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GENETICS, OMICS, AND TISSUE REGENERATION

CASE REPORT: CLINICAL CASE

Comprehensive Genetic Testing for Coexisting Marfan and Loeys-Dietz Syndromes in Hereditary Thoracic Aortic Disease

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ABSTRACT

Hereditary thoracic aortic disease (HTAD) is a rare heritable condition with several subtypes, including Marfan syndrome (MFS), vascular Ehlers-Danlos syndrome, and Loeys-Dietz syndrome (LDS). Although MFS is the most common type of HTAD caused by mutations in *FBN1*, differentiation from other conditions such as LDS is crucial due to the varying clinical courses. We report the case of a family history of early-onset ascending aortic dissection initially diagnosed as MFS based on a pathogenic variant of *FBN1*. However, comprehensive genetic testing using next-generation sequencing and array-comparative genomic hybridization revealed a copy number variation (a large deletion) in *SMAD3*, resulting in a diagnosis of the coexistence of MFS and LDS. These results significantly altered the screening and follow-up strategies. This case highlights the importance of comprehensive genetic testing, not only for assessing single nucleotide polymorphisms but also for evaluating copy number variations to ensure accurate differentiation and management of HTAD. (JACC Case Rep. 2024;29:102731) © 2024 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

HISTORY OF PRESENTATION

The patient was a 42-year-old woman attending our outpatient care clinic without any symptoms. She had visited our department 10 years before her first pregnancy for childbirth management. She is tall, with a height of 178 cm. Her arm span-to-height ratio was 1.06, and the Walker-Murdoch wrist sign was positive. Ectopia lentis was not observed. Echocardiography and enhanced computed tomography (CT) revealed mild aortic root dilation, with a maximum sinus Valsalva diameter of 35 mm (Z-score, 2.0) at the initial visit.

TAKE-HOME MESSAGES

- Multiple diseases can coexist with hereditary thoracic aortic aneurysms and aortic dissections.
- Assessment of copy number variation optimizes the diagnosis of hereditary disease.

FAMILY HISTORY

The family pedigree is shown in **Figure 1**. Her mother (II-4) developed ascending aortic dissection in her 30s and died of subarachnoid hemorrhage at the age of 49 years. Her older sister (III-3) was a 52-year-old woman who was referred to our department at the age of 42 years because of a family history of early-onset

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ABBREVIATIONS AND ACRONYMS

CT = computed tomography

NGS = next-generation sequencing

ascending aortic dissection. She was 169 cm tall, with an arm span-to-height ratio of 1.04 and a positive Walker-Murdoch wrist sign, but no ectopia lentis. Transthoracic echocardiography revealed an ascending aortic diameter of 38 mm (Z-score, 2.9). After this visit, the patient underwent annual follow-ups. At 48 years of age, a follow-up enhanced CT scan revealed a maximum ascending aortic diameter of 45 mm (Z-score, 5.4) (**Figure 2A**, Video 1), and surgery was recommended. However, she developed acute Stanford type A aortic dissection (**Figure 2B**) soon after this recommendation. An emergency Bentall procedure was performed using a mechanical valve with total arch replacement and a coronary artery bypass graft from the aorta to the right coronary artery using the great saphenous vein.

PAST MEDICAL HISTORY

She had no significant medical history and was reportedly not prescribed any medications.

