Hungarian Marfan family with large $FBN1$ deletion calls attention to copy number variation detection in the current NGS era

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Abstract: Copy number variations (CNVs) comprise about 10% of reported disease-causing mutations in Mendelian disorders. Nevertheless, pathogenic CNVs may have been under-detected due to the lack or insufficient use of appropriate detection methods. In this report, on the example of the diagnostic odyssey of a patient with Marfan syndrome (MFS) harboring a hitherto unreported 32-kb $FBN1$ deletion, we highlight the need for and the feasibility of testing for CNVs (>1 kb) in Mendelian disorders in the current next-generation sequencing (NGS) era.

Keywords: Copy number variations (CNVs); genetic testing; Marfan syndrome (MFS); $FBN1$ gene; whole-genome sequencing (WGS)

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Introduction

High-throughput next-generation sequencing (NGS) of targeted gene panels, the whole exome or even the whole genome is widely and increasingly used in the molecular diagnostics of Mendelian disorders. Standard NGS analysis pipelines are often restricted to the calling of single nucleotide variants (SNVs) and small insertions/deletions (indels), thereby missing copy number variations (CNVs) including deletions and duplications affecting more than 20 bp (1). However, pathogenic CNVs account for about 10% of disease-causing variants listed in the Human Gene Mutation Database (HGMD Professional 2018.1) and thus should not be missed. We believe that disease-causing CNVs tend to be under-detected in the current NGS era. In this study, we provide an example of this assumption. We investigated a family in which three members are clinically diagnosed with Marfan syndrome (MFS). Our results emphasize the need for the widespread use of NGS data to test for CNVs.

MFS is an autosomal dominant, systemic connective tissue disorder with varying clinical features, ranging from isolated traits to severe multiorgan manifestations, most frequently including the skeletal, ocular, and cardiovascular systems (2). Cardiovascular abnormalities can be life-threatening and include dilatation of the ascending aorta, which can result in dissection, mitral valve prolapse with or without regurgitation as well as tricuspid valve prolapse (3-6). MFS is caused by heterozygous pathogenic sequence variants in the $FBN1$ gene, which consists of 65 coding exons and encodes the protein fibrillin-1, a key component of elastic fibers (7,8). Pathogenic $FBN1$ sequence variants have been detected in >90% of patients fulfilling the diagnostic criteria for MFS (2). In the remaining cases, the
disease-causing FBN1 mutation may have been missed by the applied genetic testing method or the clinical phenotype belongs to a non-FBN1-related syndrome. Indeed, there are MFS-related disorders with overlapping features such as Loey-Dietz syndrome or Ehlers-Danlos syndrome vascular type, which in the absence of MFS-discriminating clinical features such as ectopia lentis can only be distinguished by genetic testing (2). As disease management and treatment guidelines differ among MFS and its related syndromes, the correct diagnosis is of clinical importance.

Clinical descriptions

In the investigated family, the index patient is a 32-year-old woman with clinically diagnosed MFS fulfilling the criteria of the revised Ghent nosology (2). She is 188 cm tall with a 195 cm arm span resulting in an arm span to height ratio of >1.05. Her joints are hypermobile and she shows positive wrist and thumb signs as well as scoliosis >20°. Other systemic manifestations include pes planus, high-arched palate with crowded teeth, atrophic striae, retrogrenathia, and myopia >3 dipters, resulting in a systemic score of 9. Her cardiovascular condition is also characteristic for MFS: according to echocardiography, the diameter of the ascending aorta, the sinus of Valsalva, and the aortic root were 46, 53 and 24 mm, respectively, resulting in a Z-score of 6.28 (9). In addition, mitral valve prolapse was detected, but without any hemodynamically relevant effect. According to international guidelines on the management of valvular heart disease (10), prophylactic Bentall surgery was carried out with implantation of Bio Valsalva.

