Journal of Multidisciplinary Healthcare Dovepress

Open Access Full Text Article ORIGINAL RESEARCH

Identification of a Missense Mutation in the *FLNC* Gene from a Chinese Family with Restrictive Cardiomyopathy

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Objective: Restrictive cardiomyopathy (RCM) is a heterogenous cardiomyopathy with various causes, and genetic variants take an important part of the pathogenesis. Whole-exome sequencing (WES) is effective to discover genes that cause genetic diseases. By using WES, we attempted to identify the genetic cause of an RCM family and clarify the clinical diagnosis of the patient and then provide a personalized treatment plan.

Materials and Methods: Blood samples were obtained from the proband and his healthy parents. WES and Sanger sequencing were performed to identify the possible pathogenic gene. Co-segregation analysis was conducted for candidate variants, and the allele frequency was checked in databases including Ensembl, Exome Aggregation Consortium (ExAC) and Human Gene Mutation Database (HGMD). Furthermore, the potential effect of variant was predicted using various-free software such as SIFT, Polyphen-2 and Mutation Taster and the conservation was tested using multiple sequence alignments by ClustalX.

Results: The proband was a 20 years old boy with severe heart failure symptoms including dyspnea, massive ascites, edema of both lower limbs and chest congestion. Echocardiography showed significant biatrial enlargement, normal left ventricular wall thickness and preserved systolic function of both ventricles. A missense mutation in *FLNC* (c.6451G>A, p.G2151S), encoded filamin-C was detected in proband by WES and Sanger sequencing, while it was not be found in his parents, we supposed that the *FLNC* mutation (c.6451G>A, p.G2151S) may be a de-novo mutation. Through multiple functional predictions, we found that it is a deleterious mutation and the mutation in filamin-C could alter its structure and normal function, contributing to RCM.

Conclusion: Here, an *FLNC* missense mutation (c.6451G>A, p.G2151S) known to be pathogenic in hypertrophic cardiomyopathy, was found to be associated with RCM, indicating the genetic overlap among cardiomyopathies. This study provides insights into Phenotype-Genotype Correlations of RCM in patients with *FLNC* mutations.

Keywords: restrictive cardiomyopathy, whole-exome sequencing, filamin-C, *FLNC*

Introduction

Restrictive cardiomyopathy (RCM) (MIM, #617047) is a rare cardiomyopathy characterized by increased myocardial stiffness which leads to diastolic dysfunction, biatrial enlargement and arrhythmias and conduction disturbances.[1] Systolic function and ventricular wall thickness are usually not affected until later stages of the disease. Heart transplantation is often required because of the poor prognosis of severe heart failure (HF). RCM is the least common myocardial disease, so the prevalence of RCM is unknown.[2] Children and adolescents are more likely to develop RCM,

Received: 7 October 2024
Accepted: 13 November 2024
Published: 20 November 2024

which account for 2–5% of pediatric cardiomyopathies. However, an increasing number of elderly HF patients with ejection fraction preservation are thought to have various forms of RCM.[2] RCM is a heterogenous cardiomyopathy in terms of pathogenesis, clinical presentation, diagnostic evaluation, treatment, and prognosis. Due to the complexity of RCM, it is difficult to accurately diagnose and identify, resulting in delayed diagnosis. It has been reported that survival rates are 82%, 80%, and 68% after diagnosis at one, two, and five years.[3] Therefore, early and accurate diagnosis is extremely important for RCM patients.

Various factors can cause RCM, and genetic variants account for part of the pathogenesis. Various types of RCM can occur due to inherited or acquired predispositions, such as infiltration, storage disease, non-infiltrative, and endomyocardial disease.[4] While most cases of RCM are acquired, approximately, 30% of cases are familial RCM, so far, pathogenic mutations of RCM have been found in 19 different genes, most of which are located on autosomes.[4] Almost all of these mutations are autosomal dominant,[5] although they can also be autosomal recessive and X-linked.[6] The majority genetic mutations associated with RCM have been identified in 10 gene encoding sarcomere proteins, including *TPM1, TNNC1, TNNI3, MYH7, MYL2, MYL3, MYBPC3, TNNT2, ACTC1* and *TTN*.[3,7] These mutations affect the thin and thick filaments as well as titin filaments. Mutations in non-sarcomeric encoding genes have also been reported, such as *DES, MYPN, ACTN2, FLNC, LMNA, TMEM87B, CRYAB, BAG3* and *DCBLD2*.[7] Although it has been found that the gene encoding sarcomere proteins are the main gene of RCM, more and more studies have reported that non-sarcomeric encoding genes are also related to RCM, which indicates that there is still a lot of room for exploration of the genetic mechanism of RCM. What's more, a significant genetic overlap exists with other cardiomyopathies, especially with hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM).[7] The reason why mutations in the same gene lead to different cardiomyopathies remain unclear. In addition to genetic modifiers, diverse environmental factors may also contribute to these phenotypical differences. There may be phenotypic continuum of sarcomeric cardiomyopathies.

