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Re-evaluation of a Fibrillin-1 Gene Variant of Uncertain Significance Using the ClinGen Guidelines

Seo Wan Kim, M.D.[1], Boyeon Kim, M.D., Ph.D.[2], Yoonjung Kim, M.D., Ph.D.[2], and Kyung-A Lee, M.D., Ph.D.[2]

[1]Department of Laboratory Medicine, Severance Hospital, Yonsei University College of Medicine, Seoul, Korea; [2]Department of Laboratory Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea

Background: Marfan syndrome (MFS) is caused by fibrillin-1 gene (*FBN1*) variants. Mutational hotspots and/or well-established critical functional domains of *FBN1* include cysteine residues, calcium-binding consensus sequences, and amino acids related to interdomain packaging. Previous guidelines for variant interpretation do not reflect the features of genes or related diseases. Using the Clinical Genome Resource (ClinGen) *FBN1* variant curation expert panel (VCEP), we re-evaluated *FBN1* germline variants reported as variants of uncertain significance (VUSs).

Methods: We re-evaluated 26 VUSs in *FBN1* reported in 161 patients with MFS. We checked the variants in the Human Genome Mutation Database, ClinVar, and VarSome databases and assessed their allele frequencies using the gnomAD database. Patients' clinical information was reviewed.

Results: Four missense variants affecting cysteines (c.460T>C, c.1006T>C, c.5330G>C, and c.8020T>C) were reclassified as likely pathogenic and were assigned PM1_strong or PM1. Two intronic variants were reclassified as benign by granting BA1 (stand-alone). Four missense variants were reclassified as likely benign. BP5 criteria were applied in cases with an alternate molecular basis for disease, one of which (c.7231G>A) was discovered alongside a pathogenic *de novo COL3A1* variant (c.1988G>T, p.Gly633Val).

Conclusions: Considering the high penetrance of *FBN1* variants and clinical variability of MFS, the detection of pathogenic variants is important. The ClinGen *FBN1* VCEP encompasses mutational hotspots and/or well-established critical functional domains and adjusts the criteria specifically for MFS; therefore, it is beneficial not only for identifying pathogenic *FBN1* variants but also for distinguishing these variants from those that cause other connective tissue disorders with overlapping clinical features.

Key Words: ClinGen, Connective tissue, Fibrillin-1, Gene frequency, Marfan syndrome, Penetrance

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Corresponding author:
Kyung-A Lee, M.D., Ph.D.
Department of Laboratory Medicine,
Yonsei University College of Medicine,
211 Eonju-ro, Gangnam-gu, Seoul 06273,
Korea
E-mail: KAL1119@yuhs.ac

INTRODUCTION

Marfan syndrome (MFS) (OMIM #154700) is a multisystem connective tissue disorder caused by fibrillin-1 gene (*FBN1*) variants in an autosomal dominant inheritance manner. Cardinal features occur in the cardiovascular, ocular, and skeletal systems [1]. *FBN1* monomers are cysteine-rich glycoproteins that aggregate to form microfibrils in the extracellular matrix [2]. *FBN1* is composed of 47 epidermal growth factor (EGF)-like domains, seven transforming growth factor β binding (TB) domains,

and two hybrid domains [3]. Among the EGF-like domains, there are 43 calcium-binding EGF (cbEGF)-like domains. All EGF-like (calcium- and non-calcium-binding) and TB domains have six and eight cysteine residues, respectively [4]. *FBN1* variants that affect cysteine residues perturb the intermolecular disulfide bridge, contributing to protein structural instability [5]. Cysteine residues, calcium-binding consensus sequences in cbEGF-like domains, and amino acids related to interdomain packaging are considered mutational hotspots and/or well-established critical functional domains of *FBN1* [6].

Genetic variants are generally classified according to the American College of Medical Genetics and Association for Molecular Pathology (ACMG/AMP) variant interpretation guidelines, which do not consider particular features of genes and gene-related diseases. Therefore, it is recommended to follow disease-specific expert group guidelines, such as those developed by the Clinical Genome Resource (ClinGen) Variant Curation Expert Panel (VCEP) [7]. The first official *FBN1* VCEP guidelines were approved in February 2022 [8].

Genetic variants can be reclassified over time as new disease insights emerge. A change in classification can have a significant impact on the care provided to patients. Using ClinGen *FBN1* expert panel specifications to the ACMG/AMP variant interpretation guidelines, we re-evaluated *FBN1* germline variants previously reported as variants of uncertain significance (VUSs) in patients referred for MFS/marfanoid features or aortic aneurysm/dissection and their family members.

