

Original article

In-frame Variants in *FLNA* Proximal Rod 1 Domain Associate With a Predominant Cardiac Valvular Phenotype

Luis Fernández,^{a,b,*} Jair Tenorio,^{a,b} Coral Polo-Vaquero,^{a,b} Elena Vallespín,^{a,b} María Palomares-Bralo,^{a,b} Sixto García-Miñaur,^{a,b} Fernando Santos-Simarro,^{a,b} Pedro Arias,^{a,b} Hernán Carnicer,^c Silvina Giannivelli,^c Juan Medina,^d Rosa Pérez-Piaya,^c Jorge Solís,^d Mónica Rodríguez,^e Alexandra Villagrà,^e Laura Rodríguez,^f Julián Nevado,^{a,b} Víctor Martínez-Glez,^{a,b} Karen E. Heath,^{a,b} and Pablo Lapunzina^{a,b,g}

^a Instituto de Genética Médica y Molecular (INGEMM), Instituto de Investigación del Hospital Universitario La Paz (IdiPAZ), Madrid, Spain

^b Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III (ISCIII), Madrid, Spain

^c Unidad de Pediatría, Hospital Universitario HM Montepríncipe, Madrid, Spain

^d Unidad de Cardiología, Hospital Universitario HM Montepríncipe, Madrid, Spain

^e Unidad de Cardiología Infantil, Hospital Universitario HM Montepríncipe, Madrid, Spain

^f Laboratorio Clínico, Hospital Universitario HM Montepríncipe, Madrid, Spain

^g Unidad de Genética Clínica, Hospital Universitario HM Montepríncipe, Madrid, Spain

Article history:

Received 6 July 2017

Accepted 2 October 2017

Available online 14 November 2017

Keywords:

X-linked disease

Cardiac valvular dysplasia

FLNA mutation

ABSTRACT

Introduction and objectives: X-linked cardiac valvular dysplasia is a rare form of male-specific congenital heart defect mainly characterized by myxomatous degeneration of the atrioventricular valves with variable hemodynamic consequences. It is caused by genetic defects in *FLNA*-encoded filamin A, a widely expressed actin-binding protein that regulates cytoskeleton organization. Filamin A loss of function has also been associated with often concurring neurologic and connective tissue manifestations, with mutations in the first half of the Rod 1 domain apparently expressing the full cardiac phenotype. We contribute to previous genotype-phenotype correlations with a multidisciplinary approach in a newly-described family.

Methods: Cardiologic, dysmorphic, and genetic evaluation of available members were complemented with transcriptional and X-chromosome inactivation studies.

Results: A novel *FLNA* mutation c.1066-3C>G cosegregated with a male-expressed, apparently isolated, cardiac phenotype with no skewed X-inactivation pattern in female carriers. This variant was shown to result in an in-frame deletion of 8 amino acid residues near the N-terminal region of the protein.

Conclusions: A nonimprinted, partial loss of function of filamin A proximal Rod 1 domain seems to be the pathogenetic mechanism of cardiac valvular dysplasia, with some cases occasionally expressing associated extracardiac manifestations.

© 2017 Sociedad Española de Cardiología. Published by Elsevier España, S.L.U. All rights reserved.

Variantes que mantienen el marco de lectura en el dominio Rod 1 proximal del gen *FLNA* se asocian con un predominio del fenotipo valvular

RESUMEN

Introducción y objetivos: La displasia valvular cardíaca ligada al cromosoma X es una cardiopatía congénita rara específica del sexo masculino y caracterizada principalmente por una degeneración mixomatosa de las válvulas auriculoventriculares con consecuencias hemodinámicas variables. Se debe a defectos genéticos en la filamina A (codificada por *FLNA*), una proteína de unión a actina de expresión ubicua que regula la organización del citoesqueleto. La pérdida de función de la filamina A también se ha asociado con manifestaciones neurológicas y del tejido conectivo a menudo simultáneas, y aparentemente las mutaciones en la primera mitad del dominio Rod 1 expresan el fenotipo cardíaco completo. En esta familia de nueva descripción, se ha contribuido a las correlaciones genotipo-fenotipo previas con un enfoque multidisciplinario.

Métodos: La evaluación cardiológica, dismórfica y genética de los miembros disponibles se complementó con estudios de la transcripción y de la inactivación del cromosoma X.

Resultados: La nueva mutación de *FLNA* c.1066-3C>G cosegregaba con un fenotipo cardíaco aparentemente aislado y expresado en los varones, sin que hubiera un sesgo en el patrón de

Palabras clave:

Enfermedad ligada al cromosoma X

Displasia valvular cardíaca

Mutación en *FLNA*

* Corresponding author: Instituto de Genética Médica y Molecular (INGEMM), Bloque Quirúrgico, Planta SS, Hospital Universitario La Paz, Paseo de la Castellana 261, 28046 Madrid, Spain.

E-mail address: lfernandezg@salud.madrid.org (L. Fernández).

inactivación del cromosoma X en las mujeres portadoras. Esta variante resultó en una delección dentro del marco de lectura de 8 residuos de aminoácidos cercanos a la región N-terminal de la proteína.

Conclusiones: La pérdida de función parcial y no sometida a impronta del dominio Rod 1 proximal de la filamina A parece ser el mecanismo patogénico de la displasia valvular cardiaca, expresada en algunos casos con manifestaciones extracardiacas.

© 2017 Sociedad Española de Cardiología. Publicado por Elsevier España, S.L.U. Todos los derechos reservados.

