COLD SPRING HARBOR
Molecular Case Studies

RESEARCH REPORT

Exome sequencing identifies de novo pathogenic variants in *FBN1* and *TRPS1* in a patient with a complex connective tissue phenotype

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Ontology terms: central hypotonia; congenital diaphragmatic hernia; fourth toe clinodactyly; hammertoe; inguinal hernia; joint laxity; long philtrum; malar flattening; pes planus; protruding ear; smooth philtrum; sparse anterior scalp hair; sparse lateral eyebrow; superior pectus carinatum; thin nail; thin upper lip vermilion; thoracic scoliosis; umbilical hernia

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Abstract Here we describe a patient who presented with a history of congenital diaphragmatic hernia, inguinal hernia, and recurrent umbilical hernia. She also has joint laxity, hypotonia, and dysmorphic features. A unifying diagnosis was not identified based on her clinical phenotype. As part of her evaluation through the Undiagnosed Diseases Network, trio whole-exome sequencing was performed. Pathogenic variants in *FBN1* and *TRPS1* were identified as causing two distinct autosomal dominant conditions, each with de novo inheritance. Fibrillin 1 (*FBN1*) mutations are associated with Marfan syndrome and a spectrum of similar phenotypes. *TRPS1* mutations are associated with trichorhinophalangeal syndrome types I and III. Features of both conditions are evident in the patient reported here. Discrepant features of the conditions (e.g., stature) and the young age of the patient may have made a clinical diagnosis more difficult in the absence of exome-wide genetic testing.

[Supplemental material is available for this article.]

INTRODUCTION

There are more than 200 genetic disorders of connective tissue, which may affect skin, eyes and musculoskeletal, cardiovascular, and pulmonary systems. They can present with common features including joint laxity, hypotonia, short stature, and hernias (Jobling et al. 2014). Some common inherited disorders of the connective tissue include Ehlers–Danlos syndrome, osteogenesis imperfecta, Marfan syndrome, and cutis laxa. Diagnosis of these syndromes can be difficult because of the variability and overlapping phenotypes.

We report the case of a female child suspected of having a connective tissue disorder based on findings of multiple hernias, hypotonia, and failure to thrive.

RESULTS

Clinical Presentation

A Caucasian female, age 3 yr, was referred to the Stanford Center for Undiagnosed Diseases with a suspected connective tissue disorder. The pregnancy and family history were unremarkable. Her father was 31 yr of age at the time of her birth, and her mother was 28 yr. She had been referred for genetic evaluation at 6 mo because of dysmorphic features, failure to thrive, and clinodactyly of her fourth toes bilaterally (Fig. 1; Supplemental Figs. S3A–H). Macrocephaly (head circumference 52.2 cm, 97th percentile), pes planus, pectus carinatum, scoliosis, and hypotonia were noted. Chest X-ray and abdominal magnetic resonance imaging (MRI) at 15 mo due to a history of gastroesophageal reflux disease (GERD) and poor weight gain revealed a right-sided diaphragmatic hernia, which was surgically repaired. Umbilical hernia was noted at 20 mo, and bilateral inguinal hernias were diagnosed at 2 yr.

She had a normal ophthalmology exam at 16 mo of age, ruling out myopia and ectopia lentis. An echocardiogram done at 17 mo of age was reported as normal, ruling out mitral valve prolapse and aortic dilatation (aortic root *Z*-score = 2.44) (Fig. 2). Because she had features that were not specific to one genetic condition, a broad molecular genetic analysis was desired.

