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CASE REPORT

An integration into the diagnostic workflow in a pediatric patient suspected of having Marfan syndrome

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Abstract

Background: The genetic approach to Marfan syndrome (MFS) has evolved over the last few decades, as has our understanding of the variants' potential structural and functional consequences. It has been proposed that next-generation sequencing be used to improve genetic diagnosis and patient management. To this end, we used a targeted NGS custom panel to perform genetic analysis in a patient with MFS and his or her family members.

Case presentation: Here, we describe a novel germ-line heterozygous missense variant (transversion c.5371 T > A) found in exon 43 of the FBN1 gene of a patient (proband) with MFS. *FBN1* (ENSG0000166147) and *TGFB2* (ENSG0000166147) were included in a targeted sequencing panel for MFS (ENSG0000163513). This new variant c.5371 T > A was identified only in the proband, not in unaffected family members or healthy controls.

Conclusions: Given the massive amount of data generated by gene panel analysis, clinical interpretation of genetic variants may become more difficult. As a result, 3D modeling and multidisciplinary approaches should be used in the analysis and annotation of observed variants. The analysis of the protein's conformational structure in relation to the identified variant could provide useful information. These data can be used to classify observed variants (pathogenic vs non-pathogenic) linked to the MFS phenotype, as well as to track disease progression and potential target treatments.

Keyword: *FBN1* gene, Marfan syndrome, New pathogenic variant, 3D modeling

Background

Marfan syndrome (OMIM 154,700) is a genetic connective tissue disorder with an autosomal dominant inheritance pattern that affects the ocular, skeletal, and cardiovascular systems (prevalence 2–3/10000). [1] MFS is caused by pathogenic variants in the *FBN1* gene (OMIM 134,797), which alter the FBN1 protein and cause misfolding. [2, 3]

The *FBN1* gene is found on the long arm of chromosome 15 (15q21.1) and is relatively large (237.5 kb), with 65 exons in its coding sequence. [4] *FBN1* encodes profibrillin, which is converted to asprosin and fibrillin-1, a large glycoprotein of about 350 kDa (2871 amino acids) with multiple functional domains called fibrillin-1, which consists primarily of 6-cysteine epidermal growth factor (EGF)-like and calcium-binding EGF motifs, with a 8-cysteine motifs (TGF-beta binding/8-Cys) intermixed. [5] Different pathogenic variants have been identified in association with MFS, which have almost complete penetrance but phenotypic variability both within and between families. Almost 2000 pathogenic variants (missense, alternative splicing, out-of-frame deletions and

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deletions) are involved in defective protein folding, secretion, assembly, and degradation, resulting in weakened connective tissues. [4, 6] Furthermore, deletions of the entire gene have been reported, resulting in a decrease in the amount of protein produced. [7]

In subjects with MFS, fibrillin-1 levels are lower in tissues like blood vessels, lungs, skin, and ocular ligaments, which contributes to the functional reduction of microfibrils. [8]

Even within families, MFS clinical variability is noticeable in terms of age of onset, severity, and number of clinical manifestations. Except for neonatal forms of MFS [9, 10] and truncating variants with a more severe aortic phenotype, no strong genotype–phenotype correlation between MFS and FBN1 variants has been reported to date. [11, 12]

Pathogenic variants in the *FBN1* gene have been reported in a variety of clinically distinct syndromes, including Weill–Marchesani syndrome, Shprintzen–Goldberg syndrome, acromelic dysplasia associated with family aortic aneurysms, and a wide range of skeletal, connective, and cutaneous tissue anomalies [13]. It is becoming clear that the expanded concept of fibrillinopathies entails complex mechanisms, such as tissue-specific fibrillin microfibril microenvironments, collaborative relationships between the structures of fibrillin microfibril networks, and biological functions like growth factor signaling regulation [14] . Here, we report on a novel germ-line heterozygous missense variant (transversion c.5371 T > A) identified in exon 43 of the *FBN1* gene of patient (proband) with clinical suspect of MFS.