Abbreviations

PVNH: periventricular nodular heterotopia
XCVD: X-linked cardiac valvular dysplasia

INTRODUCTION

Multivalvular heart disease is the combination of stenotic and/or regurgitant lesions of 2 or more cardiac valves. It is a highly prevalent clinical condition among patients with an underlying valvular disease, affecting approximately 20% of patients with congenital valve defects and 15% of patients undergoing valvular surgery.¹ In the PARTNER trials, the incidence of concomitant moderate-to-severe mitral regurgitation in patients with severe aortic stenosis was approximately 20%, and the incidence of moderate-to-severe tricuspid regurgitation was 27%; however, many of these valvular heart diseases were also secondary to hemodynamic alterations, ischemic heart disease, and ventricular dysfunction.¹ The complexity of determining the etiology of multivalvular disease hampers diagnosis and patient management.

X-linked cardiac valvular dysplasia (XCVD, MIM 314400), also known as polyvalvar myxomatous valve degeneration, is a rare disorder caused by mutations in the filamin A gene (*FLNA*). These genetic defects underlie early-onset, progressive myxomatous mitral and tricuspid valve deterioration that presents as valve thickening and dysfunction followed by secondary chamber remodeling and ultimately heart failure. Only a few cases and families have been described to date with this genetic disorder, some of whom were retrospectively diagnosed.²

We report a family with 5 individuals confirmed or suspected to have XCVD due to a novel mutation, c.1066-3C>G, in intron 7 of *FLNA*. This variant abolishes the canonical splice acceptor site, resulting in the in-frame deletion of 8 amino acids of the proximal Rod 1 domain. Thus, this mutation may result in partial loss of function of filamin A, consistent with most previously reported XCVD-associated variants. We review previous XCVD cases and speculate about their different phenotypes, depending on the functional effect, the impact of the variants, and the filamin A topology.

Patients

A complete pedigree of the family is shown in [Figure 1](#).

Index case IV-1. This patient was diagnosed prenatally with abnormally thick cardiac valves by ultrasound and fetal echocardiography ([Video 1 of the supplementary material](#) and [Video 2 of the supplementary material](#)). There was moderate mitral regurgitation and severe tricuspid regurgitation leading to an aneurysmatic dilatation of the right atrium. The pulmonary and the aortic valves were also dysplastic and apparently small. He was born at term (39 weeks) with a birth weight of 3950 g and severe neonatal distress, requiring extracorporeal membrane oxygenation. On the first day of life, echocardiography showed a large dilatation of the right atrium, severe tricuspid insufficiency due to a dysplastic

valve, moderate mitral insufficiency with a dysplastic mitral valve, a 6-mm atrial septal defect with right-left shunt, dilatation of the right ventricle, and left ventricular hypertrophy. The aortic and pulmonary valves were also dysplastic with regurgitation. The pulmonary artery was moderately dilated and the ductus was large with bidirectional flow, predominantly left-right. There was also a slight pericardial effusion. The clinical behavior was of a patient with hypoplastic left heart with functional aortic atresia and a patent ductus dependency. No features of a connective tissue disorder were reported on clinical examination. He underwent cardiac surgery to repair both valves but died intraoperatively. Neither autopsy nor previous brain ultrasound revealed no neuronal migration disorder. Standard karyotype was normal.

Affected relative III-4. This is a 27-year-old man with a congenital heart defect. He was born at term with respiratory distress. A heart murmur was detected at 2 months of age. Postnatal echocardiography confirmed that all valves were thickened and dystrophic, with moderate tricuspid regurgitation, mild mitral and pulmonary regurgitation, and mild aortic incompetence ([Video 3 of the supplementary material](#)). Clinical examination showed no joint hypermobility or skin hyperextensibility. Brain magnetic resonance imaging showed no neuronal migration disorder.

Affected relative III-3. This individual was born at term after an apparently normal pregnancy and delivery. Soon after birth, he developed cyanosis and respiratory distress and died on the first day of life, more than 30 years ago. No other data are available.

Affected relative III-5. This child was born preterm (37 weeks) after a normal pregnancy and delivery. He remained in neonatal intensive care for 2 weeks due to prematurity and abnormal feeding. He underwent cardiac surgery in another country and, after 2 months of indolent clinical course, died after a respiratory illness. The family reported that the neonate had abnormal cardiac valves with cavity dilatation and large heart.

Affected relative II-3. This neonate was born at term, in a home birth more than 60 years ago. The only information reported by the family is that the boy had cardiac murmurs and failure to thrive. No other data are available.

Echocardiography was performed on asymptomatic female relatives (III-2, II-2, II-4, III-6), yielding normal results in all of them except for a 58-year-old woman, II-2, who showed mild pulmonary regurgitation. On clinical examination, there was no joint hypermobility or skin hyperextensibility. No brain magnetic resonance imaging or ultrasound was performed.

METHODS

Genetic Analysis

All participants provided informed consent for the studies conducted and ethical approval was obtained from the respective institutions. The *FLNA* coding sequence and intron-exon boundaries (NM_001456.3) were amplified and sequenced using genomic DNA extracted from peripheral blood lymphocytes of the mother of the proband (III-2) as no sample could be obtained from the index case. Familial segregation studies were subsequently performed.