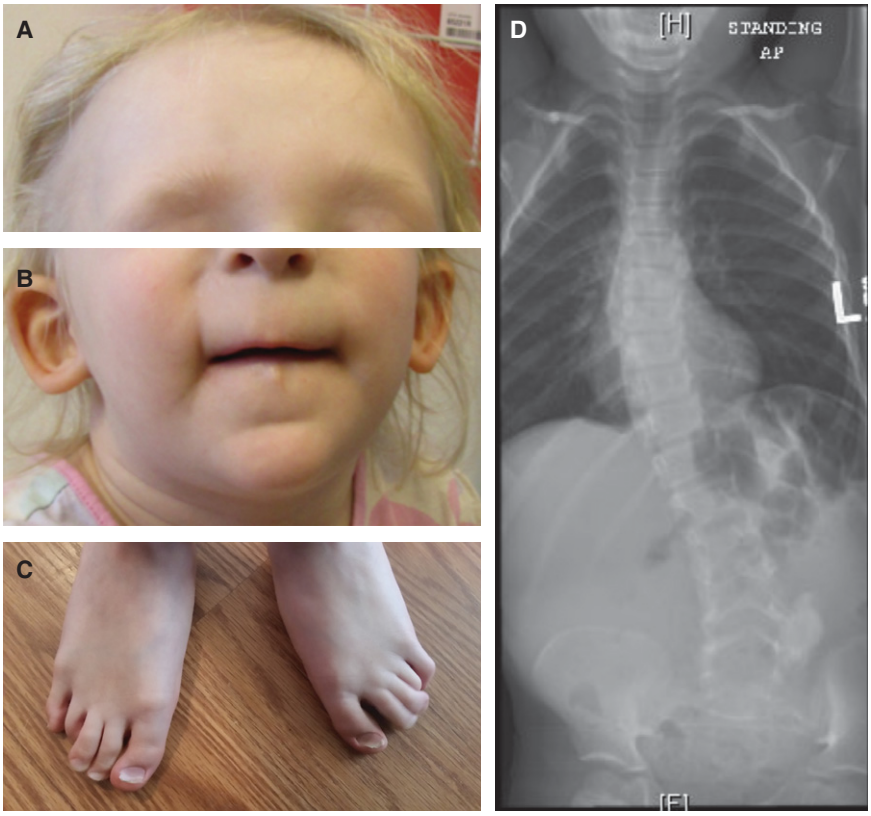

Figure 1. Clinical features. (*A*) Sparse scalp hair with receding frontotemporal hairline and thin eyebrows. (*B*) Protruding ears, thin upper lip, long flat philtrum, and horizontal groove on chin. (*C*) Clinodactyly and hammer toes. (*D*) Scoliosis radiograph.