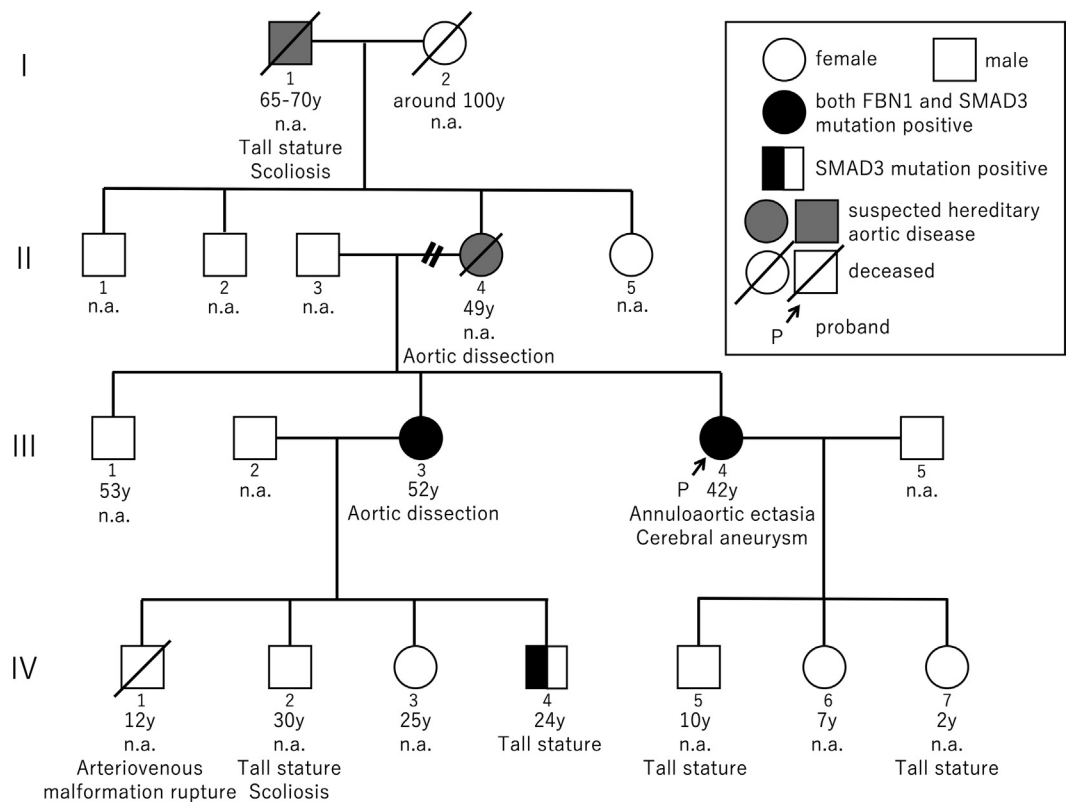
DIFFERENTIAL DIAGNOSIS

Based on her family history of early-onset ascending aortic dissection and physical examination findings, syndromic hereditary thoracic aortic disease (HTAD) including Marfan syndrome (MFS) was strongly suspected.

INVESTIGATION

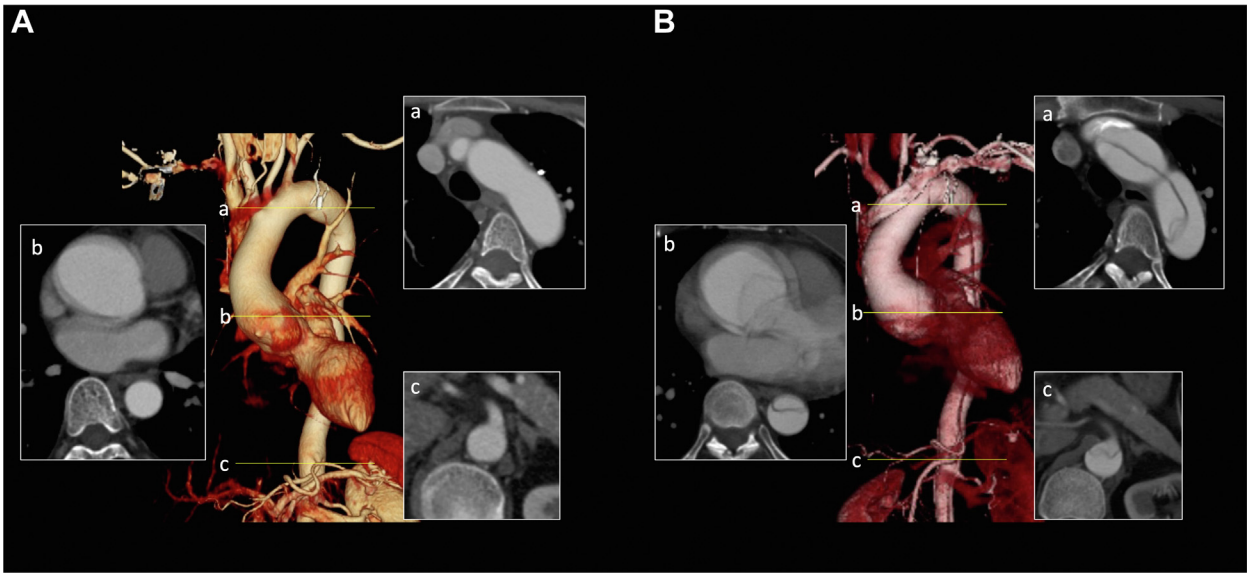
Genetic testing using Sanger sequencing of *FBN1* revealed a variant (c.2364T>G, p.Phe788Leu) (**Figure 3**) that was classified as likely pathogenic