MATERIALS AND METHODS

We re-evaluated 26 VUSs in 161 patients referred to Gangnam Severance Hospital, Seoul, Korea, for *FBN1* testing between March 1, 2013, and March 31, 2022, according to the *FBN1* VCEP guidelines (Fig. 1). We used the Human Genome Mutation Database (HGMD, Professional release 2022.1, Institute of Medical Genetics, Cardiff, UK; https://www.hgmd.cf.ac.uk/ac/index.php) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and VarSome (https://varsome.com/) databases to determine whether the variants had been reported. Allele frequencies in subpopulation groups were investigated using the gnomAD da-

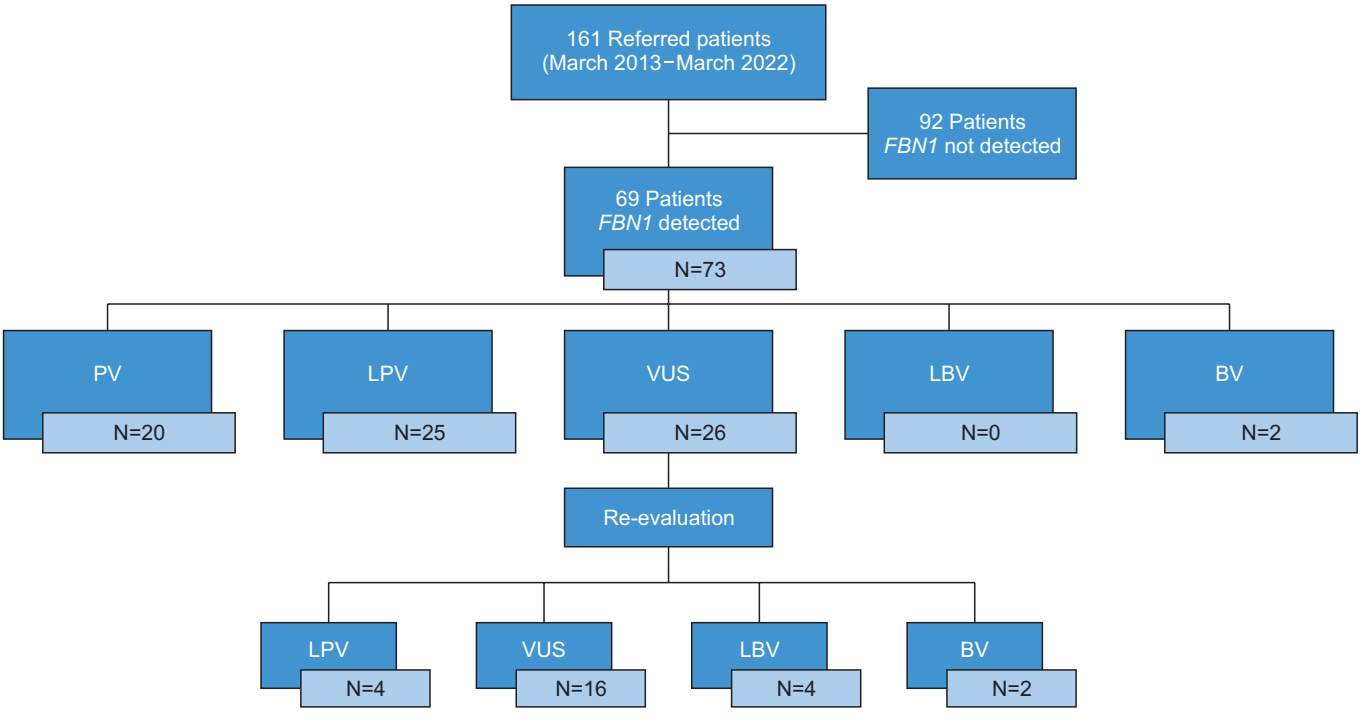

Fig. 1. Classification of reported *FBN1* variants according to the ACMG/AMP guidelines. In this study, 161 patients diagnosed as having MFS, suspected of having MFS (presenting marfanoid features), or with aortic aneurysm/dissection were enrolled. Seventy-three *FBN1* variants were identified in 69 of these patients. The ACMG/AMP guidelines were used to classify the 73 variants as PV, LPV, VUS, LBV, or BV. Among 26 VUSs, 24 different types were reported in 26 patients.
Abbreviations: ACMG/AMP, American College of Medical Genetics and Association for Molecular Pathology; MFS, marfan syndrome; PV, pathogenic variant; LPV, likely pathogenic variant; VUS, variant of uncertain significance; LBV, likely benign variant; BV, benign variant.