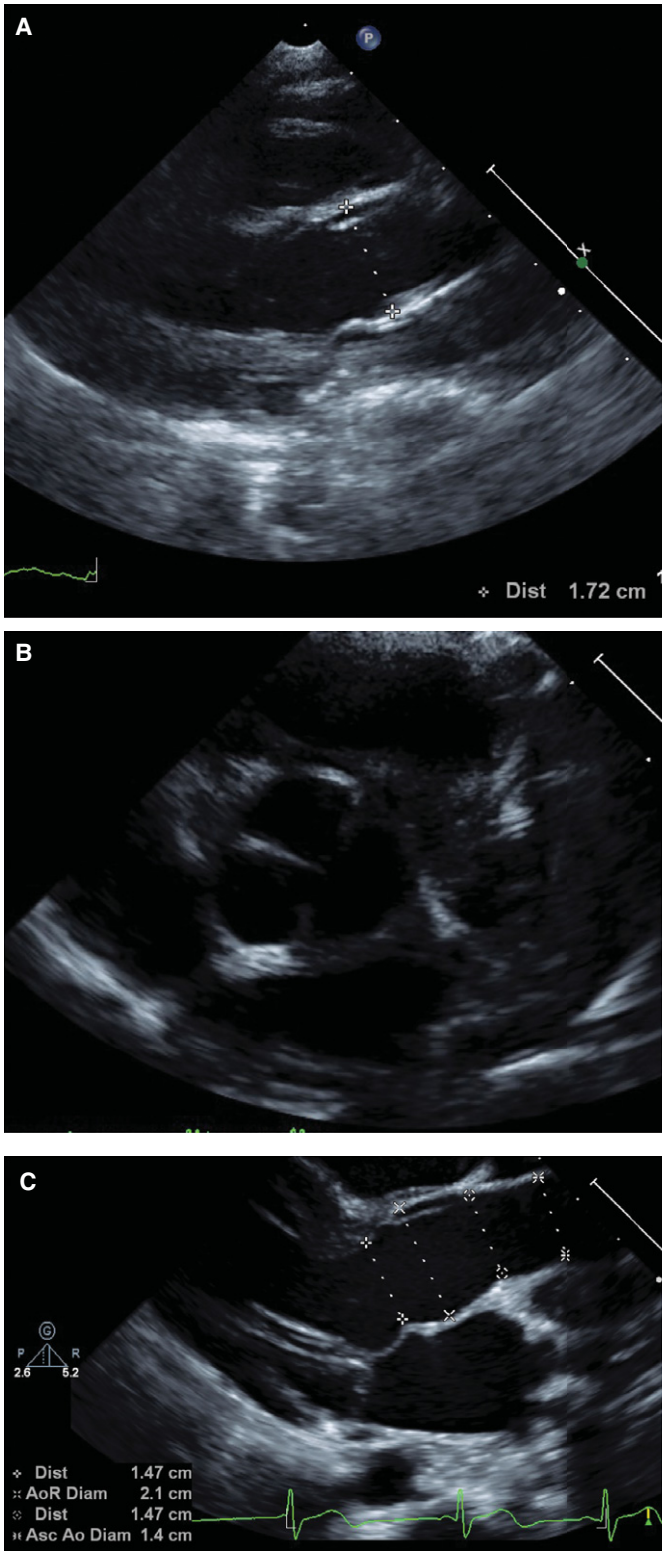

Figure 2. Echocardiogram images. (*A*) Aortic root at 17 mo, before Undiagnosed Diseases Network (UDN) evaluation. (*B*) Trileaflet aortic valve at 17 mo. (*C*) Aortic root at 4 yr and 3 mo.

Table 1. Exome-sequencing metrics

	Proband	Mother	Father
Bases covered at ≥20×	35,296,438	35,322,751	35,239,444
Average coverage	124×	134×	110×
Number of reads	126,028,542	131,764,296	111,657,880
Percent of reads mapped	94.48	95.24	94.21

Genomic Analyses

Previous genetic testing for this patient included a normal array comparative genomic hybridization ([arr(1-22,X)x2]) as well as negative Sotos syndrome (*NSD1* gene, OMIM [Online Mendelian Inheritance in Man] 606681) sequencing and deletion/duplication analysis. Insurance authorization for whole-exome sequencing (WES) was denied.

The patient applied to the Undiagnosed Diseases Network (UDN) and was enrolled at the Stanford clinical site. The UDN is a National Institutes of Health (NIH) sponsored clinical research network, the goal of which is to diagnose disease in patients who do not have a clinical or molecular diagnosis (Gahl et al. 2015, 2016). As part of the research evaluation, whole-exome or genome sequencing is often performed in addition to an on-site clinical visit with multiple subspecialists to refine understanding of the phenotype. After her enrollment in the UDN, blood samples were obtained from the patient, her mother, and her father. DNA was extracted and sent to the UDN exome-sequencing core at Baylor Miraca Genetics Laboratories for trio WES. A summary of the exome-sequencing statistics for each family member is presented in Table 1. A tiered annotated output of all sequence variant calls in the patient is included in Supplemental Table 1, as given by the Sequence to Medical Phenotypes pipeline (Dewey et al. 2015). Alignment and variant filtering identified two high-quality de novo pathogenic variants in disease genes related to the clinical phenotype (Table 2).

A heterozygous stop-gain variant c.4786C>T (p.R1596X) in the fibrillin 1 (*FBN1*; OMIM 134797) gene was detected. Pathogenic variants in the *FBN1* gene lead to Marfan syndrome (MFS; OMIM 154700) and similar disorders (MASS phenotype, mitral valve prolapse

Table 2. Pathogenic variants identified by trio whole-exome sequencing

Gene	*FBN1*	*TRPS1*
Chromosome position	15:48758017	8:116616566
HGVS DNA reference	NM_000138: c.4786C>T	NM_014112: c.1630C>T
HGVS protein reference	p.R1596X	p.R544X
Variant type	Nonsense	Nonsense
Predicted effect	Stop codon	Stop codon
dbSNP/dbVar ID	rs113871094	N/A
Genotype	Heterozygous	Heterozygous
ClinVar ID	36082	N/A
Parent of origin	De novo	De novo
ExAC allele frequency	Not present	Not present
Gene loss-of-function intolerance (pLI)	1.00	0.99