The new variant was classified as PM5, PP2, PP3 BP4, PP4 based on the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG) guidelines, as well as the fact that four other causative variants have been described in the same position [15]. By comparing the effects of all amino acids in the 1791codon (neutral variants, SNPs, and non-synonymous variants) and a 3D protein structure using a comparative approach of all known variants, bioinformatics analysis was carried out.

A 3-dimensional modeling was performed because this variant was not previously reported, as well as changes on the molecular level—the loss of disulfide bridges of the EGF-like 29 domain between TB7 and TB8, (TB8). Based on the results of 3D modeling and genetic and phenotypic data, this variant is strongly indicated as a fully causative pathogenetic variant.

Case presentation

Our Medical Genetics Unit received a referral for a 10-year-old female patient who was suspected of having MFS. MFS was not present in the family. She was the second child of non-consanguineous parents and was born after a physiological pregnancy via spontaneous delivery. During the neonatal and infant period, no clinical problems were referred. She was diagnosed with juvenile idiopathic scoliosis at the age of six and treated with a Milwaukee brace. Furthermore, at the age of six, 2D transthoracic echocardiography revealed mitral valve prolapse and a normal aortic root diameter. When the patient was 10, a general examination revealed a marfanoid habitus with slender limbs and a thin body (BMI < 3°ct). She was measured at 159 cm in height (75rd–90th percentile), 27.5 kg in weight (3rd–10th percentile), and 53.5 cm in occipital frontal circumference (OFC) (50th percentile). She showed an arm span-to-height ratio (L/D) of 1.053, chest deformity (pectus excavatum), kyphoscoliosis, and plain flat feet (pes planus). Additionally, the patient showed a positive wrist (Walker's sign) and thumb (Steinberg) sign. She had a dolichofacial shape, a high-arched palate, and a malocclusion of the teeth. Out of an unconfirmed TSH increase, laboratory tests were within normal limits (complete blood count, urea and electrolytes, calcium, magnesium, and phosphate, liver function tests, creatine kinase, homocysteine). Her total systemic score was 9 based on the revised Ghent nosology, which took into account the previously noted mitral valve prolapse. At the age of ten, a new transthoracic echocardiography revealed the prolapse of both mitral valve leaflets with mild mitral regurgitation, as well as a 25-mm aortic root with a Z-Score of 1.78. At the age of ten, an ophthalmology examination revealed mild bilateral astigmatism (< 1 diopter) as well as a "slit lamp" evaluation. Based on anamnestic data, pre-test genetic counseling, objective examination (based on the presence of a systemic score > 7), and consideration of all possible differential diagnoses, including MFS, a genetic test was performed using a targeted sequencing panel that included two genes that predispose to MFS, *FBN1* and *TGFBR2*. After obtaining informed consent, genomic DNA from three members of the family was extracted. All 65 coding exons and flanking intronic regions including splice sites of *FBN1* were analyzed using a specific targeted sequencing panel designed to include two genes predisposing to MFS, *FBN1* (ENSG0000166147) and *TGFB2* (ENSG0000163513).

