA	7	-0.101
48702532	hsa-miR-6892-5p	rs111347704	U[A/G]AGGGA	A/G	1	CCCUUAA	15	-0.113
48701854	hsa-miR-2909	rs187707102	UUAGG[G/A]C	G/A	1	CCCUAAA	5	-0.032
48702192	hsa-miR-605-5p	rs113212828	[A/G]AAUCCC	A/G	1	GGAUUUA	2	0.028
48702887	hsa-miR-2392	rs118055959	AGG[A/G]UGG	A/G	1	CCAUCCU	5	-0.135
48702891	hsa-miR-3622b-5p	rs13276615	[G/T]GCAUGG	G/T	0	CAUGCCA	4	-0.114
48701259	hsa-miR-6736-3p	rs139309317	CAGCU[C/T]C	C/T	0	GAGCUGA	3	-0.083
48701379	hsa-miR-4499	rs201540123	AGACU[G/A]A	G/A	1	UCAGUCUA	6	-0.109
48702435	hsa-miR-501-3p	rs149912461	[A/G]UGCACC	A/G	1	GUGCAUA	2	-0.04
48702443	hsa-miR-4296	rs182347826	UGUGG[G/T]C	G/T	0	GCCCACA	7	-0.101
48700902	hsa-miR-33a-3p	rs77809319	A[A/G]UGUUU	A/G	1	AAACAUU	12	-0.102
48702990	hsa-miR-4293	rs12220909	AGC[C/G]UGA	C/G	0	UCAGGCU	19	-0.202
48703130	hsa-miR-6874-3p	rs187466447	[A/G]GUUCUG	A/G	1	AGAACUA	3	-0.233