HGVS, Human Genome Variation Society; dbSNP, Database for Short Genetic Variations; dbVar, Database of Genomic Structural Variation; N/A, not available; ExAC, Exome Aggregation Consortium; pLI, probability of loss-of-function intolerance.

syndrome, ectopia lentis syndrome). This variant has been previously reported as disease causing (Loeys et al. 2001) for MFS, a well-recognized autosomal dominant condition involving the connective tissue. Primary characteristics affect the ocular, skeletal, and cardiovascular systems (Dietz 2014). The principal diagnostic clinical features include aortic root enlargement and ectopia lentis (Loeys et al. 2010). A 29-yr-old patient with the p.R1596X variant has a reported diagnosis of classic MFS according to the original Ghent nosology, with major involvement of the ocular and cardiovascular systems and the dura (Loeys et al. 2001). Thoracic aortic aneurysms and aortic dissections (TAADs) have also been associated with this variant in one ClinVar submission (SCV000233837.2).

A heterozygous nonsense variant c.1630C>T (p.R544X) in the *TRPS1* gene (OMIM 604386) was also detected in the patient. The *TRPS1* gene is a zinc finger transcription factor, and is associated with trichorhinophalangeal syndrome, types I (TRPS1; OMIM 190350) and III. Trichorhinophalangeal syndrome type I (OMIM 190350) is an autosomal dominant condition that also affects the skeletal system, with prominent dysmorphic craniofacial features. Dysmorphic findings include sparse hair, bulbous tip of the nose, long flat philtrum, thin upper vermilion border, and protruding ears (Momeni et al. 2000). Missense mutations in the GATA zinc finger domain (exon 6) have a more severe phenotype of TRPS type III, whereas mutations leading to haploinsufficiency (frameshift, nonsense, or splicing) cause TRPS type I (Ludecke et al. 2001). TRPS type II is due to a contiguous gene deletion encompassing the *TRPS1* and *EXT1* genes (Ludecke et al. 1995). The p.R544X variant has been previously reported and associated with TRPS1 in one patient (Ito et al. 2013). Allele frequency of this variant is assumed to be very low, as it is absent from the 1000 Genomes Project, the Exome Variant Server, National Heart, Lung, and Blood Institute (NHLBI) GO Exome Sequencing Project (ESP), and Exome Aggregation Consortium (ExAC) databases (http://evs.gs.washington.edu/EVS/) (The 1000 Genomes Project Consortium 2015; Lek et al. 2016). The *TRPS1* gene encodes a 1281-amino acid protein with seven exons. Important motifs include exon 6, which encodes a presumptive GATA DNA-binding zinc finger, and an IKAROS-like zinc-finger motif in the carboxyl terminus, which enables protein–protein interactions (Momeni et al. 2000; Ludecke et al. 2001). R544X is in exon 4 of the *TRPS1* gene and is predicted to result in a stop codon. Because of the location of this variant in the TRPS1 protein and the nonsense effect of the variant, nonsense-mediated decay of the resulting mutant protein is likely (Richards et al. 2015). The probability of loss-of-function intolerance (pLI) score for *TRPS1* is 0.99. Reported germline *TRPS1* mutations number at least 55, not including gross deletions and rearrangements typically associated with trichorhinophalangeal syndrome type II (Human Gene Mutation Database [HGMD], ClinVar). Nonsense variants in *TRPS1* have been reported as pathogenic (17/55 in HGMD, 5/14 in ClinVar). Therefore, the *TRPS1* p.R544X variant was classified as pathogenic based on the nonsense/null variant, de novo inheritance, low allele frequency in the general population, and multiple lines of computational evidence supporting a deleterious effect (Richards et al. 2015).