The variant was confirmed by bidirectional sequencing (Fig. 1), and the prediction of the functional effect of the c.5371 T > A variant on the *FBN1* gene was performed using the "SNAP2" a trained classifier based on a neural network tool (https://www.rostlab.org/services/snap/). SNAP2 produce a heatmap of prediction scores effect of each variant from the wild-type amino acid (*x*-axis) to any other amino acid (*y*-axis) (Fig. 2). The scores are reported in Table 1 [15]. Furthermore, in consideration
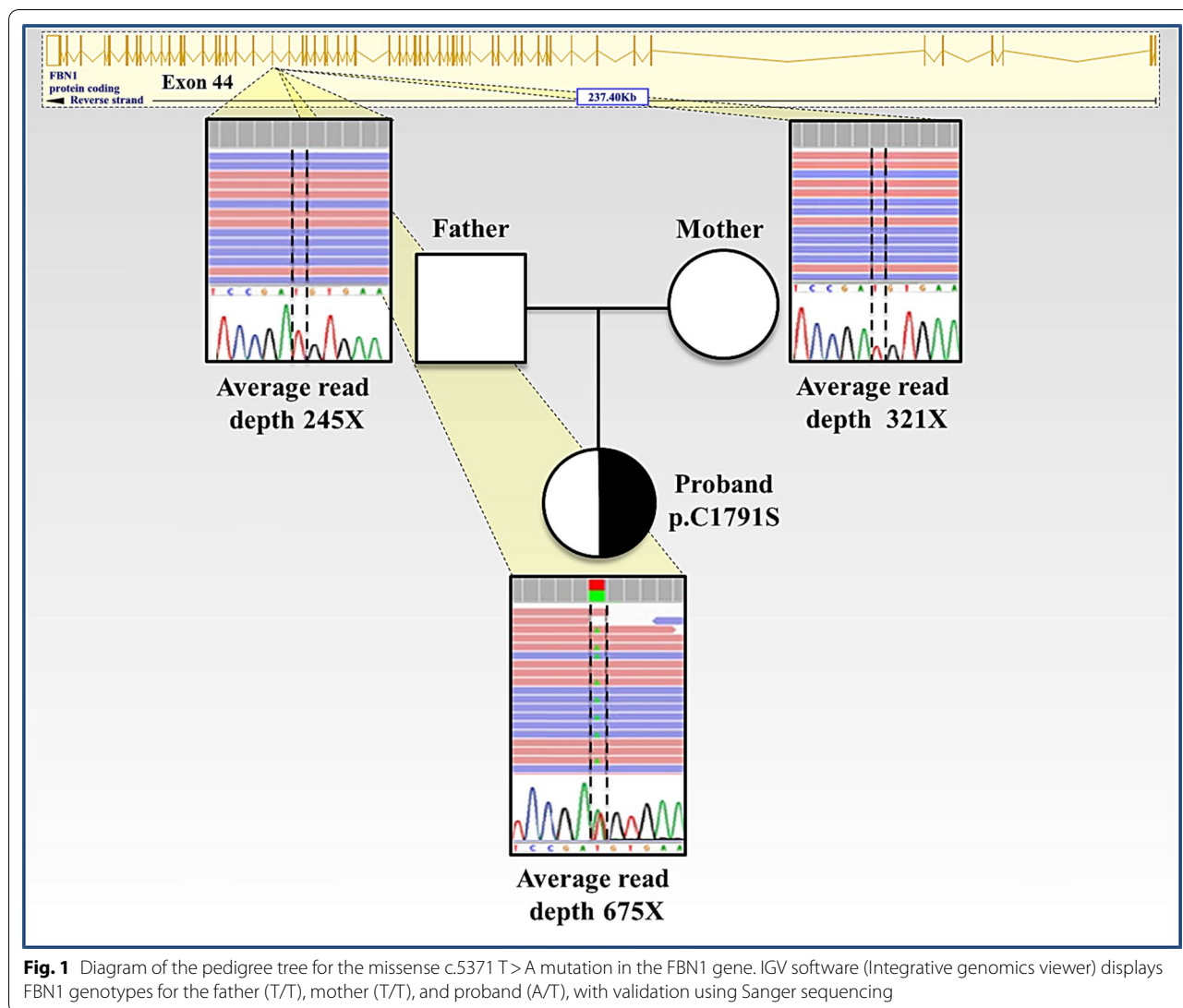
Fig. 1 Diagram of the pedigree tree for the missense c.5371 T > A mutation in the FBN1 gene. IGV software (Integrative genomics viewer) displays FBN1 genotypes for the father (T/T), mother (T/T), and proband (A/T), with validation using Sanger sequencing