Table 3. 46 SNPs are creating to new target sits

Target sites created by SNPs and INDELs in miRNA seeds								
Location	**miR ID**	**dbSNP ID**	**miR Seed**	**Allele**	**Wobble base pair**	**miRSite**	**Conservation**	**context+ score change**
48702468	hsa-miR-4301	rs184176277	[C/A]CCACUA	C/A	0	AGUGGUA	13	-0.09
48701404	hsa-miR-6889-5p	rs146254801	C[G/A]GGGAG	G/A	1	CUCCCUG	2	-0.054
48701490	hsa-miR-6787-3p	rs12941300	[C/A]UCAGCU	C/A	0	AGCUGAU	3	-0.031
48701490	hsa-miR-6787-3p	rs149068598	CUCAG[C/A]U	C/A	0	AGCUGAU	3	-0.031
48701637	hsa-miR-550b-3p	rs187080847	CUUA[C/T]UC	C/T	0	AAUAAGA	2	0.04
48702420	hsa-miR-4290	rs182483446	G[C/T]CCUCC	C/T	0	GGAGGAC	11	-0.01
48702660	hsa-miR-412-5p	rs115831106	GGU[C/T]GAC	C/T	0	UCAACCA	19	-0.095
48703023	hsa-miR-6879-3p	rs201379160	GUCA[C/T]CC	C/T	0	AGGUGAC	9	-0.217
48703023	hsa-miR-6879-3p	rs74814065	GUCACC[C/T]	C/T	0	AGGUGAC	9	-0.217
48702210	hsa-miR-3157-5p	rs141770574	U[C/T]AGCCA	C/T	0	GGCUAAA	5	0.004
48702450	hsa-miR-4691-3p	rs181585480	CAG[C/T]CAC	C/T	0	GUGACUG	12	0.009
48702769	hsa-miR-488-5p	rs199722070	CCA[G/A]AUA	G/A	1	UAUUUGG	13	-0.029
48702769	hsa-miR-488-5p	rs186200318	CC[A/G]GAUA	A/G	1	UAUUUGG	13	-0.029
48702396	hsa-miR-6850-3p	rs2955230	C[C/T]GGCCG	C/T	0	GGCCAGA	2	0.007
48701659	hsa-miR-4315	rs143523766	C[G/A]CUUUC	G/A	1	GAAAGCA	2	-0.113
48701659	hsa-miR-4315	rs147167801	[C/T]GCUUUC	C/T	0	GAAAGCA	2	-0.113
48702198	hsa-miR-6746-5p	rs116362087	C[G/A]GGAGA	G/A	1	UCUCCUG	9	-0.068
48702976	hsa-miR-4305	rs138201016	CUAGA[C/A]A	C/A	0	UGUGUAG	6	-0.145
48702976	hsa-miR-4305	rs149597993	CUAG[A/G]CA	A/G	1	UGUGUAG	6	-0.145
48702976	hsa-miR-4305	rs4636784	CUA[G/C]ACA	G/C	0	UGUGUAG	6	-0.145
48702081	hsa-miR-3692-5p	rs185691679	CUGCU[G/A]G	G/A	1	UAGCAGA	3	-0.048
48701522	hsa-miR-1255b-5p	rs189709980	[G/C]GAUGAG	G/C	0	CUCAUAC	2	-0.042
48701522	hsa-miR-1255b-5p	rs139535532	G[G/T]AUGAG	G/T	0	CUCAUAC	2	-0.042
48701919	hsa-miR-1268a	rs28599926	GGGC[G/A]UG	G/A	1	CAUGCCC	8	-0.085
48701917	hsa-miR-1237-5p	rs113909793	GGGGGC[G/A]	G/A	1	UGCCCCC	7	-0.176
48702949	hsa-miR-505-3p	rs143213653	[G/A]UCAACA	G/A	1	UGUUGAU	10	-0.117
48701472	hsa-miR-4477a	rs34026740	UAUUAA[G/A]	G/A	1	UUUAAUA	2	0.218
48702860	hsa-miR-518a-3p	rs200532946	AAAGC[G/A]C	G/A	1	GUGCUUU	14	-0.153
48702860	hsa-miR-518a-3p	rs199969520	AAAGC[G/A]C	G/A	1	GUGCUUU	14	-0.153
48703031	hsa-miR-4538	rs187724662	A[G/C]CUUGG	G/C	0	CAAGGUA	18	-0.128
48700688	hsa-miR-551a	rs187195064	C[G/A]ACCCA	G/A	1	GGGUUGA	9	-0.683
48702323	hsa-miR-3655	rs146400503	CUUGU[C/T]G	C/T	0	CAACAAG	14	-0.046
48700555	hsa-miR-145-3p	rs190323149	GAUU[C/T]CU	C/T	0	AGAAAUCA	11	-0.36
48702880	hsa-miR-5589-5p	rs202101735	GCUGG[G/A]U	G/A	1	UCCAGCA	4	-0.14
48701067	hsa-miR-124-3p	rs34059726	AA[G/T]GCAC	G/T	0	UGCAUUA	9	-0.103
48700732	hsa-miR-6805-5p	rs201627498	AGGGGG[C/T]	C/T	0	ACCCCCU	2	-0.146
48702100	hsa-miR-548ap-3p	rs150751643	AAAAC[C/A]A	C/A	0	UUGUUUUA	7	0.35
48701428	hsa-miR-548t-3p	rs73872515	AAA[A/C]CCA	A/C	0	UGGGUUU	8	-0.048
48702880	hsa-miR-3690	rs183044496	[C/G]CUGGAC	C/G	0	UCCAGCA	4	-0.132
48700583	hsa-miR-6717-5p	rs150596480	GGC[G/A]AUG	G/A	1	AUUGCCA	9	-0.161
48700583	hsa-miR-6717-5p	rs117650137	GG[C/T]GAUG	C/T	0	AUUGCCA	9	-0.161
48702387	hsa-miR-6717-5p	rs150596480	GGC[G/A]AUG	G/A	1	AUCACCA	7	0.008
48702387	hsa-miR-6717-5p	rs117650137	GG[C/T]GAUG	C/T	0	AUCACCA	7	0.008
48702860	hsa-miR-518d-3p	rs73602910	AAAGC[G/A]C	G/A	1	GUGCUUU	14	-0.153
48702980	hsa-miR-4731-3p	rs66507245	ACACA[A/T]G	A/T	0	CAUGUGU	3	-0.168
48702199	hsa-miR-4695-5p	rs79637190	AGGAG[G/A]C	G/A	1	GUCUCCU	8	-0.122