Whole-exome sequencing and Sanger sequencing of the parental samples did not detect the c.4786C>T *FBN1* variant or the c.1630C>T *TRPS1* variant. De novo mutations are assumed, although the possibility of cryptic somatic or germline mosaicism in either parent cannot be excluded. These variants were confirmed in the proband and absent in parents by Sanger sequencing (Supplemental Fig. S1A–F), whereas no variant reads were mapped in either parent for either mutation as visualized in the Integrative Genomics Viewer (Supplemental Fig. S2A–F).

DISCUSSION

We report on a female with a suspected connective tissue disorder who was found to have two pathogenic de novo variants by trio WES causing two autosomal dominant syndromes:

Marfan syndrome and TRPS1. Both pathogenic variants have been previously reported to be associated with these conditions, but to our knowledge this is the first report of these conditions appearing in the same individual.

The *TRPS1* R544X variant found in our patient was previously reported in a 5-yr-old female who presented with hair growth retardation (Ito et al. 2013). Upon examination she also had a pear-shaped nose, thin upper lip, thin toenails, and brachydactyly of the big toes. These features are similar to that of our patient (Table 3). Immunohistochemical staining of a skin sample from the previously reported patient with the TRPS1 R544X variant showed low immunoreactivity with anti-TRPS1 antibody, suggesting haploinsufficiency (Ito et al. 2013). This is consistent with genotype–phenotype correlations for *TRPS1* missense mutations leading to haploinsufficiency (Ludecke et al. 2001). A characteristic skeletal finding of TRPS1 is cone-shaped epiphyses of the phalanges, which typically present after 2 yr. These were seen in the previously reported patient, but were not investigated in our patient. Without whole-exome sequencing, this subtle dysmorphic diagnosis might not have been appreciated.

Table 3. Correlation of clinical phenotype using Human Phenotype Ontology (HPO)

System	Patient	Marfan syndrome	Trichorhinophalangeal syndrome type 1
Skeletal	Joint laxity[a]	Joint laxity	
	Pectus carinatum[b]	Pectus excavatum[c]/ carinatum	Pectus carinatum
	Thumb sign	Thumb sign	
	Scoliosis[d]	Scoliosis	Scoliosis
	Pes planus[e]	Pes planus	Pes planus
	Height = 97.0 cm (31st percentile by CDC, 17th percentile by WHO)	Bone overgrowth/ tall stature[f]	Short stature[g]
	Hammertoe[h]		
	Toe clinodactyly[i]		
	Hammertoe arachnodactyly[j]	Short fingers[k]	
	Unknown		Cone-shaped epiphysis[l] (>2 yr)
Ocular	Negative exam at 3 yr	Ectopia lentis[m], myopia[n]	
Cardiovascular	Aortic root dilated (*Z* = 3.24 at 4 yr 3 mo)	Aortic dilatation[o]	
	Normal mitral and tricuspid valves at 4 yr 3 mo	Mitral[p]/tricuspid valve prolapse[q]	
Connective tissue	Congenital diaphragmatic[r], umbilical[s], and inguinal hernias[t]	Hernias[u]	
Hair, nails	Sparse scalp hair[v]		Sparse scalp hair
	Sparse eyebrows[w]		Sparse eyebrows
	Thin nails[x]		Thin nails
Craniofacial	Full nasal tip		Bulbous nose[y]
	Long,[z] flat[aa] philtrum		Long, flat philtrum
	Thin upper lip vermilion[bb]		Thin upper lip vermilion
	Protruding ears[cc]		Protruding ears
	Malar hypoplasia[dd]	Malar hypoplasia	
Muscular	Hypotonia[ee]		Hypotonia

CDC, Centers for Disease Control and Prevention; WHO, World Health Organization.
HPO IDs: [a]0002761; [b]0000768; [c]0000767; [d]0002650; [e]0001763; [f]0000098; [g]0004322; [h]0001765; [i]0001863; [j]0001166; [k]0009381; [l]0010579; [m]0001083; [n]0000545 [o]0002616; [p]0001634; [q]0001704; [r]0000776; [s]0001537; [t]0000023; [u]0100790; [v]0002209; [w]0000535; [x]0001816; [y]0000414; [z]0000343; [aa]0000319; [bb]0000219; [cc]0000411; [dd]0000272; [ee]0008947.