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tabase v2.1.1 (https://gnomad.broadinstitute.org/). The Rare Exome Variant Ensemble Learner (REVEL) score was determined by *in silico* analysis [9]. Clinical information, including patients' systemic score, phenotype, ophthalmic records, surgery history, imaging, family history, and pedigree, was investigated. For genetic testing, the patients provided informed written consent for specimen collection and genetic analysis. This study was approved with a waiver of informed consent by the Institutional Review Board (IRB) of Gangnam Severance Hospital (approval number: IRB-3-2023-0030).

RESULTS

Among the 26 re-evaluated VUSs (of 24 types), there were 20 missense variants (76.9%), five intronic variants (19.2%), and one synonymous variant (3.8%). Among the 24 types of VUSs, seven (29.2%) and 11 (45.8%) were reported in HGMD and ClinVar, respectively. Conflicting interpretations of pathogenicity were found for seven variants (c.3043G>A, c.4211-10C>T, c.4313G>A, c.5596A>G, c.6932G>A, c.7231G>A, and c.7241G>A) in ClinVar.

After re-evaluation based on the ClinGen guidelines [8], four missense variants (c.460T>C, c.1006T>C, c.5330G>C, and c.8020T>C) were reclassified as likely pathogenic variants (LPVs). For variant c.460T>C, p.Cys154Arg, the following criteria were assigned: PM1, PM5, PM2_supportive, PP2, and PP3. PM5 was applied because a variant previously reported as an LPV (c.461G>C, p.Cys154Ser; PM1, PS1, PM2_supportive, PP3, and PP2) existed. For c.1006T>C, p.Cys336Arg, PM1, PM5, PM2_supportive, PP2, and PP3 were assigned. PM5 was applied because an LPV (c.1007G>C, p.Cys336Ser; PM1, PM5, PM2_supportive, PP2, and PP3) had been reported. Variant c.1006T>C, p.Cys336Arg was classified as a pathogenic variant (PV; 1-star) and disease-causing mutation (DM) in ClinVar and HGMD, respectively. The c.1007G>C, p.Cys336Ser variant has been reported in aortic dissection (type A case, HGMD accession: CM2115828) [10]. For variant c.5330G>C, p.Cys1777Ser, PM1_strong, PM2_supportive, PP2, and PP3 criteria were assigned. PM1_strong, PM2_supportive, PP2, and PP3 were assigned to the last variant, c.8020T>C, p.Cys2674Arg, which is classified as a PV/LPV (2-star) in ClinVar.

Two (c.1837+35C>G and c.4211-10C>T) and four (c.4313G>A, p.Ser1438Asn; c.6932G>A, p.Arg2311His; c.7231G>A, p.Asp2411Asn; and c.7241G>A, p.Arg2414Gln) variants were reclassified as benign variant (BV) and likely benign variant (LBV), respectively. All six variants were assigned BV/LBV upon adding the BS1 or BA1 criteria according to the allele frequency cut-off specified in the ClinGen guidelines. After re-evaluation, 16 variants remained VUSs (Table 1).

DISCUSSION

The *FBN1* gene has high penetrance in MFS, but there is considerable variability in onset age, tissue distribution, and clinical severity in patients and affected family members [11, 12]. Aortic aneurysms/dissections are the most critical MFS manifestations. Asymptomatic patients or patients with a small aortic diameter can also experience aortic rupture [13]. Owing to this clinical variability and unpredictability, there is significant need for timely detection of pathogenic *FBN1* variants to allow taking preventive measures.

The most remarkable adaptation in the *FBN1* VCEP is PM1. PM1_strong and PM1 are granted according to the type of affected domain or residue. The ClinGen recommendations specify that PM1_strong and PM5 should not be concomitantly applied. Furthermore, the maximum strength of PM2 is limited to the "supportive" level (PM2_supportive). Another noteworthy difference is that PP5 is not applicable. These caveats preclude interpreting *FBN1* variants as being excessively pathogenic.