FIGURE 1 Pedigree of the Patient



The patient age and characteristics are indicated below. n.a. = no genomic assessment was performed.

FIGURE 2 **Enhanced CT Scans Before and After Dissection in Patient III-3**



(A) Follow-up enhanced computed tomography (CT) before dissection revealed a maximum ascending aortic diameter of 45 mm, for which surgical intervention was recommended. (B) At the onset of Stanford type A aortic dissection, the dissection extended from the aortic root to the abdominal aorta.

according to the American College of Medical Genetics and Genomics standards and guidelines (PM1 + PP2 + PM2 + PP3).[1] The patient was diagnosed with MFS. It was deemed possible for her to deliver vaginally under pain management; however, her labor was prolonged, and a cesarean delivery was performed. Eventually, she successfully gave birth to a son. She has been followed-up annually and, at the ages of 35 and 40 years, she had one daughter and another daughter, respectively, also via cesarean delivery.

Ten years after the diagnosis of MFS, her nephew (IV-4) also exhibited tall stature and extended upper limbs suggestive of MFS. He exhibited a positive Walker-Murdoch wrist sign, but no ectopia lentis. With the family's consent, we performed targeted next-generation sequencing (NGS) using hybrid capture to analyze *FBN1*, *TGFBR1*, *TGFBR2*, *ACTA2*, *COL3A1*, *EFEMP2*, *FBN2*, *FLNA*, *MYH11*, *MYLK*, *SLC2A10*, *SMAD3*, *TGFB2*, and *TGFB3*; however, no *FBN1* mutation was found. Through a detailed survey using Integrative Genomics Viewer, a tool for the visual exploration of genomic data, a copy number variation (CNV) involving a large deletion encompassing exons 2-9 of *SMAD3*, the causative gene of Loeys-Dietz syndrome (LDS), was suspected (**Figure 4A**). We validated this CNV using array-comparative genomic hybridization (array-CGH) analysis, which revealed a monozygotic deletion in the Chr15q22.33-q23 region encompassing approximately 510-560 kilobase pairs, including *SMAD3* (**Figure 4B**). This led to the definitive diagnosis of LDS. Subsequently, NGS was performed again on the proband (III-5) and her older sister (III-3), confirming both the *FBN1* c.2364T>G mutation and the large deletion of *SMAD3* and resulting in a definitive diagnosis of the coexistence of MFS and LDS.