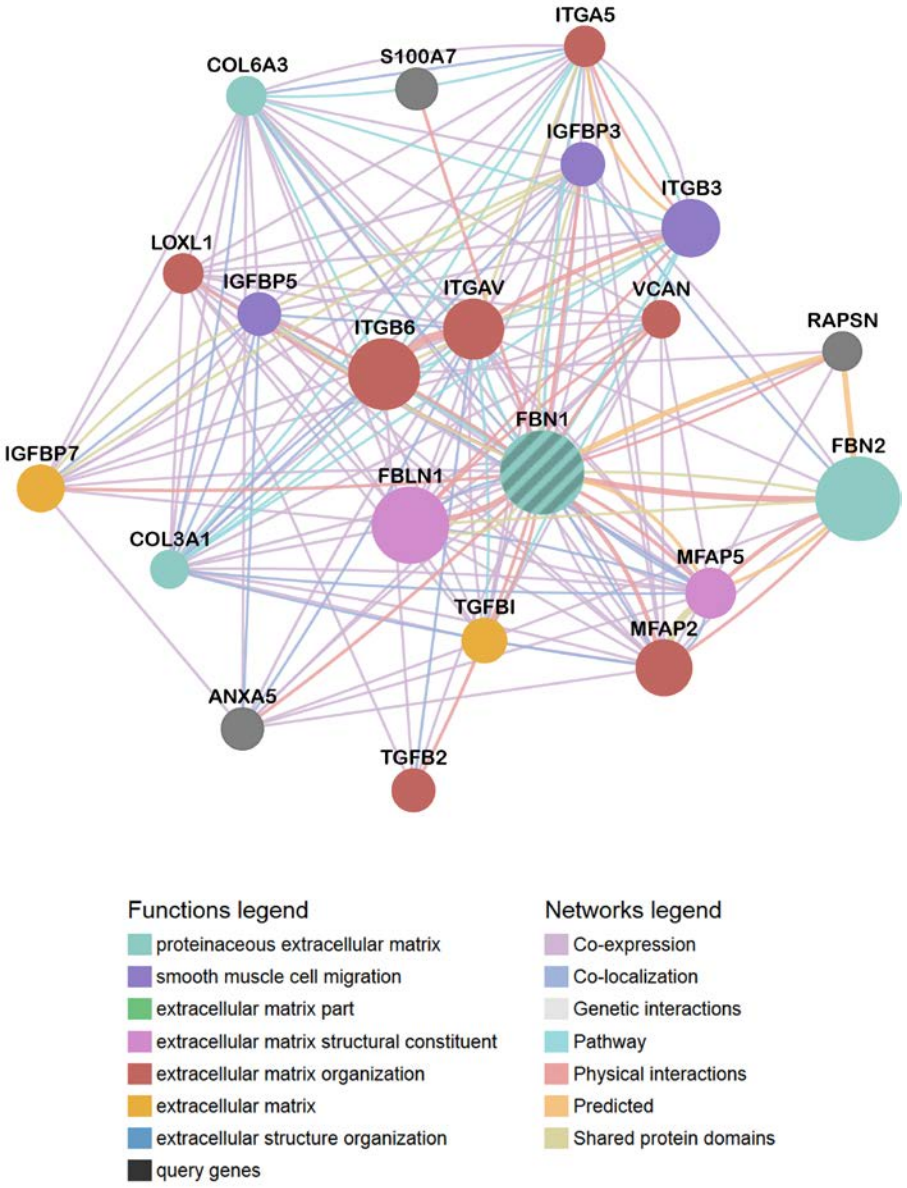


Figure. Shows functions and interaction of FBN1 with other genes predicted using Gene MANIA software [28]

4. Conclusion

This work presenting multiple damaging SNPs that affect FBN1 gene and subsequently cause alteration and truncation of the gene product:using of the damaging ns-SNPs predicted on this work may be helpful in early diagnosis and on screening of FBN1 related disorders which put a highlight to next generation sequencing technology when investigating patients with FBN1 related disorders.

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