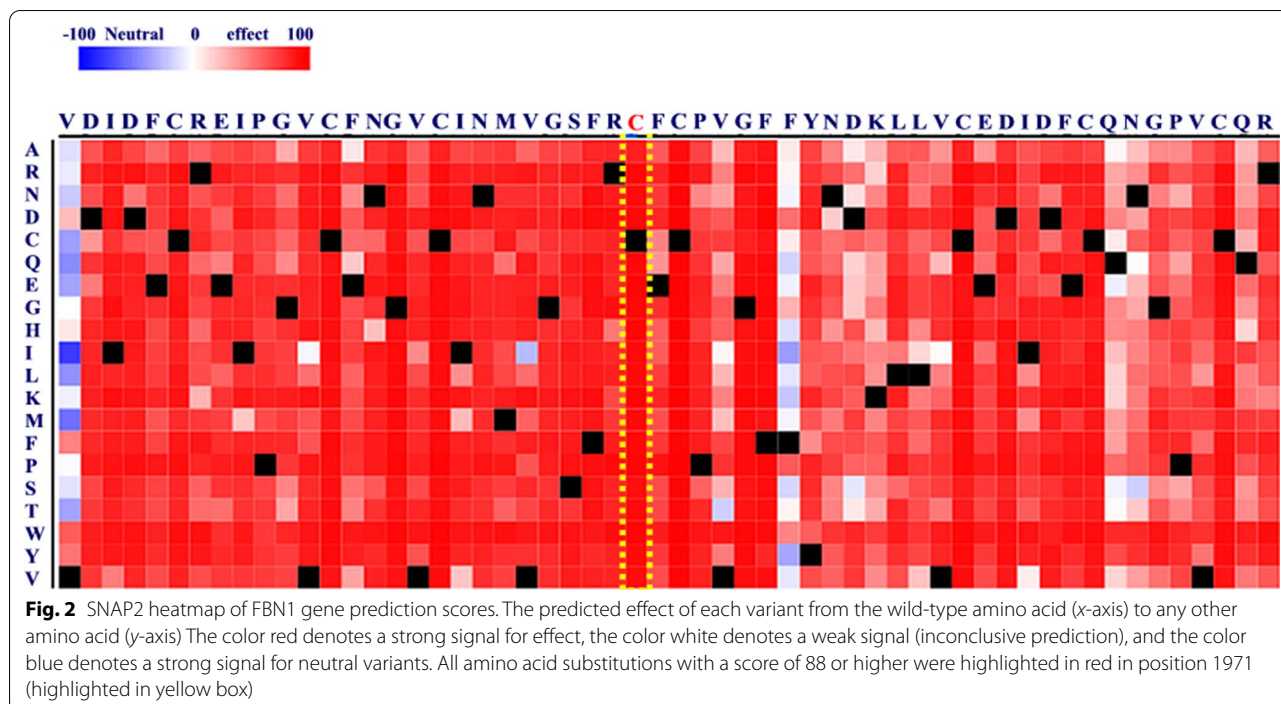
that this variation has not been reported before, structural characteristics such as secondary structure, accessibility to solvents and, if available, annotations on known functional residues, patterns, homologous regions are considered [16]. At the protein level, the consequences of the p.Cys1791Ser substitution on FBN1 tertiary structure were evaluated using Phyre2 software (Protein Homology-fold Recognition Server; www.sbg.bio.ic.ac.uk/phyre2/) analyzing, in particular, the amino acid sequence between the TB7 and TB8 domain (AA 1500–2119) [17].