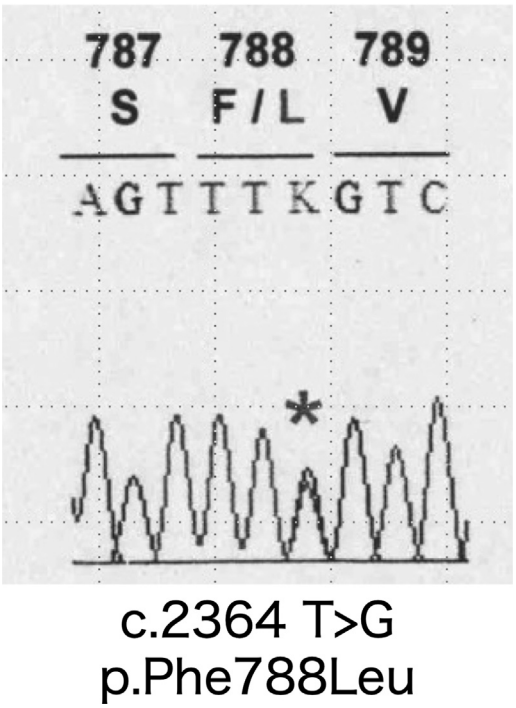
MANAGEMENT AND FOLLOW-UP

Brain magnetic resonance imaging-based screening, as part of the LDS evaluation, revealed multiple cerebral aneurysms (**Figure 5**) in the proband (III-5). Her latest CT scan showed progressive dilation of the sinus of Valsalva to 39 mm (Z-score, 3.2). In cases of double genetic mutations associated with MFS and LDS, the patient is scheduled for stringent follow-up and early invasive interventions.

DISCUSSION

This report describes a patient with a family history of early-onset ascending aortic dissection who was

FIGURE 3 Sanger Sequencing on *FBN1* Gene



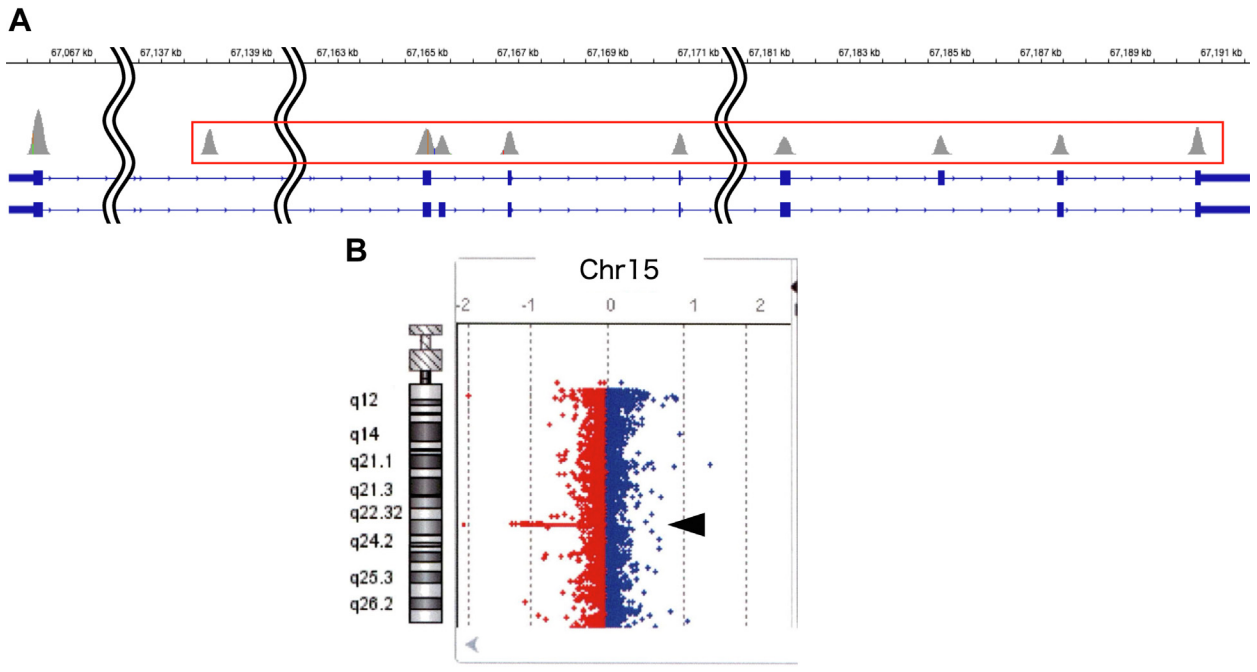
Genetic testing by Sanger sequencing in 2014 revealed an *FBN1* mutation on exon 20 (c.2364T>G, p.Phe788Leu).

definitively diagnosed with coexisting MFS and LDS; the initial diagnosis was MFS based on the identification of a pathogenic variant of *FBN1* using Sanger sequencing. However, further comprehensive genetic testing using NGS and array-CGH analysis revealed an additional large heterozygous deletion in *SMAD3*.