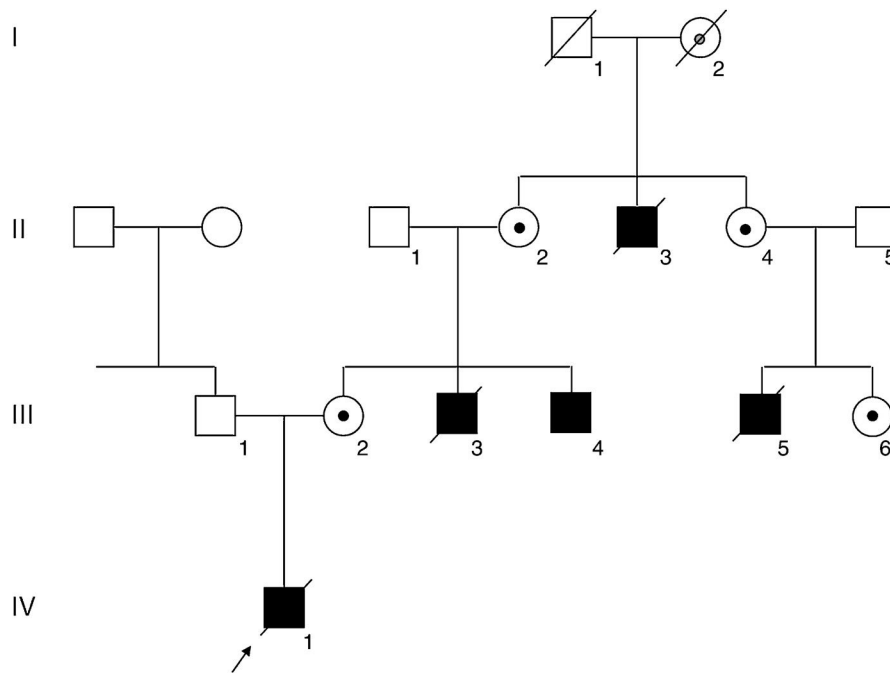


Figure 1. Pedigree of the family. Squares and circles depict male and female members, respectively. Affected members are shown in black, and the deceased members are crossed with a slash. An arrow indicates the index patient (IV-1). The *FLNA* mutation was confirmed in the living affected male III-4 and in carrier females II-2, II-4, III-2, and III-6, depicted with a black dot. Obligate carrier I-2 is depicted with a gray dot.

RNA Studies

Peripheral blood from the living affected male, III-4, was collected in PAXgene blood RNA tubes (PreAnalytiX GmbH, Qiagen NV/Becton Dickinson & company) for RNA extraction. RNA was extracted using the blood RNA kit (PreAnalytiX GmbH) and complementary DNA (cDNA) synthesis was performed using the High Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific), both according to the manufacturer's instructions. cDNA was amplified using an amplicon that contains *FLNA* exons 6–9 (NM_001456.3), using the oligonucleotides: 5'-ACACCAGGAG-GAGGCAAAG-3' located across the exon 6 and 7 junction, and 5'-GCTCTACCGTGCCTTCTGT-3' in exon 9. The products were visualized by electrophoresis and subsequently sequenced.

X-chromosome Inactivation

X-chromosome inactivation pattern was determined using genomic DNA from the lymphocytes of available female carriers by means of an indirect methylation quantitative assay in the androgen receptor gene, as previously described.³

Bioinformatic Analysis

Variants with a minor allele frequency below 0.1% were filtered according to available data in the gnomAD browser.⁴ *In silico* pathogenicity predictions were determined using Alamut Visual V2.9.0 including the following splicing simulation tools: SpliceSiteFinder-like, MaxEntScan, NNSPLICE, GeneSplicer and Human Splicing Finder. Variant classification was made according to the ACMG standards and guidelines for the interpretation of sequence variants.⁵

RESULTS

A heterozygous intronic variant, c.1066-3C>G, was detected in *FLNA* in the mother of the index case (III-2, Figure 2A), at position -3 from the splicing acceptor site in intron 7. This variant was absent

in more than 177600 alleles in the gnomAD database. Family members II-2, II-4, III-4, and III-6 also tested positive for the same variant, confirming segregation in all available affected and carrier members and denoting I-2 as an obligate carrier (Figure 1).

The canonical splice site acceptor in intron 7 was totally inactivated in the mutated sequence according to SpliceSiteFinder-like, MaxEntScan and GeneSplicer, and significantly weakened (76.17 vs 86.47) as predicted by Human Splicing Finder (Figure 2B). According to *in silico* splicing predictions, a cryptic acceptor site exists 24 bp downstream within exon 8, potentially resulting in the rescue of the protein reading frame (p.Val356_Gln363del). This prediction was confirmed by RNA studies from the peripheral blood of patient III-4 (Figure 2C).

The X-chromosome inactivation pattern was determined for female carriers III-2, II-2, and III-6. They all showed a random pattern of inactivation (50:50, 45:55 and 44:56, respectively). Female carrier II-4 was uninformative for the polymorphism employed in this assay.