Two variants reclassified as LPVs (c.460T>C, p.Cys154Arg and c.5330G>C, p.Cys1777Ser) were not specified in any categories of ClinVar or HGMD. Variant c.460T>C, p.Cys154Arg (PM1, PM5, PM2_supportive, PP2, and PP3) was identified in the prenatal amniocentesis sample from a woman married to a man diagnosed as having MFS. This variant substitutes cysteine for arginine in the third EGF-like domain. Another amino-acid change (c.461G>C, p.Cys154Ser) was reported in a 14-yr-old patient with ectopia lentis, dilatation of the ascending aorta, mitral valve prolapse, striae atrophica, multiple skeletal findings (pectus carinatum, reduced upper:lower or increased arm-span:height ratio, joint hypermobility, and high arched palate), dental crowding, and characteristic facial appearance (HGMD accession: CM040031) [14]. This reclassification to LPV has clinical significance in that it can help decision-making on whether to maintain a pregnancy. Variant c.5330G>C, p.Cys1777Ser (PM1_strong, PM2_supportive, PP2, and PP3) was detected in a patient with severe aortic regurgitation due to annuloectasia, dilated left ventricle, chronic dyspnea on exertion, and thumb sign. This variant substitutes cysteine for serine in the 25th cbEGF-like domain. Another amino-acid change (c.5330G>A, p.Cys1777Phe) was reported in a 35-yr-old patient diagnosed as having incomplete MFS with minimal skin, integument, skeletal, and cardiovascular involvements (HGMD acces-

Table 1. Reclassification of VUSs among *FBN1* variants

Case No.	Exon	Nucleotide substitution	Amino acid substitution	ClinVar ID	ClinVar status	ClinVar classification	VarSome classification	HGMD classification (HGMD accession)	Previous evidence by ACMG/AMP guideline	Previous classification	ClinGen guidelines' evidence	Reclassification	PopMax† (%)	REVEL score	Co-occurring LPV/PV
1	1	c.26T>A	p.Ile9Asn	N/A	N/A	N/A	VUS	DM (CM098630)	PM2, PP2, PP4, PP5	VUS	PM2_supportive, PP4, BP4	VUS	N/A	0.1759	-
2	5	c.460T>C	p.Cys154Arg	N/A	N/A	N/A	PV	N/A	PM2, PP2, PP3	VUS	PM1, PM5, PM2_supportive, PP2, PP3	LPV	N/A	0.874	-
3	9	c.1006T>C	p.Cys336Arg	548997	1-star	PV	PV	DM (CM1711819)	PM2, PP2, PP3	VUS	PM1, PM5, PM2_supportive, PP2, PP3	LPV	N/A	0.986	-
4	Intron 14	c.1837+35C>G	p.?	N/A	N/A	N/A	LBV	N/A	BP4	VUS	BA1	BV	0.1355	N/A	-
5	21	c.2579G>A	p.Gly860Glu	N/A	N/A	N/A	VUS	N/A	PP3	VUS	BS1	VUS	0.005438	0.7279	*FBN1* c.7465T>C, p.Cys2489Arg
6	24	c.3043G>A	p.Ala1015Thr	225356	1-star	VUS(3); BV(2)	VUS	DM (CM062715)	BP2	VUS	BS1	VUS	0.02718	0.46	-
7	Intron 33	c.4211-10C>T	p.?	457205	1-star	VUS(1); BV(8)	LBV	N/A	BP4	VUS	BA1	BV	0.2357	N/A	-
8	34	c.4267G>A	p.Ala1423Thr	N/A	N/A	N/A	VUS	N/A	PM2, BP4	VUS	PM2_supportive, BP4	VUS	N/A	0.303	-
9	34	c.4313G>A	p.Ser1438Asn	155792	1-star	VUS(11); LBV(1)	VUS	DM? (CM1712882) [23]	BP5	VUS	BS1, BP5	LBV	0.03007	0.446	*ACTA2* c.773G>A, p.Arg258His
10	37	c.4629C>T	p.Asp1543=	N/A	N/A	N/A	LBV	N/A	PM2, BP7	VUS	PM2_supportive, BP7	VUS	N/A	N/A	*SMAD3* c.1271_1272insAGAC, p.Ser425AspfsTer64
11	43	c.5330G>C	p.Cys1777Ser	N/A	N/A	N/A	PV	N/A	PM2, PP2, PP3	VUS	PM1_strong, PM2_supportive, PP2, PP3	LPV	N/A	0.9649	-
12	45	c.5596A>G	p.Ile1866Val	926451	1-star	VUS(1);LBV(1)	VUS	DM (CM1913014)	-	VUS	BS1	VUS	0.04525	0.3569	*FBN1* c.5728G>T, p.Gly1910Cys
13	45	c.5608G>A	p.Gly1870Arg	N/A	N/A	N/A	LPV	N/A	PM2, PP2, PP3	VUS	PM1, PM2_supportive, PP2, PP3	VUS	N/A	0.9139	-
14	Intron 55	c.6872-14A>G	p.?	418201	1-star	PV(1);LPV(1); VUS(4)	LBV	DM (CS157719)	PM2, PM6_supportive, PP3, PP1	VUS	PM2_supportive	VUS	N/A	N/A	-
15	Intron 55	c.6872-5A>G	p.?	N/A	N/A	N/A	VUS	N/A	PM2, PP3	VUS	PM2_supportive	VUS	N/A	N/A	-
16	56	c.6932G>A	p.Arg2311His	200197	1-star	VUS(2);BV(1); LBV(1)	VUS	N/A	BP4	VUS	BS1, BP4	LBV	0.02008	0.291	-