According to a previous report,[2] the risk of aortic dissection in patients with mild aortic dilatation (<45 mm) is low. However, the report also highlighted the difficulty in determining the exact aortic size before dissection. Furthermore, the 2022 American College of Cardiology/American Heart Association guidelines for the diagnosis and management of aortic disease[3] mention the potential of aortic dissection in patients with specific genetic mutations and mild aortic dilatation. Therefore, we highlight the importance of systematic genetic testing, careful follow-up, and elective surgical intervention when appropriate, particularly in patients with mild ascending aortic dilatation and a family history suggestive of HTAD.

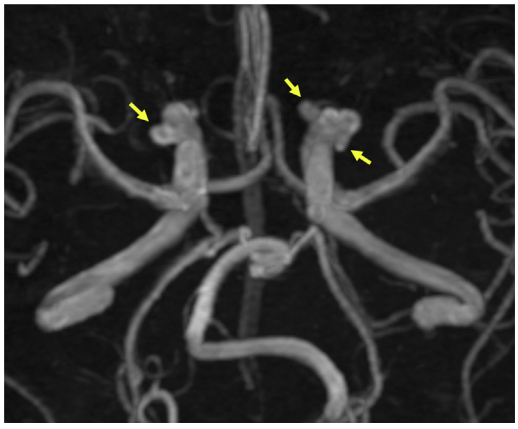
This case report presents 2 additional important insights. First, it revealed that multiple types of HTAD can coexist and that differential diagnosis is clinically important. *FBN1*, which is responsible for MFS, is one of the most commonly mutated genes in hereditary aortic diseases, accounting for more than half of all cases.[4] The current Ghent diagnostic criteria for MFS[5] emphasize aortic root enlargement and ectopia lentis as major manifestations, with significant consideration given to *FBN1* genetic findings. Given the long history of *FBN1* as a well-known gene, Sanger sequencing has been widely used to confirm *FBN1* mutations, leading to the initial diagnosis of MFS in this family. Other HTADs that complicate aortic dissection include syndromic hereditary aortic diseases such as LDS, which is caused by mutations in *TGFBR1*, *TGFBR2*, *SMAD3*, and *TGFB2*, *TGFB3*, and vascular-type Ehlers-Danlos syndrome. Additionally, nonsyndromic hereditary aortic diseases include mutations in genes such as *ACTA2*, *MYH11*, *MYLK*, *LOX*, and *PRKG1*. These diseases exhibit similar phenotypes; however, LDS is suggested to cause aortic dissection earlier than that by MFS, and earlier surgical intervention is recommended.[6] Moreover, MFS is not commonly associated with cerebral aneurysms,[7] whereas LDS is linked to a higher incidence of cerebral aneurysms.[8] Detailed screening of the proband led to the discovery of cerebral aneurysms, emphasizing the need for vigilant follow-up. Furthermore, compared with single gene mutations, double mutations in different genes may result in more severe phenotypes. For example, in familial hypercholesterolemia, double mutations in the low-density lipoprotein (LDL) receptor and proprotein convertase subtilisin/kexin 9 genes lead to a more severe phenotype with elevated LDL cholesterol levels and an increased prevalence of coronary artery disease.[9] In this family, no cases of an isolated *FBN1* gene mutation or individuals older than middle age with a single *SMAD3* mutation have been identified, making it challenging to assess the specific severity. However, the proband's older sister (III-3), who harbored both *FBN1* and *SMAD3* mutations, developed aortic dissection shortly after her ascending aortic

FIGURE 4 Genetic Findings and Array-CGH Analysis



(A) Genetic findings of patient IV-4 using Integrative Genomics Viewer software. The read depth of *SMAD3* exon 2-9 (red square) was approximately 0.5 times shallower than that of other parts of analyzed exons, suggesting copy number variation. (B) Array-comparative genome hybridization analysis revealed a deletion in the Chr15q22.33-q23 region (black arrowhead), encompassing approximately 510-560 kilobase pairs, including *SMAD3*.