DISCUSSION

Both syndromic and isolated forms have been described for hereditary valve disease. Connective tissue disorders such as Marfan syndrome and more rarely Loeys-Dietz syndrome, show mitral valve prolapse as well as other valve dysfunctions as a part of a wider vascular, ocular, and skeletal clinical spectrum.⁶ Ehlers-Danlos syndrome, especially the autosomal recessive cardiac valvular form, caused by mutations in *COL1A2*, also present with left-side valve manifestations in different degrees of severity.⁷

The XCVD was the first nonsyndromic valve dystrophy to be genetically characterized by linkage analysis showing defects in filamin A as the underlying etiology.⁸ It is distinguished by early-onset bilateral multivalve dysfunction with predominant left involvement, likely due to the higher hemodynamic stress associated with mitral and aortic valves.² It is inherited in an X-linked, apparently recessive manner that typically shows complete

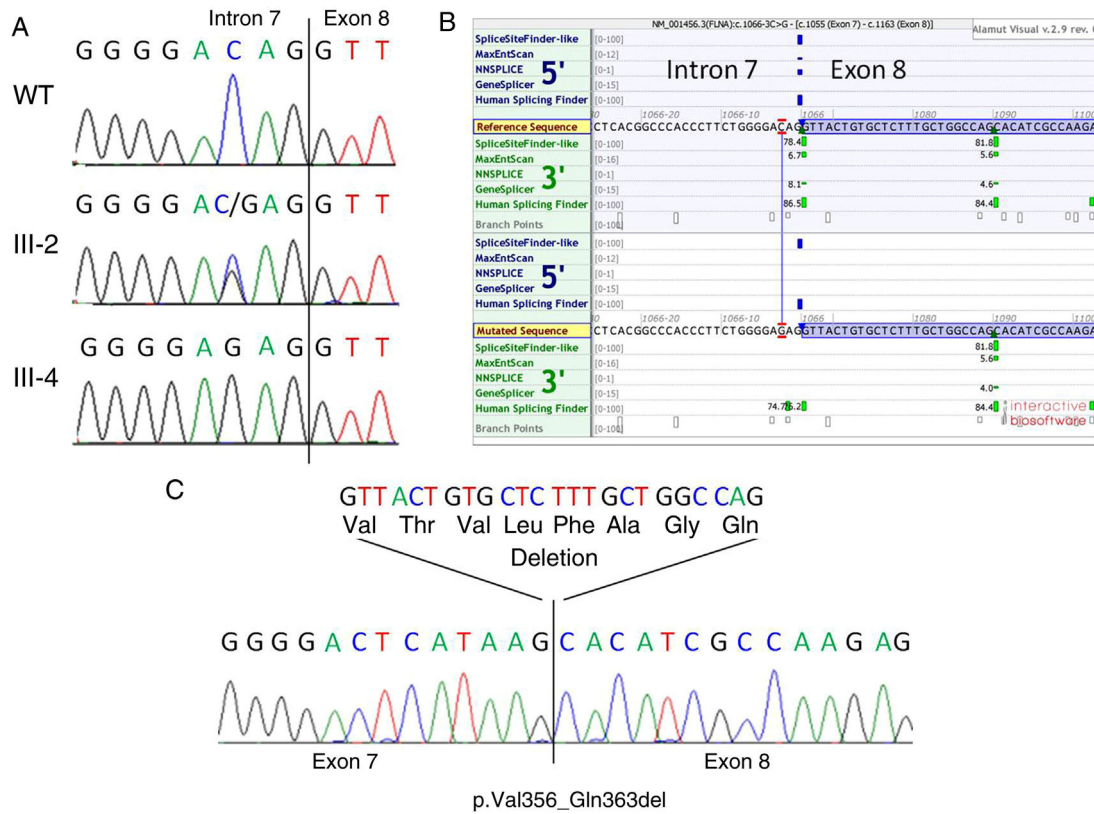


Figure 2. Genetic studies. A: Genomic sequencing chromatogram showing the c.1066-3C>G variant in a normal control (WT, upper panel), heterozygous female carrier (III-2, middle panel) and hemizygous affected male (III-4, lower panel). The intron 7-exon 8 boundary is indicated. B: *In silico* splicing predictions showing the consequence of the variant on the acceptor site of intron 7. Note that a strong cryptic acceptor site is located 24 bp downstream in exon 8. C: cDNA sequencing of patient III-4 showing that indeed the canonical splice acceptor site in intron 7 is abolished in the mutant cDNA and that the alternative downstream acceptor site in exon 8 is used. This results in the in-frame deletion of 24 bp (8 amino acids).

penetrance in males and a subclinical reduced expression among female carriers.⁸

However, *FLNA* mutations have also been associated with a wide variety of distinct genetic diseases including periventricular nodular heterotopia (PVNH, MIM 300049),⁹ FG syndrome type 2 (MIM 300321), neuronal intestinal pseudo-obstruction (MIM 300048), terminal osseous dysplasia (MIM 300244), and the otopalatodigital spectrum disorders otopalatodigital types I (MIM 311300) and II (MIM 304120), Melnick-Needles syndrome (MIM 309350) and frontometaphyseal dysplasia (MIM 305620).¹⁰ It has been proposed that total and partial loss of filamin A function result in neuronal migration disorders and valvular dystrophies, respectively, whereas a gain of function mechanism seems to be associated with skeletal dysplasias.¹⁰ Nevertheless, exceptions to this model have been observed in phenotypic outcome, tissue expression, and expected male lethality.^{11–13} Mixed phenotypes have also been described,¹⁴ including transcript-dependent opposite functional effects of single variants.^{15,16}

Severe mitral and pulmonary valve defects have been occasionally observed in patients with PVNH.^{17,18} Before these reports, bicuspid aortic valve, aortic root dilatation, and patent ductus arteriosus were the only cardiovascular manifestations that had been associated with PVNH. Moreover, an Ehlers-Danlos syndrome-like phenotype including joint hypermobility and skin hyperlaxity has also been found in patients with *FLNA*-related PVNH.^{19–21} Valvular defects and connective tissue disorders are both included in a single extracellular matrix-related etiologic spectrum and, in fact, both conditions have been concurrently observed in filamin A patients.^{22–25} Unlike skeletal dysplasias, all

these defects are apparently associated with a complete or partial loss of function of filamin A and may be part of a clinical continuum. Thus, *FLNA*-related XCVD should not be considered a nonsyndromic form of valvular dystrophy, but rather a pleiotropic disorder with variable expressivity, possibly related to the functional alteration and impact of the mutation.