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Table 1. Continued

Case No.	Exon	Nucleotide substitution	Amino acid substitution	ClinVar ID	ClinVar status	ClinVar classification	VarSome classification	HGMD classification (HGMD accession)	Previous evidence by ACMG/AMP guideline	Previous classification	ClinGen guidelines' evidence	Reclassification	PopMax† (%)	REVEL score	Co-occurring LPV/PV
17	58	c.7231G>A	p.Asp2411Asn	1502613	1-star	VUS(2);LBV(1)	VUS	N/A	-	VUS	BS1	VUS	0.005440	0.493	-
18	58	c.7231G>A	p.Asp2411Asn	1502613	1-star	VUS(2);LBV(1)	VUS	N/A	BP5	VUS	BS1, BP5	LBV	0.005440	0.493	*COL3A1* c.1988G>T, p.Gly663Val
19	58	c.7241G>A	p.Arg2414Gln	161234	1-star	VUS(10);BV(1)	VUS	DM? (CM062706) [31-33]	-	VUS	BS1	VUS	0.01505	0.4499	-
20	58	c.7241G>A	p.Arg2414Gln	161234	1-star	VUS(10);BV(1)	VUS	DM? (CM062706) [31-33]	BP2	VUS	BS1, BP2	LBV	0.01505	0.4499	*FBN1* exon 3–4 deletion (*cis*)
21	60	c.7507A>C	p.Thr2503Pro	1759237	1-star	VUS	LPV	N/A	PM2, PP2, PP3	VUS	PM2_supportive, PP2, PP3	VUS	N/A	0.9279	-
22	62	c.7759G>T	p.Gly2587Cys	N/A	N/A	N/A	LPV	N/A	PM2	VUS	PM2_supportive	VUS	N/A	0.699	-
23	63	c.8020T>C	p.Cys2674Arg	406263	2-star	PV/LPV	PV	N/A	PM2, PP2, PP3, PP5	VUS	PM1_strong, PM2_supportive, PP2, PP3	LPV	N/A	0.9959	-
24	63	c.8042T>A	p.Ile2681Lys	N/A	N/A	N/A	VUS	N/A	PM2, PP2, PP4	VUS	PM2_supportive, PP2, PP4	VUS	N/A	0.5879	-
25	63	c.8044G>T	p.Gly2682Cys	N/A	N/A	N/A	LPV	N/A	PM2, PP2, PP3	VUS	PM2_supportive, PP2, PP3	VUS	N/A	0.924	-
26	Intron 64	c.8226+121A>G	p.?	N/A	N/A	N/A	LBV	N/A	PM2	VUS	PM2_supportive	VUS	N/A	N/A	-

Co-occurring LPVs/PVs (*FBN1*, *ACTA2*, *SMAD3*, and *COL3A1*) are all inherited in an autosomal dominant manner. *ACTA2*: aortic aneurysm, familial thoracic (OMIM #611788) and multisystemic smooth muscle dysfunction syndrome (OMIM #613834); *SMAD3*: Loeys–Dietz syndrome 3 (OMIM #613795); *COL3A1*: Elers–Danlos syndrome, vascular type (OMIM #130050). HGMD variant classes are denoted as follows: DM, disease-causing mutation; DM?, likely pathogenic mutation reported to be disease-causing in the corresponding reports [23, 31-33], but where the author has indicated that there may be some degree of doubt, or subsequent evidence has come to light in the literature, calling the deleterious nature of the variant into question.