FIGURE 5 Cerebral Magnetic Resonance Imaging of Proband (III-5)



Multiple cerebral aneurysms were detected in the cavernous sinus segment of bilateral internal carotid artery (yellow arrows).

diameter reached 45 mm, suggesting a relatively early onset, whether in the context of MFS or LDS. This observation indicates that an earlier intervention is warranted for the proband.

Second, the assessment of CNV in syndromic HTAD is important. With the widespread implementation of NGS-based genetic testing, comprehensive analysis of genes related to HTAD, as mentioned above, has enabled the identification of genetic mutations in more than 80% of cases.[10] However, the commonly used short-read NGS is insufficient for detecting CNVs, and multiplex ligation-dependent probe amplification and array-CGH are considered the gold standards for CNV genotyping. In this case, as previously reported,[11] initiating array-CGH based on a detailed NGS evaluation allowed for the identification of a large heterozygous deletion in *SMAD3*.

CONCLUSIONS

Through detailed genetic testing involving short-read NGS in combination with array-CGH to assess CNVs,

we were able to diagnose a family with concurrent HTAD, MFS and LDS. Comprehensive genotypic evaluation is crucial for predicting patient prognosis and planning follow-up strategies, particularly in patients with suspected HTAD.

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REFERENCES

1. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–424.

2. Paruchuri V, Salhab KF, Kuzmik G, et al. Aortic size distribution in the general population: explaining the size paradox in aortic dissection. *Cardiology*. 2015;131:265–272.

3. Isselbacher EM, Preventza O, Hamilton Black J 3rd, et al. 2022 ACC/AHA guideline for the diagnosis and management of aortic disease: a report of the American Heart Association/American College of Cardiology Joint Committee on Clinical Practice Guidelines. *J Am Coll Cardiol*. 2022;80(24):e223–e393.

4. Akutsu K, Morisaki H, Okajima T, et al. Genetic analysis of young adult patients with aortic disease not fulfilling the diagnostic criteria for Marfan syndrome. *Circ J*. 2010;74:990–997.

5. Loeys BL, Dietz HC, Braverman AC, et al. The revised Ghent nosology for the Marfan syndrome. *J Med Genet*. 2010;47:476–485.

6. MacCarrick G, Black JH 3rd, Bowdin S, et al. Loeys-Dietz syndrome: a primer for diagnosis and management. *Genet Med*. 2014;16:576–587.

7. Vornetti G, De Martino SRM, Baroni MC, et al. Prevalence of unruptured intracranial aneurysms in patients with Marfan syndrome: a cross-sectional study and meta-analysis. *Eur Stroke J*. 2023;8:501–507.

8. Huguenard AL, Johnson GW, Osbun JW, Dacey RG, Braverman AC. Frequency of screening-detected intracranial aneurysms in patients with Loeys-Dietz syndrome. *Circulation*. 2022;146:142–143.

9. Ohta N, Hori M, Takahashi A, et al. Proprotein convertase subtilisin/kexin 9 V4I variant with LDLR mutations modifies the phenotype of familial hypercholesterolemia. *J Clin Lipidol*. 2016;10:547–555.e5.

10. Baetens M, Van Laer L, De Leeneer K, et al. Applying massive parallel sequencing to molecular diagnosis of Marfan and Loeys-Dietz syndromes. *Hum Mutat*. 2011;32:1053–1062.

11. Takeda N, Inuzuka R, Yagi H, et al. Clinical impact of copy number variation on the genetic diagnosis of syndromic aortopathies. *Circ Genom Precis Med*. 2021;14:e003458.

KEY WORDS aorta, cardiovascular disease, dissection, genetic disorders, genetics, vascular disease

APPENDIX For a supplemental video, please see the online version of this paper.