Discussion and conclusions

In this case report, we identified a novel, apparently de novo, germ-line heterozygous variant in *FBN1* gene (c.5371 T > A) in a female proband with suspect of MFS.

This missense variant determines the replacement of conserved cysteine residue with a serine and remove one of the six characteristic disulfide bridges within the EGF-like 29 domain.

Other four different amino acid substitutions in particular p.Cys1791Arg, p.Cys1791Tyr, p.Cys1791Phe, p.Cys1791Trp associated with MFS as reported in Universal Mutation Database (UMD) (http://www.umd.be/) [18] in the same position have been previously described. Despite the pathogenicity of the variants, the analysis reported in Ensembl (https://www.ensembl.org/index.html), associated with the 1791 codon, provides conflicting data. In particular, the variant p.Cys1791Trp (reported in Ensembl) is classified as pathogenetic for SIFT, probably damaging for Polyphen

Fig. 2 SNAP2 heatmap of FBN1 gene prediction scores. The predicted effect of each variant from the wild-type amino acid (*x*-axis) to any other amino acid (*y*-axis) The color red denotes a strong signal for effect, the color white denotes a weak signal (inconclusive prediction), and the color blue denotes a strong signal for neutral variants. All amino acid substitutions with a score of 88 or higher were highlighted in red in position 1971 (highlighted in yellow box)

Table 1 The effect of amino acid substitution and the associated score

| Wild-type Amino acid	Position	Variant Amino Acid	Predicted effect	Score	Expected Accuracy (%)
C	239	A	effect	88	91
C	239	R	effect	97	95
C	239	N	effect	96	95
C	239	D	effect	97	91
C	239	C	neutral	− 99	97
C	239	Q	effect	97	95
C	239	E	effect	98	95
C	239	G	effect	97	95
C	239	H	effect	98	95
C	239	I	effect	96	95
C	239	L	effect	96	95
C	239	K	effect	98	95
C	239	M	effect	96	95
C	239	F	effect	97	95
C	239	P	effect	98	95
C	239	S	effect	94	95
C	239	T	effect	96	95
C	239	W	effect	98	95
C	239	Y	effect	96	95
C	239	V	effect	95	95

and likely benign for CADD confirming the variability of results of predictive tools.

To understand and classify this novel variant, considering the possible effects of all the amino acid changes reported at position 1791, an in silico comparative analysis was performed (Fig. 3).

SNAP2 as prediction software was used. This tool is able to distinguish between the effect of neutral variants, SNPs and non-synonymous variants taking in consideration evolutionary information and comparing the effect of all amino acids in the same codon. In a second step, 3D structure of the protein was analyzed by means of a comparative approach of all known variants in codon 1791.

