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Case Report

Genome Sequencing Readily Detects a Copy Number Variant in *FBN1* in a Patient with Marfan Syndrome

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Marfan syndrome is characterized by musculoskeletal, ocular, and cardiovascular findings and results from heterozygous disease-causing variants in *FBN1* (*fibrillin-1*). Given that the majority of pathogenic variants in *FBN1* are single-nucleotide variants (SNVs) or variants smaller than 50 base pairs, routine clinical genetic testing is optimized for these variant types. Here, we present an individual with a clinical diagnosis of Marfan syndrome, in whom routine commercial testing was nondiagnostic. Genome sequencing (GS) revealed a novel heterozygous deletion of exons 36-38 in *FBN1*, highlighting the utility of GS in detecting rare complex variants in individuals who remain unsolved following routine clinical testing.

Novel Teaching Points

- Up to 5% of Marfan syndrome cases are caused by copy number or structural variants impacting *FBN1*.
- Routine clinical genetic testing can have limited ability to detect CNVs or structural variants in *FBN1*.
- Offering GS as a first-tier test will have the effect of reducing the time to diagnosis for many patients with Marfan syndrome, due to its ability to detect complex variants.

The female patient was first assessed at age 7 years, with a history of severe high myopia, aortic root dilatation (29 mm; Z-score +4.7), pectus carinatum, and reduced elbow extension. Her presentation was somewhat confounded by macrocephaly (+3.6 standard deviations), contractures of the hips, knees, ankles, and fingers, and mild, chronic CK (creatine kinase) elevation. Over time, she developed bilateral ectopia lentis, which allowed for a clinical diagnosis of Marfan syndrome using the Revised Ghent Nosology. The family history was noncontributory, and her previously healthy father was deceased due to nonmedical reasons (Fig. 1A). Clinical molecular testing consisted of a large multigene aortopathy panel and duo exome sequencing performed by Blueprint Genetics (Whole Exome Family Plus Test, v2), both of which were reported as negative. This prompted enrollment in a local GS initiative.

After the patient consented to be enrolled into the Care 4 Rare Solve project and genomic DNA was extracted from whole blood, duo polymerase chain reaction–free GS was performed on the Illumina NovaSeq 6000 platform and analyzed with the Illumina TruSight Software Suite (version 2.5.0, San Diego, CA) variant calling pipeline, which used DRAGEN (version 3.7.5). Variants were filtered and prioritized based on frequency in population databases, inheritance, pathogenicity, and relatedness to patient's phenotype, and they were classified following American College of Medical Genetics and Genomics (ACMG) guidelines. This analysis revealed a heterozygous 4773 base pair deletion on chromosome 15 from position g.48758368_48763141 in the proband, but not in her unaffected mother (Fig. 1B). This variant overlaps exons 36-38 out of a total of 66 exons of *FBN1* (NM_000138.4) and is predicted to result in the in-frame deletion of 135 amino acids (p.(Asp1446_Ser1583del)), which encodes the calcium-binding epidermal growth factor domains 22 and 23. This deletion is novel in GnomAD (v4.0) and DGV and has not been reported to date in the literature or ClinVar. The presence of this deletion was confirmed in the proband by breakpoint polymerase chain reaction (primer_

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F_common 5′-AGTTGTTACTCTGCCCGTCTG -3′, primer_R_deletion 5′-GGTGGAGTGGTCATGGCTATTTA-3′, primer_R_wildtype 5′-ATCACCGTCCTGGTGCATAC -3′) and was absent from all other familial samples tested, following recruitment of the paternal grandparents into the study (Fig. 1C). These results suggest that this deletion was *de novo* in the proband or was inherited from her father, who was not known to be clinically affected. Therefore, the American College of Medical Genetics and Genomics codes PVS1_strong (in-frame loss-of-function variant), PM2_supporting (absent from population databases), and PP4 (phenotype highly specific) were applied to this variant, for a final classification of Likely Pathogenic.[1]

Discussion

Marfan syndrome is caused by pathogenic variants in *FBN1* (*fibrillin-1*), which encodes a cysteine-rich glycoprotein that functions as a structural protein within the extracellular matrix, providing connective tissue support throughout the body. Upwards of 60% of missense variants occur within the calcium-binding epidermal growth factor-like domains, with 63% of these being variants that substitute conserved cysteine residues, highlighting the importance of these residues in the function of *FBN1*.