†gnomAD subpopulation with the highest allele frequency except for Finnish, Ashkenazi Jewish, or "Other" populations. In missense variants, PP3 and BP4 were granted in cases with REVEL scores of >0.75 and <0.326, respectively.

Abbreviations: HGMD, human genome mutation database; ACMG/AMP, American College of Medical Genetics and Association for Molecular Pathology; REVEL, rare exome variant ensemble learner; N/A, not applicable or no reports; VUS, variant of uncertain significance; LPV, likely pathogenic variant; PV, pathogenic variant; LBV, likely benign variant; BV, benign variant.

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sion: CM074863) [15]. Another amino-acid change c.5330G>A, p.Cys1777Tyr had been classified as an LPV (PM1, PM2, PP2, PP3, and PP5) based on the ACMG/AMP guidelines (HGMD accession: CM205331) [16]. The multitude of reports on the same amino acid indicates the importance of this hotspot.

The two variants reclassified as BVs (c.1837+35C>G, c.4211-10C>T), both intronic, showed the highest allele frequencies in the East Asian subpopulation (c.1837+35C>G; 0.1355% in gnomAD, 0.1044% in Exome Aggregation Consortium [ExAc], c.4211-10C>T; 0.2357% in gnomAD, 0.2892% in ExAc) and were granted BA1 (>0.1% in gnomAD and ExAc). Although ClinVar showed conflicting interpretations of pathogenicity for variant c.4211-10C>T (one VUS and eight BVs), the only report of a VUS was curated in 2018, prior to the publication of the ClinGen guidelines. A considerably larger number of studies reported this variant as benign (ClinVar submitters: Invitae; accession SCV000627906.6, Color Diagnotics, LLC DBA Color Health, accession SCV000903673.1, Illumina Laboratory Services, Illumina, accessions SCV001276079.1, SCV001276081.1, SCV001276080.1, SCV001276083.1, SCV001276084.1, SCV001276085.1).

Four missense variants (c.4313G>A, p.Ser1438Asn; c.6932G>A, p.Arg2311His; c.7231G>A, p.Asp2411Asn; and c.7241G>A, p.Arg2414Gln) were granted BS1 by applying the allele frequency cut-off specified in the ClinGen guidelines (>0.005%). The BP4 criteria were granted to c.6932G>A, p.Arg2311His as the REVEL score (0.291) was below the discriminatory cut-off value (0.326) in the ClinGen guidelines.

The BP5 criteria were applied in two cases that presented an alternate molecular basis for disease and did not show conspicuous features of MFS. In case 9 (c.4313G>A, p.Ser1438Asn), involving a 34-yr-old male patient with chronic type III/B aortic dissection, patent ductus arteriosus (PDA), pulmonary artery hypertension, cardiomegaly, and recurrent occlusive right renal infarctions, a pathogenic missense variant (c.773G>A, p.Arg258His) in the smooth muscle aortic alpha-actin gene (*ACTA2*) co-occurred. Mutations in *ACTA2* are responsible for hereditary thoracic aortic disease (OMIM #611788) and multisystemic smooth muscle dysfunction syndrome (OMIM #613834) [17-20]. The *ACTA2* p.Arg258His variant is classified as PV in ClinVar (2-star) and is closely associated with PDA, pulmonary hypertension, and increased severity of vascular disease, including early-onset stroke [18, 21, 22]. Yet, HGMD and ClinVar refer to this *FBN1* c.4313G>A as DM? (likely pathogenic mutation reported to be disease-causing in the corresponding reports) [23] and as variant that harbor conflicting pathogenicity interpretations (11 VUSs and one LBV), respectively. The patient in case 18 (c.7231G>A, p.Asp2411Asn), who was referred for traumatic thoracic aortic injury with a history of recurrent pneumothorax and hemoptysis, had a *de novo* pathogenic missense variant in *COL3A1* (c.1988G>T, pGly633Val). *COL3A1* is the causative gene of vascular Ehlers–Danlos syndrome (vEDS, OMIM #130050). Affected people are frequently short in stature and typically have fragile blood vessels/organs that easily rupture (e.g., hemoptysis and pneumothorax [collapsed lung]). Another distinctive trait of vEDS is hip dislocation [24-26]. Our patient was short in stature and had acetabular dysplasia in both hips. The *COL3A1* variant p.Gly633Val is classified as LPV/PV in ClinVar (2-star) and has been reported in vEDS patients [27, 28].