Our results show how a single amino acidic change could induce considerable variation on the protein structure and stability due to crucial role of disulfide bridge between residues 1777 and 1791. As highlighted in Fig. 3, the new variant p.Cys1791Ser (panel B) determines an overall lengthening of the region between the TB7 domain and TB8 by 8%, comparable to the effect of the variant p.Cys1791Phe (Fig. 3 panel C). This fibrillin stretch is compatible with a limited amount of fibrillin in extracellular matrix with long, smooth, and thin fibers in the extracellular area. Furthermore, amino acidic change in Thr or Arg (respectively, Fig. 3 panel E and F) seems to be the least tolerated. As highlighted into yellow circle, these two pathogenic variants induced a TB8 module misfolding, with a rotation of about 90° and consequent deleterious effects on the packing of global fibrillin
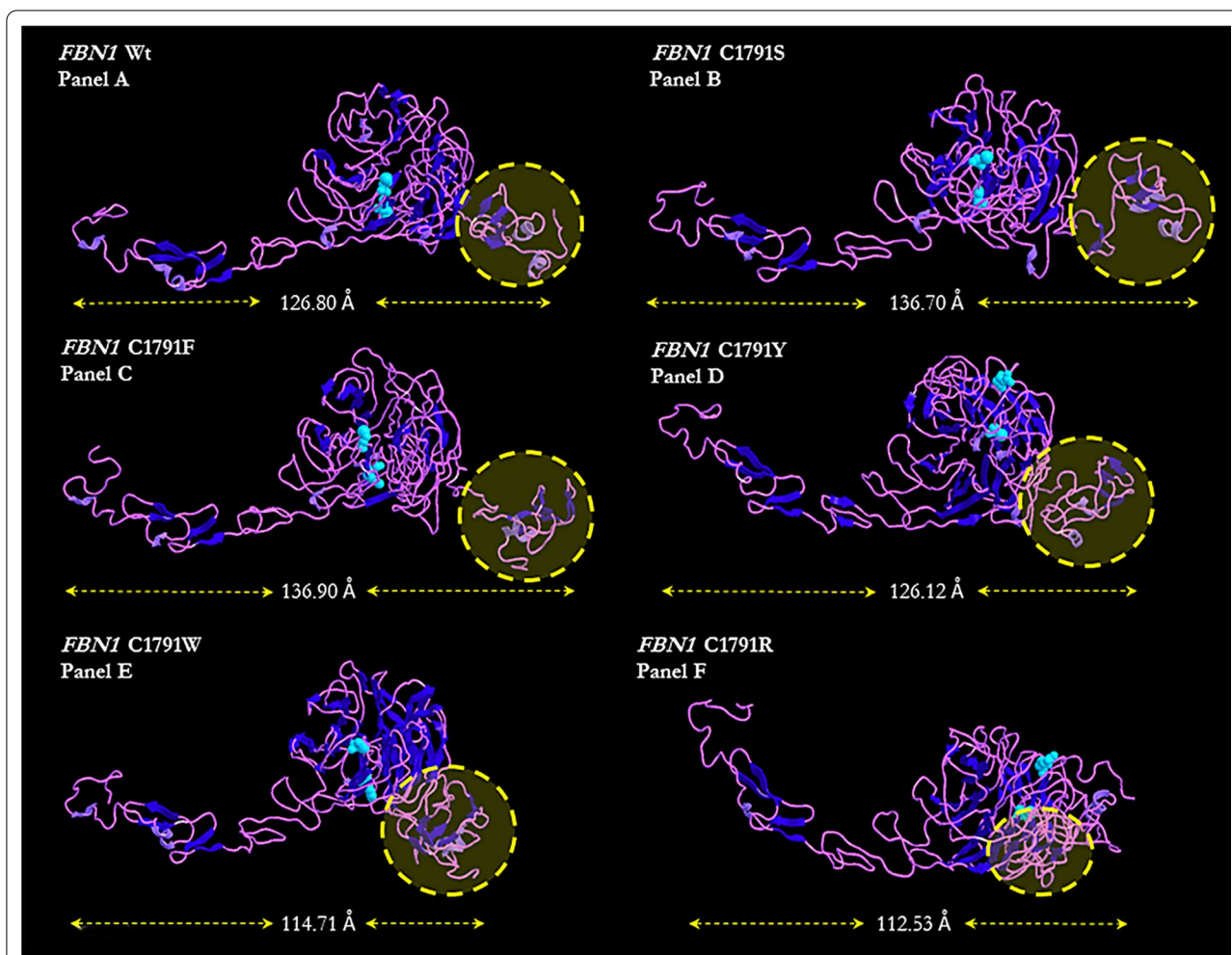
Fig. 3 The structure of the missense pathogenic variant in the amino acid sequence between the TB7 and TB8 domains was analyzed (AA 1500–2119). The variant p.Cys1791Ser found in the EGF-like domains 29 interferes with the formation of a disulfide bond between the two cysteines. (Residues 1777 and 1791 were highlighted in blue space filling mode.) The new pathogenic variant p.Cys1791Ser (panel B) causes an 8 per cent lengthening of the region between the TB6 and TB8 domains. The total length of the analyzed amino acid fragment changes from 126.80 Å in the wild-type protein to 136.70 Å in the mutant protein. The effect of the new variant is comparable to that of the variant p.Cys1791Phe (panel C), which causes an elongation of the analyzed region. The amino acidic changes to Thr and Arg (panels E and F) appear to be the least tolerated. These two pathogenic variants caused TB8 module misfolding with a rotation of about 90°, as indicated by the yellow circle. The final variant, p.Cys1791Tyr (panel D), appears to have no effect on protein length while causing a critical misfolding of the EGF-like 29 domain