Given that SNVs and indels smaller than 50 base pairs in *FBN1* are the most frequent causes of Marfan syndrome, routine testing typically consists of a multi-gene next-generation sequencing (NGS) panel, thereby maximizing the detection of these types of variants. The genes associated with Loeys-Dietz syndrome are typically part of these panels, as 5%-10% of individuals who meet the Ghent criteria for Marfan syndrome may carry variants in *TGFB2*, *TGFBR2*, *TGFBR3,* or *SMAD3*.[2-4] However, targeted gene panels are ill-suited for the detection of copy number variants (CNVs) for a number of reasons. For example, given that intronic regions are not covered by gene panels, many bioinformatic algorithms such as paired-end mapping and split-read detection will miss any CNVs with breakpoints located within introns. In addition, non-

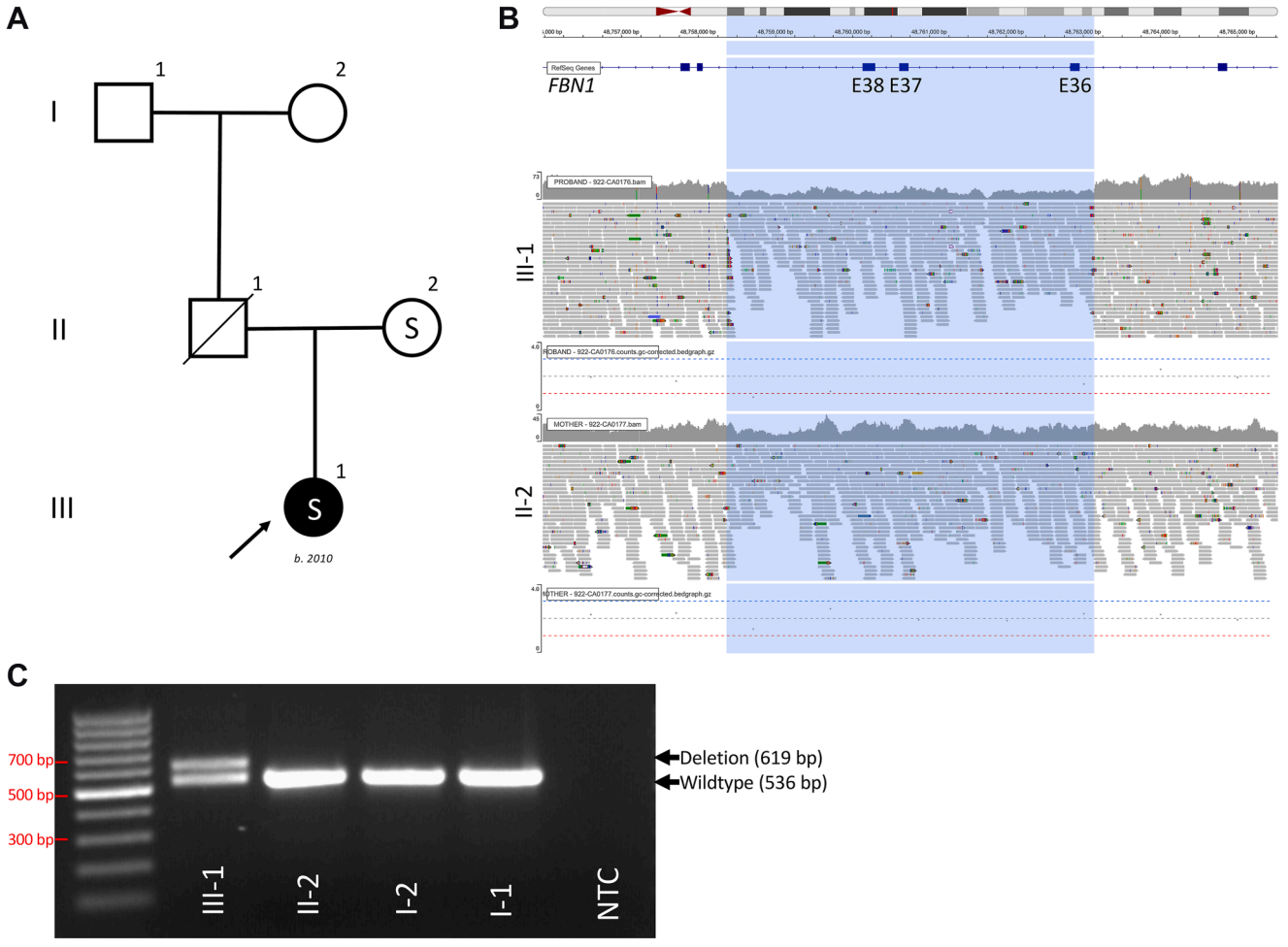

Figure 1. (**A**) Pedigree of the affected family. **Black filled symbols** indicate clinically affected individuals, whereas **open symbols** indicate unaffected individuals. Symbols marked with an "S" indicate individuals who were submitted for whole-genome sequencing. The proband is indicated with an **arrow**. (**B**) Identification of copy number variant. Integrated Genome Viewer (IGV) illustrating the read alignment and coverage tracks at 15q21.1 following duo short-read whole-genome sequencing. A heterozygous copy number deletion encompassing portions of the RefSeq gene *FBN1* (NM_000138.4) was identified in individual III-1 (**blue highlighted region**), whereas individual II-2 had normal dosage in this region. (**C**) Validation of copy number variant in family members. Allele-specific polymerase chain reaction electrophoresis confirmed the presence of the deletion in III-1 and the absence of the variant in unaffected family members (I-1, I-2, and II-2). bp, base pair.