Growth alterations leading to abnormal height is a significant feature in both TRPS1 and MFS. Short stature in TRPS1 is progressive with age and is therefore more pronounced in adulthood (Ludecke et al. 2001). In contradistinction, long bone overgrowth in patients with Marfan syndrome often leads to absolute or relative increased height as well as arachnodactyly. In TRPS1, growth retardation is due to premature closure of the growth plates. *TRPS1* is involved in regulation of chondrocyte and perichondrium development by repressing expression of RUNX2 (Napierala et al. 2008). Studies of *FBN1* in the skeletal pathway suggest involvement of the TGFβ and BMP pathways (Quarto et al. 2012a,b). Typically for children with MFS, tall stature (height ≥ 97th percentile, or >3 SD above the mean) is a common presenting feature (Lipscomb et al. 1997; Faivre et al. 2009). TGF-β is up-regulated in MFS, which recruits and proliferates osteoprogenitors, but down-regulates BMP; this inhibits osteoblast proliferation, differentiation, and mineralization. The height of our patient (97.0 cm: 31st percentile by the Centers for Disease Control and Prevention [CDC]; 17th percentile by the World Health Organization [WHO]) may reflect epistasis between *FBN1*, promoting TGF-β-related chondrogenesis, and *TRPS1* haploinsufficiency, leading to growth retardation through premature closure of epiphyses. This perhaps confused the potential clinical/phenotypic diagnosis of Marfan syndrome before genetic testing was initiated.

The FBN1 R1596X variant was reported in a family with phenotypic features similar to our patient (De Backer et al. 2007). A 4-yr-old male presented with inguinal hernia at age 2 yr and with flat feet. He had pectus excavatum, arachnodactyly, and tall stature (103 cm, 97th percentile), and an echocardiogram showed dilation of the proximal aorta (*Z*-score = 3.2). Six other members of the family were subsequently found to have the same *FBN1* variant, with limited expression of the disease (3/7 fulfilled the Ghent criteria). RNA expression studies in this family were done on two sisters: one satisfied the Ghent criteria (proband's mother), and the other did not. The authors found that they had similar expression and concluded that was not the cause for the intrafamilial variation.

Clinical diagnosis of Marfan syndrome in children has been noted to be more difficult as compared to adults because of the delay in onset of clinical features (De Backer et al. 2007). This is especially true in the absence of ocular or cardiovascular features. Revised Ghent diagnostic criteria for MFS combine family history, medical history, physical exam, and molecular *FBN1* analysis (Loeys et al. 2010). The skeletal findings, such as bone overgrowth and joint laxity, are also clinically assessed in the revised Ghent criteria as part of the Systemic Score, where features are assigned a value and a total value score greater than or equal to 7 can be included as diagnostic criteria (Loeys et al. 2010). In the absence of a family history, the diagnosis is made when aortic root enlargement or ectopia lentis is present in addition to other findings. Our patient had a normal ocular exam and presented with no identified cardiovascular features. Using the systemic score for MFS, our patient presented with the thumb sign, pectus carinatum, pes planus, and scoliosis; this gave a systemic score of 5. Therefore, she did not overtly meet clinical criteria for MFS upon presentation to the UDN. Echocardiogram performed during the UDN evaluation, but after WES results were available, revealed mild aortic root dilation (*Z*-score = 3.24), aortic annulus (*Z*-score = 4.13), and sinotubular junction (*Z*-score = 3.03) (The Marfan Foundation; https://www.marfan.org/dx/zscore). These findings combined with the known pathogenic *FBN1* variant allowed the diagnosis of MFS to be established. *FBN1* variants and MFS have been reported in association with congenital diaphragmatic hernia (CDH), which was present in our patient (Petersons et al. 2003; Faivre et al. 2009; Jetley et al. 2009; Beck et al. 2015). However, this characteristic is not part of the revised Ghent diagnostic criteria or systemic score. When the diagnosis of MFS in people under the age of 20 yr is suspected, a suggestion is to use "nonspecific connective tissue disorder" or "potential MFS" until aortic root dilation is present (*Z*-score ≥ 3.0) (Dietz 2014). Also, the Kid-Short Marfan Score (SMS) diagnostic tool can be used, but this also relies heavily on cardiac, ocular, and family history features (Mueller