Both the patient’s mother and sister show similar characteristics with a systemic score of 9 as well and conspicuous cardiovascular manifestations. The patient’s father does not fulfill the diagnostic criteria for MFS, but shares some MFS features such as reduced upper segment to lower segment and increased arm span to height ratios, tall stature, hypermobile joints, and dolichocephaly. Some phenotypic characteristics of the patient’s deceased grandparents are also known, of which the patient’s maternal grandfather showed myopia >3 dipters and a mild mitral valve prolapse. The other grandparents’ known phenotypes are not related to MFS.

Molecular genetic testing

The index patient underwent genetic testing for MFS and related cardiovascular diseases on genomic DNA level. First, NGS of a targeted gene panel was performed by means of PCR amplification of all coding exons and flanking intronic regions of the genes FBN1, TGFB1, TGFB2, SMAD3, TGFB2, TGFB3, ACTA2, COL3A1, MYH11, and SKI. Amplicons were analyzed on a MiSeq Personal Sequencer (Illumina, San Diego, CA, USA) (11). This approach revealed no pathogenic sequence variants in the analyzed amplicons. As gene panel NGS is restricted to selected amplicons/genes and limited in the detection of deletions and duplications affecting more than 20 bp (1), we subsequently carried out 60x PE150 PCR-free whole-genome sequencing (WGS) on a HiSeq X Ten platform (Illumina, San Diego, CA, USA) as previously described (12). WGS can not only identify sequence variants throughout the genome but has also the advantage of the most continuous coverage, improving both SNV and CNV detections (1).

Based on WGS, no pathogenic SNVs and small indels were detected in the ten previously analyzed genes or further genes related to the patient’s clinical features including ADAMTS14, BGN, CBS, COL1A1, COL1A2, COL4A5, COL5A1, COL5A2, COL9A1, COL9A2, COL11A1, COL11A2, EFEMP2, ELN, FBN2, FLNA, FOXE3, GLA, JAG1, LOX, LTBP2, MAT2A, MED12, MFAP5, MYLK, NOTCH1, PLOD1, PROK1, PTEN, SLC2A10, SMAD2, SMAD4, and TNXB. Subsequently, the WGS data were analyzed for CNVs using the software Nexus Copy Number (BioDiscovery, El Segundo, CA, USA), revealing a 31,956-bp deletion of the FBN1 gene (NM_000138.4:c.164+13846_442+1334del). Due to the advantage of WGS, the deletion breakpoints could be identified at base-pair level. The deletion was confirmed by multiplex ligation-dependent probe amplification (MLPA, P065/P066, MRC-Holland, Amsterdam, the Netherlands) (13,14) as well as by standard PCR with a 407-bp amplicon spanning the deletion breakpoints followed by Sanger sequencing (primer_F 5’-AGGAACGATTTGAACAGATATAGT-3’, primer_R 5’-CAGCAAACGACTTGCTATAGT-3’) (Figure 1). At protein level, the deletion of coding exons 2-4 is predicted to lead to a frameshift and a premature termination codon [NP_000129.3:p.(Pro56Cysfs*3)]. Thus, nonsense mediated mRNA decay may take place resulting in functional haploinsufficiency (15). Testing of the index patient’s first-degree relatives for the detected FBN1 deletion by MLPA and Sanger sequencing revealed that the deletion is present in the patient’s sister and mother but absent in the father, as expected based on clinical features (Figure 2).
Discussion

This work exemplifies the importance of testing for CNVs in Mendelian disorders because they may be missed by standard NGS mutation screening. One of the advantages of an appropriate NGS methodology is that it allows not only the detection of SNVs and small indels but also of CNVs in a single assay. However, not all NGS applications are equally suited for CNV detection (1,16). While PCR-free WGS data facilitates CNV detection and enables CNV characterization at base-pair level due to relatively even read depth and continuous coverage (1,12), the
enrichment bias and gaps of whole-exome sequencing (1,17) or targeted sequencing data introduce variable read depth and uneven coverage, hampering CNV detection (Figure 3). Consequently, CNV detection should be considered in the choice and data analysis pipeline of comprehensive NGS.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: Ethical approval for this study was obtained from the Scientific and Research Ethical Committee of the Medical Research Council of Hungary (ETT-TUKEB, 12751-3/2017-EKU). Written informed consent was obtained from the patient for publication of this manuscript and any accompanying images.

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