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Case Report

Novel *FLNC* Gene Variant Associated with Hypertrophic Cardiomyopathy

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Abstract

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiomyopathy, affecting approximately 1 in 500 people. It is the most common genetic cardiomyopathy inherited as a Mendelian trait in approximately 50% of patients, mainly due to pathogenic variants in genes encoding sarcomeric proteins. Mutations in the sarcomeric protein filamin C (*FLNC*) gene, with a cytogenetic localization on 7q32.1, have been linked to hypertrophic cardiomyopathy, as they have been determined to increase the risk of ventricular arrhythmia and sudden death. We present the case of a patient with HCM recognized by magnetic resonance imaging and echocardiography with a family history of cardiopathies. The molecular study in this patient was performed by next-generation sequencing on the Illumina MiniSeq instrument, comparing the results with international databases. In genetic studies, a novel mutation in the protein *FLNC* was detected. It is heterozygous, missence type. It is a variant where Cytosine is changed by timina at position 6305 of the *FLNC* gene. This produces the change of the amino acid proline by leucine at position 2102 of the Filamin C protein. The

rare variant is located in Ig-like domain 19 within the ROD2 domain. This variant report suggests that there may indeed be a direct relationship between *FLNC* variants, mainly the ROD2 domain, and HCM. We think this new result should be considered for future genetic counseling of families affected by this type of cardiomyopathy.

Keywords
Hypertrophic cardiomyopathy; sarcomeric protein filamin C gene; ROD2 domain

1. Introduction

Familial heart disease is one of the leading causes of sudden death and heart failure. The fact that it is inherited puts family members at risk from childhood to age. These diseases can have different characteristics due to their genetic heterogeneity and variable clinical presentation, which makes diagnosis and prognosis difficult. They may have the exact etiology and present a variable phenotype due to phenomena such as reduced penetrance and variable expressivity, or they may present the same phenotype but have different genetic causes (locus heterogeneity) [1].

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiomyopathy, affecting approximately 1 in 500 people, although recent research suggests an even higher prevalence. The prognosis is usually favorable but variable, with sudden cardiac death (SCD) and severe congestive heart failure being the most serious outcomes. HCM is a diagnosis of exclusion; secondary causes of left ventricular hypertrophy (LVH), such as systemic hypertension, valvular and subvalvular aortic stenosis, and infiltrative cardiomyopathies must be excluded [2]. It is the most common genetic cardiomyopathy inherited as a Mendelian trait in approximately 50% of patients, mainly due to pathogenic variants in genes encoding sarcomeric proteins [3].

Mutations in the sarcomeric protein filamin C (*FLNC*) gene (with a cytogenetic localization on 7q32.1) have been linked to hypertrophic cardiomyopathy (HCM) (OMIM * 617047), as they have been determined to increase the risk of ventricular arrhythmia and sudden death [4].

Filamin C protein is expressed in striated muscle and is localized around the Z-disc, sarcolemma, myotendinous junction, and intercalated disks. Its primary function is to maintain the structural integrity of the sarcomere [5]. Filamin C contains an N-terminal actin-binding domain (ABD) and 24 C-terminal immunoglobulin (Ig)-like domains, which are responsible for protein dimerization and interacting with myotilin and FATZ-1 at Z-discs [6]. Filamin C plays a role in myofibril maintenance and myogenesis in cardiac and skeletal muscles, as demonstrated in human and animal models [7].

The *FLCN* gene has ~29.5 kb of genomic DNA containing 47 coding exons (NM_001458.4) [8]. Only a few publications on cohorts with *FLNC* variants have been published, and the pathophysiology of *FLNC*-related cardiomyopathy is poorly understood [9].

Genetic studies of cardiomyopathies began in Panama in 2019 to characterize the genome of patients suffering from these diseases.

This study aims to report a family with HCM and a new potentially pathogenic variant of the *FLNC* gene that will be useful for future population-based studies of this type of pathology.

2. Materials and Methods

The patient was referred from the cardiology department, where he had undergone several previous tests. The patient was evaluated at the Genetics Office of the Medical Genetics Service of the National Institute of Medical Genetics and Genomics in Panama City. The medical record was created. During the interview, a genealogical tree was established, and the patient was informed about the possible genetic origin of his disease.

Genomic DNA was isolated from peripheral blood leukocytes. DNA extraction and purification are performed with the QIAamp® genomic DNA Kit. Subsequently, the concentration and quality of DNA are evaluated by spectrometry and fluorometry (EPOCH and Qubit).

The sequencing reaction mixture is prepared with a capture technology-based assay (SOPHiA EXTENDED CARDIO SOLUTION™) followed by next-generation sequencing on the Illumina MiniSeq instrument.