A previous study reported cases in two independent families in which *FBN1* and *FBN2* variants co-occurred [29]. In this study, dual variant-carrying individuals, including probands, manifested combinational or synergistic MFS- and congenital contractural arachnodactyly (CCA)-associated features. Additionally, family members who exclusively had *FBN2* LPVs showed a relevant CCA-associated feature. In light of these findings, it is reasonable to regard the exemplified cases as two diseases segregating independently. In our two patients (cases 9 and 18), there was meager evidence suggesting MFS-associated features other than aortic dissection/injury. Instead, certain features associated with the PVs detected in *ACTA2* and *COL3A1* were relatively prominent.

There is a possibility that the MFS-associated phenotype in the proband may be atypical or of late onset, and if a family member with the same *FBN1* variant clearly exhibits the MFS-associated phenotype, there is room for reconsideration regarding the applicability of BP5. Nonetheless, in case 18 (co-occurrence with a *COL3A1* PV), one of the asymptomatic parents (patient's father) carried the same *FBN1* variant, providing a convincing argument for applying BP5. Further familial tests may result in a different interpretation.

Multiple factors can render a variant to be classified as VUS rather than LPV or LBV. The VUS in case 5 (c.2579G>A, p.Gly860Glu) co-occurring with an LPV in *FBN1* (c.7465T>C, p.Cys2489Arg; PM1_strong, PM2_supportive, PP2 and PP3) and the VUS in case 12 (c.5596A>G, p.Ile1866Val) co-occurring with an LPV in *FBN1* (c.5728G>T, p.Gly1910Cys; PM1, PM2_supportive, PP2, PP3, PM6_supportive) are representative examples. The LPV in *FBN1* c.7465T>C, p.Cys2489Arg has been reported in patients with MFS [30] and is classified as PV (1-star) in ClinVar. As the positional relationship (in *cis* or *trans*) between co-occurring LPV/PVs and VUSs is generally undeter-

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mined and there are insufficient data to compare the severity between co-occurring cases and cases with a known pathogenic variant alone, the assignment of BP2 criteria is limited. If the aforementioned information is available, a VUS can be reclassified as an LBV by adding the BP2 criteria. Throughout our examination, we were able to assign BP2 to a single case where co-occurring LPV was found to be *in cis* relationship. An example of this is the *FBN1* c.7241G>A, p.Arg2414Gln (case 20), which is classified as DM? in HGMD [31-33], and as a variant with conflicting interpretations of pathogenicity (10 VUS and one BV) in ClinVar. However, in the case of our patient, his 18-year-old son exhibited a mild MFS phenotype (e.g., tall stature (187cm), scoliosis, and pectus excavatum) and both the *FBN1* c.7241G>A and LPV *FBN1* exon 3-4 deletion were detected by familial test, indicating that they were *in cis* relationship.

Many cases lack pathogenic data, such as information on co-segregation (PP1), detailed review of the phenotype including systemic score and family history (PP4), functional analysis (PS3), and parental genetic analyses (PS2 and PM6, *de novo*). For instance, c.5608G>A, p.Gly1870Arg detected in case 13 may have been elevated to LPV by adding the co-segregation score PP1 if the identical variant had been confirmed in two tall brothers. Functional studies can help strengthen the evidence of pathogenicity in borderline cases [34-36].

To the best of our knowledge, this is the first study to re-evaluate *FBN1* VUSs according to the ClinGen guidelines using data from real patients in a single center. Provided that the prognosis, clinical phenotypes of extra-cardiovascular organs, and locations of aortic involvement differ among similar connective tissue disorders [37], confirming the presence of a pathogenic *FBN1* variant will serve as a starting point for patients' treatment plans and disease prognoses. Accordingly, it is highly recommended to adhere to the ClinGen *FBN1* VCEP.

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AUTHOR CONTRIBUTIONS

Kim SW and Lee KA conceptualized and designed the study. Kim SW collected the data, conducted the evaluation, and wrote the original manuscript. Kim BY and Kim YJ reviewed and commented on the manuscript. Lee KA supervised the study and finalized the manuscript. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST

None declared.

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