et al. 2013). Patients similar to ours, with pathogenic de novo *FBN1* variants and a milder skeletal phenotype, will likely remain undiagnosed in childhood based on clinical features alone. This is problematic, as the first diagnostic manifestation is skeletal for a quarter of children with *FBN1* mutations (Faivre et al. 2009). Importantly, the clinical diagnosis of MFS has implications for ongoing surveillance and treatment to avoid aortic catastrophe.

There was no family history for our patient of Marfan syndrome or specific phenotypic findings suggesting TRPS1. This of course is consistent with the de novo nature of the variants. Approximately 25% of patients with MFS have a de novo variant (Dietz 2014). Because of the small number of reported pathogenic *TRPS1* variants (approximately 55), the frequency of de novo variants has yet to be determined. Although the p.R544X was assumed to be de novo in the previous report (Ito et al. 2013), those parents did not have molecular analysis to confirm. Half of the *TRPS1* variants reported by Momeni et al. (2000) were sporadic (3/6). Thirty TRPS1 cases studied by Ludecke et al. (2001) were isolated, but causative *TRPS1* variants were found in only 24 of those cases, and only 15 cases had mother and father available for analysis. Though rare, multiple prior reports have found more than one pathogenic de novo variant on WES (Tunovic et al. 2014; Yoo et al. 2015; Gahl et al. 2016). With the increase in clinical WES, multiple de novo pathogenic variants are likely to be more common than previously appreciated.

Genome sequencing has allowed more accurate analysis of the frequency of de novo mutations (Conrad et al. 2011; Kong et al. 2012; Francioli et al. 2015). Paternal age is a known factor for de novo mutations during DNA replication in spermatogenesis and has been confirmed with these recent genome investigations; however, the father of our patient was 31 yr at the time of her birth. Clinical guidelines suggest that paternal age of >40 yr reaches significance for increased risk of pathogenic de novo variants to offspring in autosomal dominant genetic conditions (Toriello and Meck 2008). The annual mutation rate is estimated to increase by one to two mutations per year with paternal age (Kong et al. 2012; Francioli et al. 2015). The overall mutation rates based on whole-genome studies are estimated at $0.97–1.20 \times 10^{-8}$ per nucleotide per generation (Conrad et al. 2011; Kong et al. 2012). Most de novo mutations will take place in noncoding regions; Francioli et al. found that only 1.22% of de novo mutations in studied genomes were in exonic regions. The estimated prevalence of MFS is 1/5000–1/10,000 (Dietz 2014). The prevalence of clinically recognized TRPS1 is low; approximately 100 cases have been published (Orphanet Report Series 2016). The prevalence of TRPS1 is estimated to be 1/1,000,000. Therefore, the likelihood of a co-occurrence of MFS and TRPS1 is 1/5,000,000,000–1/10,000,000,000, indicating our patient may be unique among the world's population (7.4 billion) (World Population Clock; http://www.worldometers.info/world-population/, accessed July 7, 2016); the likelihood of de novo mutations causing these specific phenotypes would be even lower. WES presents a unique advantage over single gene sequencing and gene panels to interrogate the exome for these rare de novo variants responsible for clinical disease.

This is the first report, to our knowledge, of MFS and TRPS type 1 appearing in the same individual, along with molecular discovery of *FBN1* and *TRPS1* de novo pathogenic variants by WES. The expected phenotypes of Marfan syndrome and trichorhinophalangeal syndrome type 1 partially overlap (e.g., scoliosis, pectus carinatum, and pes planus are common to both) yet are distinct in others (e.g., skeletal and craniofacial features), which made it difficult to arrive at a clinical diagnosis prior to WES. Because of the overlapping or subtle characteristics of these conditions in our patient (height, multiple and recurrent hernias, joint laxity, hypotonia, and dysmorphic features), clinical diagnoses were only possible after genetic evaluation through WES. Additionally, if targeted testing for connective tissue disease had been done, then the diagnosis of TRPS1 may not have been appreciated. This case highlights the importance and utility of WES for undiagnosed genetic disease in thoroughly identifying multiple conditions and allowing management of life-threatening sequellae.