The coding and splice regions (±5 bp) of 128 most relevant genes (470 kb target region) associated with arrhythmias (e.g., long QT/short QT syndrome or Brugada syndrome) and cardiomyopathies are analyzed. Alignment with the reference genome [GRC37.12 (HG19)] is performed.

Pathogenicity of variants was determined according to current American College of Medical Genetics and Genomics guidelines (pathogenic, likely pathogenic, uncertain significance (VUS), likely benign, or benign) [10]. The filtering and analysis of the genetic variants found were performed using the bioinformatics tools SOPHiA DDM® (Switzerland), Franklin by Genoox, and Varsome.

The Institutional Ethics Committee of the Caja del Seguro Social approved the research project "Genetic variants in Panamanian patients with hereditary heart disease at the National Institute of Medical Genetics and Genomics, Ciudad de La Salud." Code: CIEI-CSS-PVS-003-2024. This Ethics Committee Report applies to publications arising from this project.

3. Results

A 59-year-old patient with an early history of cardiac problems. At the age of 16, a left bundle branch block was accidentally detected on an electrocardiogram during a medical check-up. Since the age of 33, he began to experience symptoms of dyspnea, palpitations, fatigue, weakness, and sweating during moderate physical exertion. The patient has a medical background of arterial hypertension treated with ACE Inhibitors.

Initially, acute coronary syndrome was suspected, so an electrocardiogram and cardiac enzyme were performed. Electrocardiogram detected a sinus rhythm, and Peguero-Lopresti criteria for ventricular hypertrofy was found (40 mm). The Cornnel voltage and Sokolov-Lyon criterion were also positive. The patient showed severe asymmetric left ventricular hypertrophy with a cardiac mass index of 146 g/m^2, the maximum parietal thickness was septal (24 mm), and the patient also had severe hypertrophy of papillary muscles. Non-wall motion abnormalities were found. Non-left ventricular outflow tract obstruction was observed. Left Ventricular ejection fraction was average at 58%, but the longitudinal strain (SLG) was reduced in basal segments of anterolateral walls (-13%). (Figure 1). The echocardiogram showed atrial dilatation (52 ml/m^2), diastolic dysfunction, and elevated diastolic ventricular pressure.

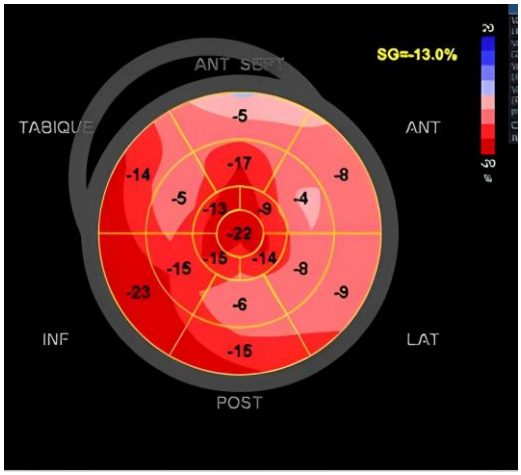

Figure 1 The longitudinal strain (SLG) was reduced in basal segments of anterolateral walls. (-13%).

In cardiac magnetic resonance imaging (MRI), ventricular hypertrophy was confirmed. In the T2 sequence, there was no evidence of myocardial edema, and no perfusion defects were observed. There was a significant late enhancement with patchy distribution in the septal wall, and myocardial fibrosis was detected in 38% of the myocardial mass (Figure 2).

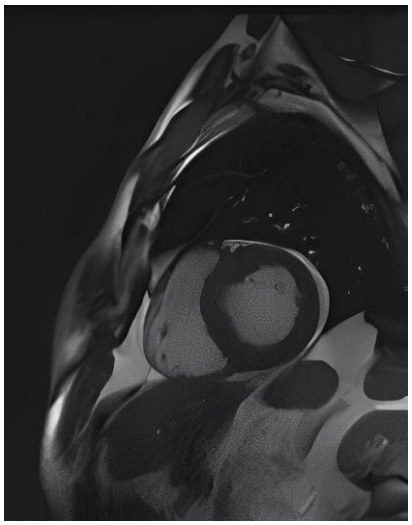

Figure 2 Cardiac MRI showed a significant late enhancement with patchy distribution in the septal wall, and myocardial fibrosis was detected in 38%.

Due to the patient's symptomatology and family history, he was referred to the medical genetics service to complement his study.