Table 1. Utility of various methodologies used for copy number variant detection

Technology	Resolution	Clinical utility	Advantages	Limitations
MLPA	Single exon	Targeted gene	Phenotype-driven High-throughput Relatively quick Useful for pseudogenes	Precise breakpoints not known
CMA	> 50,000 bp	Genome-wide assay	Wide search space Can detect AOH	Cannot detect balanced rearrangements Precise breakpoints not known Limited utility for some clinical indications Increased risk of VUSs
Gene panels	Variable	Targeted gene(s)	Phenotype-driven	Precise breakpoints not known Increased risk of VUSs
Short-read GS	> 1 bp	Genome-wide assay	Wide search space Breakpoints known Robust bioinformatics Can detect AOH	Some SVs challenging to detect with current bioinformatic tools Increased risk of VUSs and secondary findings
Long-read GS	> 1 bp	Genome-wide assay	Wide search space Can detect methylation Easier to detect SVs Breakpoints known Phasing Easier to sequence repetitive regions Can detect AOH	Not routinely used clinically Limited bioinformatic tools Higher costs (currently) Requires high-quality DNA Increased risk of VUSs and secondary findings
Optical genome mapping	> 500 bp	Genome-wide assay	Wide search space Easier to detect SVs	Precise breakpoints not known Not routinely used clinically DNA treatment considerations Increased risk of VUSs

AOH, absence of heterozygosity; bp, base pair; CMA, chromosomal microarray; GS, genome sequencing; MLPA, multiplex ligation-dependent probe amplification; SV, structural variant; VUS, variant of uncertain significance.

uniform coverage among targeted regions can confound bioinformatic tools relying upon read depth to determine copy number, which is especially true for small CNVs involving only a single or partial exon. Ultimately, this means that a diagnosis may be missed in up to 5% of individuals with Marfan syndrome who have a CNV either partially or wholly impacting *FBN1*.[3,4] With that in mind, methods that are able to detect CNVs greater than 50 base pairs in size should be considered in cases of Marfan syndrome for which routine panel testing is nondiagnostic (Table 1).

Multiplex ligation-dependent probe amplification (MLPA) has proven to be an effective means of detecting CNVs impacting exonic regions, but it is unable to delineate breakpoints, and many clinical labs do not have kits readily available for genes for which CNVs are not a common mechanism of disease. Similarly, chromosomal microarray (CMA) is capable of detecting genomic imbalances, but intragenic CNVs, such as the one identified in this case, would remain undetected due to the lower resolution of CMA. In addition, aortopathies would not meet the criteria for CMA testing in many centres. On the other hand, GS is a feasible alternative methodology that is becoming increasingly accessible, and as illustrated in this case, it is capable of detecting a relatively small multi-exon heterozygous deletion in *FBN1* in an individual clinically diagnosed with Marfan syndrome. Indeed, as costs for GS decrease, and bioinformatic tools improve, its utility as a first-tier testing option in patients diagnosed with clinically homogeneous diseases will increase. This is particularly true given its ability to detect the full spectrum of causative variants without requiring extensive orthogonal or reflex testing, including even more complex variants, such as copy neutral inversion events, the likes of which have been detected recently in a family with Marfan syndrome.[5] In centres where this approach may be cost prohibitive, we minimally recommend GS as a second-tier test in the approximately 5% of Marfan cases that remain undiagnosed following alternative testing strategies.

In summary, we report the detection of a novel CNV in *FBN1* using GS, in an individual clinically diagnosed with Marfan syndrome. The broader implementation of GS as a first-tier test should ultimately reduce the time to diagnosis for many patients, as demonstrated in the resolution of our patient's 6-year diagnostic odyssey.

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Data Availability

Care4Rare Canada deposits multi-omic data in a national platform, Genomics4RD (genomics4rd.ca), to facilitate data sharing. Phenotypic and DNA/RNA sequencing data from this family are made available through a controlled access request to Genomics4RD (genomics4rd@cheo.on.ca).

Ethics Statement

This study has been approved by the Conjoint Health Research Ethics Board (CHREB) (REB18-1486).

Patient Consent

The authors confirm that a patient consent form has been obtained for this article.

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Disclosures

The authors have no conflicts of interest to disclose.

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