Indeed, among previously reported cases with XCVD, connective tissue manifestations and even PVNH seem to be common in patients in whom those features were examined (Table). Marfanoid skeletal features are routinely tested when congenital valvular defects are found, whereas PVNH, even in the absence of epilepsy or seizures, can only be ruled out by brain magnetic resonance imaging. On the other hand, the only families apparently showing PVNH have unspecified *FLNA* mutations not revealing the affected domain or the impact of the mutation (Table).

Nevertheless, the individuals reported in our study have no extracardiac, neurologic, skeletal, or cutaneous manifestations. The index patient was born with an atrial septal defect, a previously reported malformation in association with defects in filamin A. Notably, the 2 other patients with XCVD and this cardiac defect carried both the same mutation, also in Ig-like repeat 1 within the Rod 1 domain (Table). Vascular defects present in our index patient have also been described in patients with PVNH, and in general, cardiovascular defects are common in *FLNA*-associated skeletal dysplasias.¹⁴

Filamin A is a ubiquitous protein that stabilizes actin filaments and links them to membrane glycoproteins. It has also been reported to interact with many other proteins such as integrins, transmembrane receptor complexes, and second messengers, with

Table

Summary of Cardiac and Extracardiac Phenotypes and Genetic Findings in Patients With X-linked Cardiac Valvular Dysplasia

| Family | FLNA Rod 1 Ig-like repeat | Predicted effect | Male phenotype | | | | | Female phenotype | Reference |
|--------|---------------------------|-----------------------|--|---|------|--|--|------------------------------|------------------------------------|
| | | | Valvular | Cardiac | CNS | Connective tissue | Other | | |
| 1 | ND | ND | Insufficiency | Cardiomegaly Hypertrophy PVC | | | | Leg pain Headaches | Monteleone and Fagan ²⁶ |
| 2 | 4 | Nonsynonymous variant | Prolapse Thickening Regurgitation | | | | | Regurgitation | Kyndt et al. ⁸ |
| 3 | 1* | Nonsynonymous variant | Prolapse Thickening Insufficiency Degeneration BAV | ASD Cardiomegaly Dilatation | | | | | Kyndt et al. ⁸ |
| 4 | 5 | Nonsynonymous variant | Thickening Dystrophy Insufficiency | | | | | | Kyndt et al. ⁸ |
| 5 | 5-7 | In-frame deletion | Thickening Regurgitation Insufficiency Stenosis | | | | | Mild insufficiency | Kyndt et al. ⁸ |
| 6 | 1* | Nonsynonymous variant | Prolapse Dysplasia Stenosis BAV | ASD Cardiomegaly SupraVT | | Joint hypermobility | VUR after pelvicaiectasis | | Bernstein et al. ²³ |
| 7 | NA | NA | Prolapse Regurgitation | | PVNH | Joint hypermobility Skin hyperextensibility Pectus excavatum | | Regurgitation PVNH DCM | Hoashi et al. ²⁴ |
| 8 | NA | NA | Prolapse Regurgitation Insufficiency | | PVNH | Joint hypermobility | Recurrent left ureteropelvic junction obstruction | Aortic aneurysm | Ma et al. ²⁵ |
| 9 | 4 | In-frame deletion | Thickening Insufficiency | Dilatation | | Joint hypermobility Skin hyperextensibility Papyraceous atrophic scar Scoliosis Bilateral myopia Bilateral inguinal hernia | | Skin hyperextensibility | Ritelli et al. ²² |
| 10 | 1 | In-frame deletion | Dysplasia Regurgitation Insufficiency | ASD PDA Dilatation Hypertrophy | | | | Mild regurgitation | This case |

ASD, atrial septal defect; BAV, bicuspid aortic valve; CNS, central nervous system; DCM, dilated cardiomyopathy; NA, not available; ND, not determined; PDA, patent ductus arteriosus; PVC, premature ventricular contractions; PVNH, periventricular nodular heterotopia; SupraVT, supraventricular tachycardia; VUR, vesicoureteral reflux.

* Both families carried the same variant.

a suggested role in signal transduction. Structurally, the conserved actin-binding domain at the N-terminus of the protein sequence is followed by 24 homologous Ig-like repeats of 96 amino acid residues each. This main structure is separated by 2 hinge domains into Rod 1 (repeats 1–15) and Rod 2 (repeats 16–23), which are followed by the C-terminal dimerization domain (repeat 24)^{27,28} (Figure 3).