structure. These two pathogenic variants might also alter the volume of the calcium-binding pocket of the adjacent EGF-like domain with a consequent endoplasmic reticulum retention of the protein and an increase of fibrillin peptides proteolysis. Finally, the last variant p.Cys1791Tyr seems to have no effect on the length of the protein while produce a critical misfolding of the EGF-like 29 domain.

Evidence from our case and published data strongly supported that the p.Cys1791Ser is a new pathogenic variant of MFS, present only in the proband and absent in the other unaffected family members and healthy controls. The causal role of this variant is supported from the substitution in EGF-like domains 29 of *FBN1* gene that plays a critical role in the pathogenesis of MFS producing different structural and functional consequences. The mutant residue substitutes a conserved 157 cysteine residue, with a serine removing one of the six characteristic disulfide bridges. The variant, in addition to a conformational change of the same domain, induces also a misfolding of neighboring domains causing a possible anomalous elongation of microfibrils [19, 20].

Only a broad integrated approach (such as data collection, phenotypic characteristics evaluation,

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bioinformatics approach, and protein modeling) can help in understanding molecular mechanisms and genotype–phenotype relationships.

Using simple prediction software, the conflicting data could be discovered. As a result, combining analysis and the creation of protein structural models may be advantageous. The visualization of three-dimensional models provides useful information on possible protein domain conformational changes those are directly related to pathogenicity and metabolic pathway modifications. These changes were linked to the clinical and/or phenotypic manifestations [21, 22].

This study emphasizes the importance of close collaboration between clinicians and laboratories throughout the diagnostic process, from clinical suspect to genetic variant identification, interpretation, and clinical contextualization, with the unavoidable introduction of functional investigations into the diagnostic workflow. Diagnostic algorithms are used in situations where the diagnosis is not immediately clear in Clinical Genetics Genetic Syndrome "gestalt." Diagnoses still start with the recognition of a clinical situation based on observation of symptoms and signs, even with the current understanding of a greater variety of genetic pathologies and the recent understanding of the molecular basis of many of these illnesses. Even though some signs unquestionably match clinical hypotheses, they are still only speculative hypotheses [23, 24].

Author contributions
All persons who meet authorship agreement criteria are listed as authors and all authors certify that they have participated in the concept, design, analysis, writing and revision of work. MO contributed to original draft preparation. MT was involved in data curation and reviewing and editing. MR contributed to draft editing. CP was involved in conceptualization, draft editing, and investigation. MTP contributed to draft editing. NB was involved in reviewing, editing, and data analysis. MDM contributed to supervision. MC was involved in conceptualization, bioinformatics analysis, and original draft preparation. All authors read and approved the final manuscript.

Availability of data and material
All the data and executed analyses are available and can be provided upon official request from the requesting institution.

Declarations

Ethics approval and consent to participate
The study was carried out in accordance with the 2013 Revised Declaration of Helsinki of the World Medical Association. Appropriate informed consent for sequencing analysis was obtained from all family members. Parental approval was also obtained for the inclusion of the genetic data in a scientific publication. The informed consent used has been approved by the Ethics Committee "Cardarelli-Santobono" which specifies the possible use for experimentation and biomedical research activities that take place at the A.O.R.N. A. Cardarelli and A.O.R.N. Santobono-Pausilipon. There is no additional ethics statement or approval required for this study because no animals were used in it.

Consent to publish
According to the informed consent, the patient or study participant agrees to the publication of their personal information for scientific reports.

Competing interests
The authors declare no potential conflicts of interest with respect to research, authorship, and/or publication of this article.

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