We estimate the risk of sudden cardiac death at 4%, and therapy with beta-blockers was started. A follow-up was carried out at the electrophysiology clinic. Months later, the patient presented typical paroxysmal atrial flutter, and the tricuspid cavus isthmus ablation was performed. Due to the extensive myocardial fibrosis (38%), the medical staff decided to implant a cardiac defibrillator for primary prevention.

Next-generation sequencing detected a likely pathogenic variant in the *FLNC* gene. Table 1.

Table 1 Novel mutation detected in *FLNC* gene.

Gene	Protein	Transcript	c-notation	p-Notation	Exon	protein-coding domains	Family history	Variant type
FLNC	Filamin C	NM_001458	c.6305C > T	p.(Pro2102Leu)	38	ROD2	yes	missense

The novel mutation in the protein is heterozygous missense type. It is a variant where Timina changes cytosine at position 6305 of the FLNC gene, which produces the change of the amino acid proline by leucine at position 2102 of the Filamin C protein. The mutation is located in Ig-like domain 19 within the ROD2 domain. Figure 3.

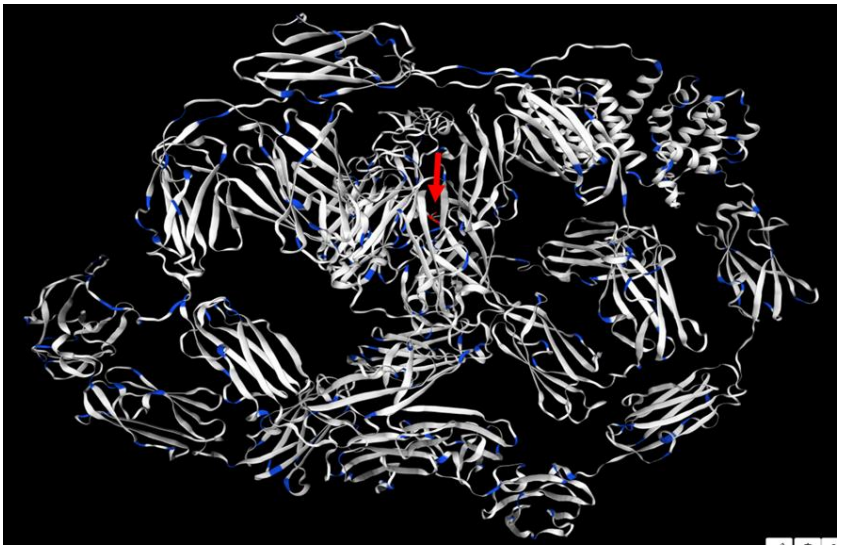

Figure 3 Swiss model of the *FLNC* protein structure. The location of the mutation (highlighted in red) within the structure is indicated by the arrow.

The next-generation sequencing results are compared with different international databases. Figure 4 and Figure 5.