Among the 9 previously described families with XCVD, mutations in *FLNA* were reported in 8 of them (patients of the family described by Monteleone and Fagan in 1969 were not tested).²⁶ In 2 of the 8 *FLNA*-positive families, the mutation/protein change is not specified,^{24,25} while in the remaining 6, all variants are located in the Ig repeats 1 to 7, within the Rod 1 domain (p.Gly288Arg, c.1829-1G>C, p.Pro637Gln, p.Val711Asp, p.Val761_Gln943del)^{8,22,23,29} (Figure 3). All these variants likely work as hypomorphic alleles by maintaining the reading frame of a presumably noncritical

domain and thus may preserve some residual protein activity since they have been observed in hemizygous living males. The variant c.1829-1G>C was predicted to abolish the splicing acceptor site of intron 12, with a suggested subsequent activation of a cryptic acceptor site 15 bp downstream in exon 13, which would result in an in-frame deletion of 5 consecutive residues (p.Phe611_Gly615del).²² Of note, a variant with the same predicted splicing effect was previously associated with PVNH and mild mitral valve dysfunction.¹⁸

The variant detected in our family was also predicted to destroy the canonical splicing acceptor site of intron 7, with the use of an alternative site located 24 bp downstream in exon 8. In our case, the splicing alteration was confirmed by RNA studies, resulting in an in-frame deletion of 8 conserved amino acid residues (p.Val356_Gln363del). In-frame variants located in proximal Rod 1 domain of *FLNA* are considered to be associated with an isolated

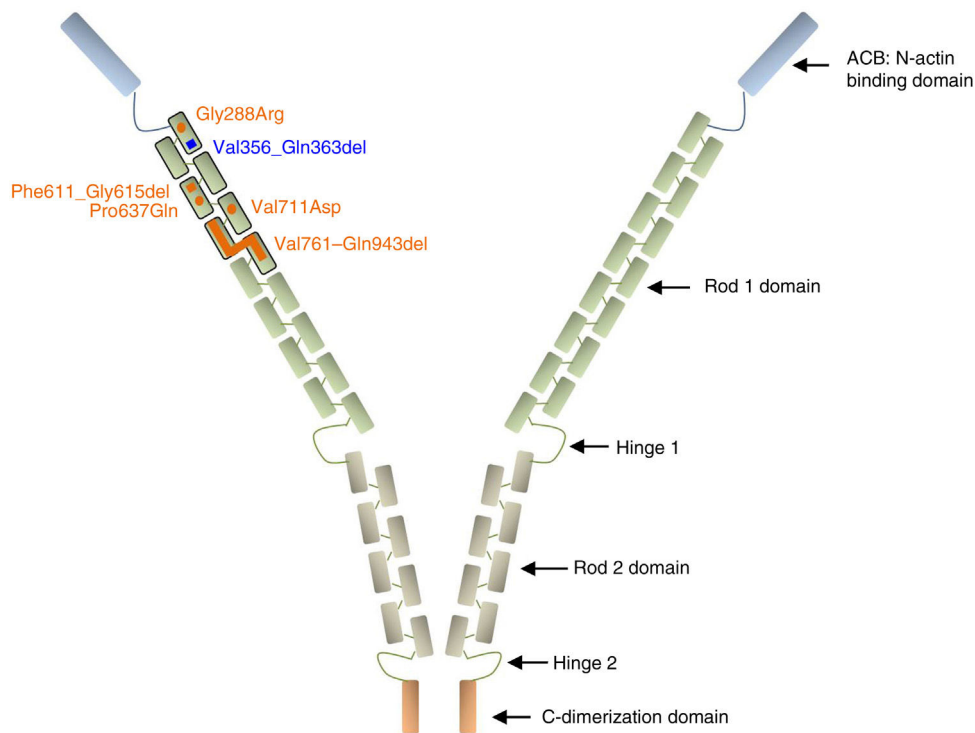


Figure 3. Schematic representation of the filamin A protein. Nonsynonymous variants are represented with circles and intragenic deletions with bars. All XCVD reported changes are depicted in orange except the one described in this report, which is shown in blue. Note that all alterations are clustered in the first 7 Ig-like repeats of the Rod 1 protein domain. References for each variant are cited in the text.

cardiac phenotype in affected male individuals. However, extracardiac findings in those patients suggest a rather continuous spectrum between partial and total loss of function variants (Table).

Cryptic acceptor sites, downstream of the canonical splice acceptor sites, are involved in the molecular mechanism of many human disorders. The use of these alternative sites to rescue the reading frame has been observed for other genes in which a partial loss of function may be better tolerated such as *COL1A1* and *COL1A2* in Ehlers-Danlos syndrome type VII vs osteogenesis imperfecta,³⁰ *COL5A1* in Ehlers-Danlos syndrome types I and II,³¹ *DMD* in Duchenne and Becker muscular dystrophies,³² and *LAMA2* in autosomal recessive limb-girdle vs congenital muscular dystrophy.³³ However, we cannot explain why patients with the 5-residue deletion in *FLNA* repeat 4 expressed such severe extracardiac manifestations, apparently observed even in their female ancestor, whereas the 4-exon deletion lacking repeats 5 to 7 and the present 8-residue deletion in repeat 1 are apparently restricted to an isolated cardiac phenotype. Different partner-dependent interactions in these regions, as well as other genetic factors, may be responsible for the observed phenotypes.

A role of skewed X-chromosome inactivation in the modulation of phenotypic expression in female carriers of *FLNA* pathogenic variants has also been proposed. However, an extremely skewed X-chromosome inactivation toward the mutated allele has only been persistently observed in *FLNA*-related skeletal dysplasias with congenital systemic involvement, which is consistent with a cell requirement of filamin A function during development.¹⁰ In loss of function alterations, however, no specific pattern has been described in association with the degree of disease manifestation.⁹ We observed a random X-chromosome inactivation pattern in 3 female carriers, all of whom were asymptomatic and one of them had mild, subclinical valve dysfunction. Thus, there seems to be no clinical correlation between phenotypic expression and X-chromosome inactivation pattern, supporting previous observations.

Notwithstanding the foregoing, it should be noted that the X-chromosome inactivation process can be tissue-dependent and its determination in genomic DNA from lymphocytes may not be applicable to affected tissues.