METHODS

Whole-Exome Sequencing

For the paired-end precapture library procedure, genome DNA was fragmented by sonication and ligated to the Illumina multiplexing paired-end (PE) adapters. The adapter-ligated DNA was the polymerase chain reaction (PCR) amplified using primers with sequencing barcodes (indexes). For target enrichment/exome capture procedure, the precapture library was enriched by hybridizing to biotin-labeled VCRome 2.1 in-solution exome probes (Bainbridge et al. 2011) for 64–72 h at 47°C. Additional probes for more than 2600 Mendelian disease genes were also included in the capture in order to improve the exome coverage. For massively parallel sequencing, the postcapture library DNA was subjected to sequence analysis on Illumina HiSeq platform for 100-bp paired-end reads. The following quality control metrics of the sequencing data are generally achieved: >70% of reads aligned to target, >95% target base covered at >20×, >85% target base covered at >40×, mean coverage of target bases >100×. Single-nucleotide polymorphism (SNP) concordance to genotype array: >99%. As a quality control measure, DNA is also analyzed by a SNP array (Illumina Human Exome-12v1 array). The SNP data are compared with the WES data to ensure correct sample identification and to assess sequencing quality.

Data Analysis and Interpretation by Mercury 1.0

The output data from Illumina HiSeq are converted from BCI files to FASTQ by Illumina CASAVA 1.8 software, and mapped by BWA (Li and Durbin 2009) to the reference haploid human genome sequence (Genome Reference Consortium human genome build 37, human genome 19). The variant calls are performed using Atlas-SNP and Atlas-indel developed in-house by the Baylor College of Medicine Human Genome Sequencing Center (BCM HGSC) (Yang et al. 2013). The variant annotations are performed using in-house developed software: HGSC-SNP-anno and HGSC-indel-anno. Synonymous variants, intronic variants not affecting splice site, and common benign variants are excluded from interpretation unless they were previously reported as pathogenic variants. The variants were interpreted according to American College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al. 2015) and patient phenotypes. Variants related to patient phenotypes are confirmed by Sanger sequencing for patient and parents.

ADDITIONAL INFORMATION

Data Deposition and Access

Whole-exome sequencing data have been submitted to the Database of Genotypes and Phenotypes (dbGaP; https://www.ncbi.nlm.nih.gov/gap) per NIH study protocol and patient consent (accession number phs001232.v1.p1). The *FBN1* and *TRPS1* variants have been submitted to ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) under accession numbers SCV000328213.1 and SCV000328214.1. Patient-derived biospecimens are available by contacting the corresponding author.

Ethics Statement

The clinical trial was approved by the Institutional Review Board of Stanford University and the NIH (NIH Study Reference Number 15-HG-0130), and written informed consent and assent was obtained from the patient and family members.

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D.B.Z. and M.T.W. analyzed and interpreted the data and drafted and critically reviewed the manuscript. M.W. and C.M.E. generated, analyzed, and interpreted the sequencing data and drafted the manuscript. A.D. obtained consent from the patient and family and critically reviewed the manuscript. J.K. obtained images for the manuscript and critically reviewed the manuscript. L.F. critically reviewed the manuscript. D.W. analyzed and interpreted the sequencing data and critically reviewed the manuscript. P.A.Z. and J.A.B. obtained consent from the patient and family, collected the clinical data, and critically reviewed the manuscript. M.A.M. and E.F. collected and provided the clinical data and critically reviewed the manuscript. J.A.B., P.G.F., E.A.A., M.T.W., and members of the Undiagnosed Diseases Network conceived of the study and critically reviewed the manuscript.

Competing Interest Statement

E.A.A. and M.T.W. report holding stock in Personalis, Inc.

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