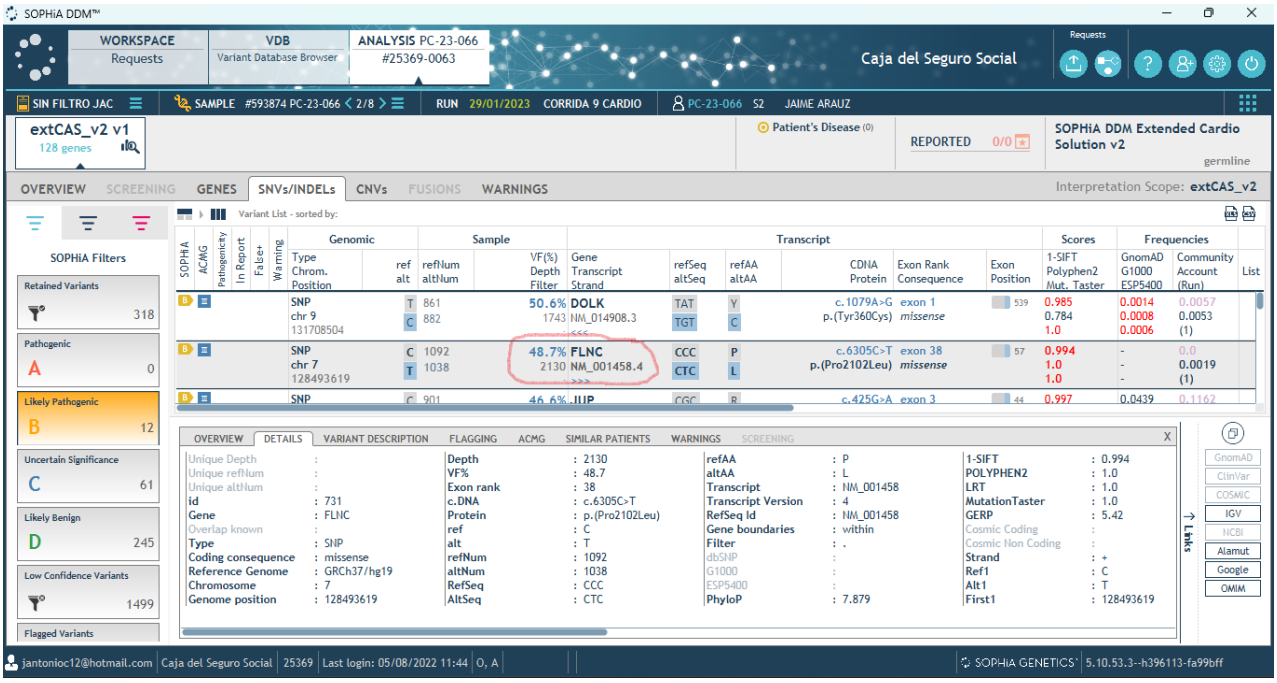

Figure 4 Result of next-generation sequencing compared in the SOPHIA database. Note the mutation in the *FLNC* gene (circulated) was reported as possibly pathogenic. This mutation detected by sequencing is the only one that corresponds to the patient's phenotype. (hypertrophic cardiomyopathy).

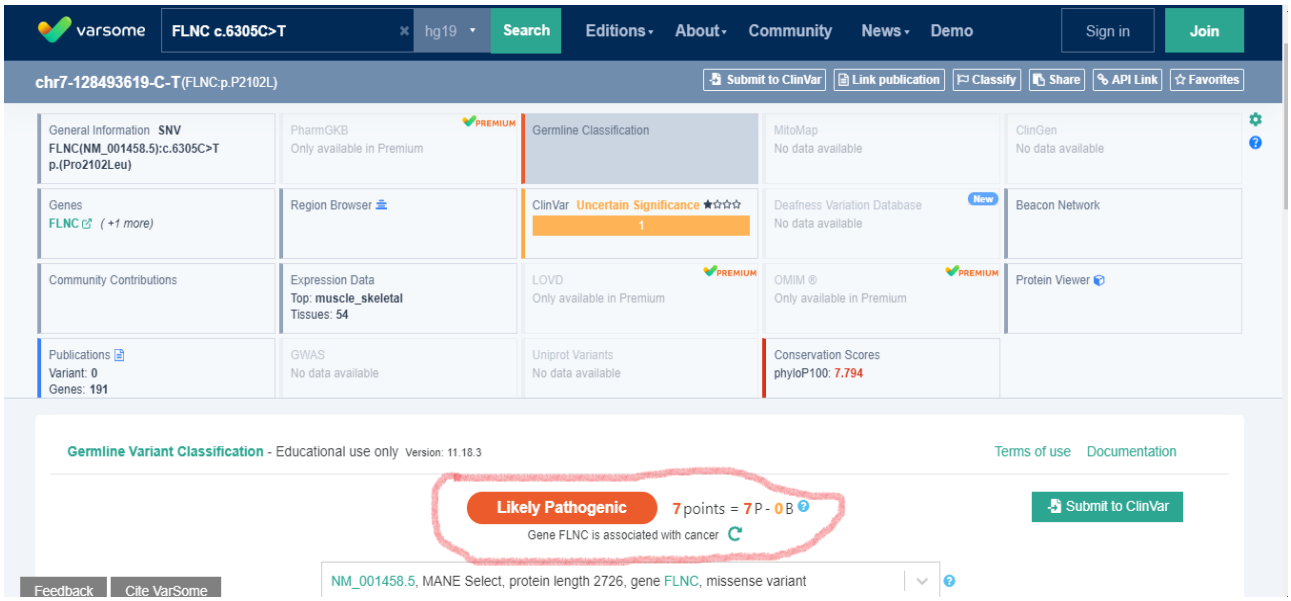

Figure 5 Result of the search in the VARSOME database. The mutation in the *FLNC* gene is reported as possibly pathogenic.

3.1 Family Segregation of This FLNC Variant

This is a large family from a rural area of Panama. The propositus has eight living siblings (4 males and 4 females) who range in age from 63 to 46 years old. There was a brother who died a year after birth. The cause of death is unknown. In this family, according to the survey conducted, there were

no cases of adoption, gamete donation, or any other possibility of false paternity. As we will see below, the segregation of the genetic variant suggests that it occurred through the paternal route.

The father of the propositus died at the age of 87 due to heart disease; two paternal uncles also died due to heart disease (89 and 79 years old, respectively). In the paternal line, there was a grandnephew of the father of the purpositus who died of sudden death at the age of 40 due to heart problems. However, none of the propositus' eight siblings suffer from any heart disease. One of the limitations of this study is that the Propositus' siblings could not be studied because they do not have health insurance to cover the costs of genetic testing.