Filamin A is known to bind several partner proteins besides actin filaments, thus probably explaining the reported diversity in the resulting phenotypes. Valvular phenotypic expression and possibly other soft tissue manifestations of *FLNA*-related disorders are thought to be associated with the interaction of filamin A with members of the TGF- β signaling pathway, which is already implicated in connective tissue disorders showing aortic root aneurysms and mitral valve prolapse.³⁴ However, most of the interacting proteins identified to date do not bind to the N-terminal region of the Rod 1 domain, where mutations associated with XCVD have been described. Recently, a pathogenic role of these mutations has been functionally characterized as reducing cell spreading and migration capacities by means of an impaired Rho-GTPase signaling network modifying actin remodeling pathways that are important for cellular responses to mechanical stress, cell-extracellular matrix interactions, and epithelial-mesenchymal transformation.³⁵

CONCLUSIONS

Our findings support a role of in-frame variants in repeats 1–7 of the Rod 1 domain of filamin A in the expression of an XCVD main phenotype that can also include variably expressed connective tissue and possibly neurologic manifestations. An apparently isolated XCVD phenotype should prompt the search for these associated features beyond fibrillin and collagen disorders.³⁶ Identifying genetic alterations in *FLNA* in these patients will confirm their diagnosis, enhance prognosis, and improve genetic counseling to their families.

ACKNOWLEDGEMENTS

The authors wish to thank all patients and relatives for their kind support.

FUNDING

This work was partly supported by grant PI13/1450 from ISCIII (*Instituto de Salud Carlos III*) and grant SAF2015-66831-R from MINECO (*Ministerio de Economía, Industria y Competitividad*), and cofinanced by the FEDER (*Fondo Europeo de Desarrollo Regional*).

CONFLICTS OF INTEREST

None declared.

WHAT IS KNOWN ABOUT THE TOPIC?

- X-linked cardiac valvular dysplasia is a rare congenital disorder that is predominantly expressed in males. It is caused by mutations in *FLNA*, the gene encoding membrane-cytoskeletal mediator protein filamin A. Unlike other autosomal forms of valvular disease, including connective tissue disorders, it can occur in an isolated manner, although some overlap among different filamin A-related phenotypes has been observed. It is thought that the different domains, mutation effects, interacting proteins, and genetic factors are involved in this variability.

WHAT DOES THIS STUDY ADD?

- A novel mutation in *FLNA* has been found in a clinically described 3-generation family with XCVD. RNA studies in an affected male family member confirmed an in-frame deletion in the proximal Rod 1 domain and hence a partial loss of protein function as the predicted disease mechanism. Previously reported cases of XCVD have been reviewed, accounting for the predicted mutation location and protein damage when known, and extra-cardiac phenotypes when examined. A continuum of all filamin A loss of function-associated phenotypes is proposed, suggesting the need for their examination in patients with valvular heart disease.

SUPPLEMENTARY MATERIAL



Supplementary material associated with this article can be found in the online version available at <http://dx.doi.org/10.1016/j.rec.2017.10.013>

REFERENCES

1. Unger P, Clavel MA, Lindman BR, Mathieu P, Pibarot P. Pathophysiology and management of multivalvular disease. *Nat Rev Cardiol*. 2016;13:429–440.
2. Lardeux A, Kyndt F, Lecoite S, et al. Filamin-a-related myxomatous mitral valve dystrophy: genetic, echocardiographic and functional aspects. *J Cardiovasc Transl Res*. 2011;4:748–756.
3. Kubota T, Nonoyama S, Tonoki H, et al. A new assay for the analysis of X-chromosome inactivation based on methylation-specific PCR. *Hum Genet*. 1999;104:49–55.
4. gnomAD (genome Aggregation Database) browser beta. Available at: <http://gnomad.broadinstitute.org/>. Consulted 20 Sep 2017.
5. 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.
6. Judge DP, Rouf R, Habashi J, Dietz HC. Mitral valve disease in Marfan syndrome and related disorders. *J Cardiovasc Transl Res*. 2011;4:741–747.
7. Malfait F, Symoens S, Coucke P, Nunes L, De Almeida S, De Paepe A. Total absence of the alpha2(I) chain of collagen type I causes a rare form of Ehlers-Danlos syndrome with hypermobility and propensity to cardiac valvular problems. *J Med Genet*. 2006;43:e36.
8. Kyndt F, Gueffert JP, Probst V, et al. Mutations in the gene encoding filamin A as a cause for familial cardiac valvular dystrophy. *Circulation*. 2007;115:40–49.
9. Fox JW, Lamperti ED, Eksioglu YZ, et al. Mutations in filamin 1 prevent migration of cerebral cortical neurons in human periventricular heterotopia. *Neuron*. 1998;21:1315–1325.
10. Robertson SP, Twigg SR, Sutherland-Smith AJ, et al. Localized mutations in the gene encoding the cytoskeletal protein filamin A cause diverse malformations in humans. *Nat Genet*. 2003;33:487–491.
11. Sheen VL, Dixon PH, Fox JW, et al. Mutations in the X-linked filamin 1 gene cause periventricular nodular heterotopia in males as well as in females. *Hum Mol Genet*. 2001;10:1775–1783.
12. Moro F, Carozzo R, Veggiotti P, et al. Familial periventricular heterotopia: missense and distal truncating mutations of the *FLN1* gene. *Neurology*. 2002;58:916–921.
13. Hehr U, Hehr A, Uyanik G, Phelan E, Winkler J, Reardon W. A filamin A splice mutation resulting in a syndrome of facial dysmorphism, periventricular nodular heterotopia, and severe constipation reminiscent of cerebro-fronto-facial syndrome. *J Med Genet*. 2006;43:541–544.
14. Robertson SP, Jenkins ZA, Morgan T, et al. Frontometaphyseal dysplasia: mutations in *FLNA* and phenotypic diversity. *Am J Med Genet A*. 2006;140:1726–1736.
15. Zenker M, Rauch A, Winterpacht A, et al. A dual phenotype of periventricular nodular heterotopia and frontometaphyseal dysplasia in one patient caused by a single *FLNA* mutation leading to two functionally different aberrant transcripts. *Am J Hum Genet*. 2004;74:731–737.
16. Parrini E, Mei D, Pisanti MA, et al. Familial periventricular nodular heterotopia, epilepsy and Melnick-Needles Syndrome caused by a single *FLNA* mutation with combined gain of function and loss-of-function effects. *J Med Genet*. 2015;52:405–412.
17. Jefferies JL, Taylor MD, Rossano J, Belmont JW, Craigen WJ. Novel cardiac findings in periventricular nodular heterotopia. *Am J Med Genet A*. 2010;152A:165–168.
18. Reinstein E, Frenzt S, Morgan T, et al. Vascular and connective tissue anomalies associated with X-linked periventricular heterotopia due to mutations in *Filamin A*. *Eur J Hum Genet*. 2013;21:494–502.
19. Sheen VL, Jansen A, Chen MH, et al. *Filamin A* mutations cause periventricular heterotopia with Ehlers-Danlos syndrome. *Neurology*. 2005;64:254–262.
20. Gomez-Garre P, Seijo M, Gutierrez-Delgado E, et al. Ehlers-Danlos syndrome and periventricular nodular heterotopia in a Spanish family with a single *FLNA* mutation. *J Med Genet*. 2006;43:232–237.
21. Parrini E, Ramazzotti A, Dobyns WB, et al. Periventricular heterotopia: phenotypic heterogeneity and correlation with *Filamin A* mutations. *Brain*. 2006;129:1892–1906.
22. Ritelli M, Morlino S, Giacomuzzi E, et al. Ehlers-Danlos syndrome with lethal cardiac valvular dystrophy in males carrying a novel splice mutation in *FLNA*. *Am J Med Genet A*. 2017;173:169–176.
23. Bernstein JA, Bernstein D, Hehr U, Hudgins L. Familial cardiac valvulopathy due to *Filamin A* mutation. *Am J Med Genet A*. 2011;155A:2236–2241.
24. Hoashi T, Shiraishi I, Ichikawa H. Surgical experience for prolapse of both atrioventricular valves in a patient with *Filamin A* mutation. *Cardiol Young*. 2015;25:365–367.
25. Ma PH, Sachdeva R, Wilson EC, Guzzetta NA. Longitudinal Echocardiographic Evaluation of an Unusual Presentation of X-Linked Myxomatous Valvular Dystrophy Caused by *Filamin A* Mutation. *Semin Cardiothorac Vasc Anesth*. 2016;20:240–245.
26. Monteleone PL, Fagan LF. Possible X-linked congenital heart disease. *Circulation*. 1969;39:611–614.
27. Ehrlicher AJ, Nakamura F, Hartwig JH, Weitz DA, Stossel TP. Mechanical strain in actin networks regulates *FilGAP* and integrin binding to *Filamin A*. *Nature*. 2011;478:260–263.
28. Nakamura F, Stossel TP, Hartwig JH. The filamins: organizers of cell structure and function. *Cell Adh Migr*. 2011;5:160–169.
29. Newbury-Ecob RA, Zuccollo JM, Rutter N, Young ID. Sex linked valvular dysplasia. *J Med Genet*. 1993;30:873–874.
30. Byers PH, Duvic M, Atkinson M, et al. Ehlers-Danlos syndrome type VIIA and VIIB result from splice-junction mutations or genomic deletions that involve exon 6 in the *COL1A1* and *COL1A2* genes of type I collagen. *Am J Med Genet*. 1997;72:94–105.
31. Takahara K, Schwarze U, Imamura Y, et al. Order of intron removal influences multiple splice outcomes, including a two-exon skip, in a *COL5A1* acceptor-site mutation that results in abnormal pro-alpha1(V)N-propeptides and Ehlers-Danlos syndrome type I. *Am J Hum Genet*. 2002;71:451–465.
32. Tuffery-Giraud S, Chambert S, Demaille J, Claustres M. Point mutations in the dystrophin gene: evidence for frequent use of cryptic splice sites as a result of splicing defects. *Hum Mutat*. 1999;14:359–368.

33. Naom I, D'Alessandro M, Sewry CA, et al. Laminin alpha 2-chain gene mutations in two siblings presenting with limb-girdle muscular dystrophy. *Neuromuscul Disord.* 1998;8:495–501.
34. Sasaki A, Masuda Y, Ohta Y, Ikeda K, Watanabe K. Filamin associates with Smads and regulates transforming growth factor-beta signaling. *J Biol Chem.* 2001;276:17871–17877.
35. Duval D, Lardeux A, Le Tourneau T, et al. Valvular dystrophy associated filamin A mutations reveal a new role of its first repeats in small-GTPase regulation. *Biochim Biophys Acta.* 2014;1843:234–244.
36. Read RC, Thal AP, Wendt VE. Symptomatic valvular myxomatous transformation (the floppy valve syndrome). A possible forme fruste of the Marfan syndrome. *Circulation.* 1965;32:897–910.