4. Discussion

Filamin C, encoded by the *FLNC* gene, is an emerging cause of cardiac or skeletal myopathies. Previous studies demonstrated the effect of *FLNC* mutations on sarcomere structures [11-13].

The mean age of onset of HCM in *FLNC* carriers is 35.9 ± 14.8 years, which does not coincide with the onset of the first symptoms in our patient, which were earlier [9]. Dominant pathogenic variants in *FLNC* have been associated with the development of isolated cardiac phenotypes, including hypertrophic cardiomyopathy (HCM) [4].

According to our criteria, this new FLNC protein variant suggests it is likely pathogenic. Analyzing the possible segregation of this mutation in this family and taking into account [10].

We can state that this new variant meets the criteria of:

PP1 BS4 Segregation analysis

1. The *FLNC* gene was sequenced entirely (including entire introns and 5' and 3' UTRs), and no extra variant was found that could be associated with the patient's phenotype. Only the one reported by the authors. See annexes.
2. Several generations of the patient's family are affected by heart disease, including a distant relative of the patient's father, although they could not be studied genetically.

PM1 Mutational hot spot and critical and well-established functional domain

1. The protein domain, the ROD 2 domain, where the missense mutation is located, is widely described in the scientific literature as associated with hypertrophic cardiomyopathy [9, 11, 14]. Recent data on genotype-phenotype correlations between carriers of pathogenic *FLNC* variants point to a cluster of missense variants in this region and the appearance of the MHC phenotype [9, 11, 14]. This 18-21 cluster (within the ROD2 domain) interacts with Z-disk proteins, muscle development, and contraction-related proteins. Moreover, it is exciting since it is a crucial point for protein phosphorylation [15]. It has been indicated that this ROD2 subdomain is essential for *FLNC* dimerization and acquisition of secondary protein structure [11]. Thus, missense variants in the ROD2 subdomain can produce a misfolded protein with impaired cross-linking, leading to sarcomeric disorder [11, 16].

Other criteria that we believe support our suggestion that this finding is likely pathologic are as follows:

A) This variant is classified as possibly pathogenic by the following bioinformatics platforms: SOPHiA DDM® (Switzerland) and Varsome. However, Franklin by Genoox classifies it as a variant of uncertain significance. This variant is not found in CLINVAR, has not been identified as a single nucleotide polymorphism, is not found in the gnomAD Genomes and Exomes database, and has not been published in the literature.

B) Alder et al. declare, and I quote, "We considered as "likely pathogenic," unpublished missense variants with a frequency below 0.01% and unknown in our database, located in a functional domain of the protein and with pathogenicity prediction tools mainly (at least three out of four tools) in favor to a strong effect. An informative segregation analysis also supports the pathogenicity of these likely pathogenic variants, supported by an informative segregation analysis" [9]. This new variant we reported meets many of the criteria mentioned by these authors.

However, in this family, it is noteworthy that none of the siblings of the propositus seem to be affected by cardiopathies. Possible causes involved could be inheriting the gene without the pathologic variant, the phenomenon of reduced penetrance, and the less likely, but not impossible, gonadal mosaicism in the parent of the offspring in which the likely pathologic variant undergoes a second mutation and becomes a benign variant. Unfortunately, none of these hypotheses could be tested as it was impossible to study these individuals.

Although not all the databases consulted provide information on the possible pathogenicity of this mutation, the data provided in the present work suggest, once again, its possible implication as a pathogenic variant within the group of patients with HCM.

The pathophysiology of HCM linked to the genetic variants found, especially of the missense type, is poorly understood. This mutation reported in our study suggests that there may indeed be a direct relationship between *FLNC* mutations, mainly the ROD2 domain, and HCM. We think this new result should be considered for future genetic counseling of families affected by this type of cardiomyopathy.

Acknowledgments

We are grateful for the help of Dr Judith Pupo Balboa in the correct determination of the domain where the mutation was located within the *FLNC* protein.

Author Contributions

LAMR, JCE: Conceptualized and designed the research study. Reviewed and edited the manuscript. CVC: Clinical patient analysis. EMB and JSL: Wrote the draft manuscript. Clinical patient analysis. Reviewed the final versión. JCE and RGA: Were involved in supervision and funding acquisition. Reviewed and adequately modified the final versión. LSB, LAMR: Proper interpretation of results. Reviewed and edited the manuscript. All authors read and approved the final manuscript.

Competing Interests

The authors have declared that no competing interests exist.

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