RCM has an overall poor prognosis and over the years, limited improvements in outcome have been made[8] and an overview of the RCM genetic landscape is still incomplete. However, improvements in the understanding of the genetic etiology of RCM have occurred over time on account of the advances in next-generation sequencing (NGS). Here, we discovered a de novo *FLNC* mutation (c.6451G>A, p.G2151S) from a sporadic RCM patient using whole-exome sequencing (WES) and sanger sequencing, and found it to be the most likely disease-causing mutation after mutation prediction analysis.

FLNC gene, located in human chromosome 7q32, contains 48 exons, encoding filamin-C protein, which is mainly expressed in skeletal muscle and myocardium. Filamin-C is an important structural crosslinker that crosslinks actin filaments into orthogonal networks in cortical cytoplasm and participates in the anchoring of membrane proteins for the actin cytoskeleton. Filamin-C contains an N-terminal actin-binding domain, 2 hinge regions and a domain with 24 Ig-like repeats (R1-R24), which are divided into ROD1 (R1-R15) and ROD2 (R16-R24) subdomains.[9] The dimerization of filamins through their Ig-like domains 24 is essential for the proper function of filamin. Mutations in the *FLNC* gene can cause a range of cardiomyopathy, and studies have found that about 1–8% of cardiomyopathy cases involve *FLNC* gene mutations.[10] Different mutation types of *FLNC* may affect the development of RCM through different mechanisms.[9] Non-truncating mutations of *FLNC* could result in filamin-C protein misfolding and aggregation, while for truncating mutations of *FLNC*, no aggregation of filamin-C protein was found in patients with RCM. These truncating *FLNC* mutations may cause filamin-C protein haploinsufficiency, which leads to a range of phenotypes. Valdes-Mas et al also confirmed that filamin-C protein aggregates in patients with *FLNC* mutation (c.6451G>A, p.G2151S) by Immunohistochemical staining.[11] Although we did not conduct further functional studies of this mutation in our study, we speculate that filamin-C protein aggregates resulting from *FLNC* mutation are still a possible mechanism for patient with RCM.

Materials and Methods

Study Object

A 20 years old boy diagnosed with RCM, was the proband from a Chinese family (Figure 1). After carefully inquiring about the family history of the proband, it was found that the proband's grandfather had coronary heart disease,

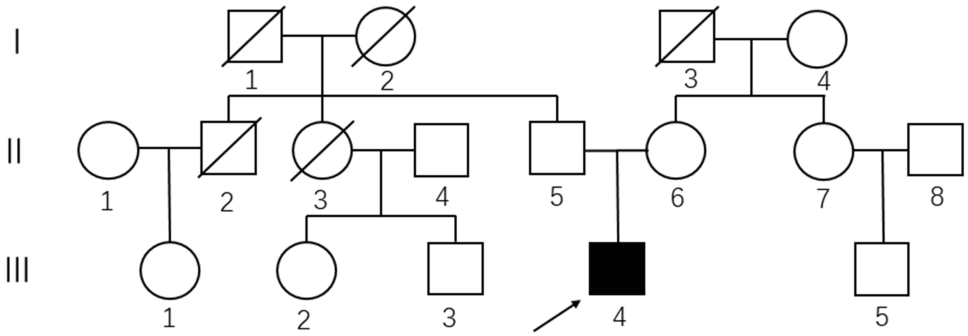

Figure 1 Pedigree of an RCM family. I, II, III refers to the family algebra of the family, respectively. Squares represent male relatives; circles represent female relatives; filled symbols indicate RCM patients; slants indicate dead members; arrow represent proband.

Parkinson's disease and cerebral infarction. Her grandmother had a history of stroke, and the rest of the family was healthy. Written informed consent was obtained by all participants.

Clinical Evaluation

Clinical evaluation included detailed medical history family history, physical examination, 12-lead ECG, two-dimensional and Doppler echocardiography. We also collected other inspection results, such as complete blood count, immune fixation electrophoresis, quantitative blood and urine light chain, quantitative blood Immunoglobulin, autoantibody and serum AFP level. Primary RCM was diagnosed based on the published criteria.[4]

Genetic Studies

Whole-Exome Sequencing

Peripheral blood samples were obtained from the patient and his healthy parents. Genomic DNA was extracted using DNeasy Blood & Tissue Kit (Cat#69506, QIAGEN, GmBH, Germany). WES was conduct for the genomic DNA of the patient. For sample to be sequenced, target captures were performed using SeqCap EZ MedExome Kit (Roche NimbleGen) and sequenced by an Illumina HiSeq X instrument (Illumina). The average sequencing depth of the exome sequencing target area was 199×, and the sequencing depth of 98.9% of the target sequence was over 20×. According to WES results, the candidate disease-causing variants were initially screened.

Sequence Analysis

We analyzed the WES results according to the following screening principles:

1. Sequencing depth ≥20×;
2. Variants with a frequency lower than 1% or never reported;
3. Variants located in the exon region or splice site region of the gene;
4. The mutation must be a non-synonymous mutation, a terminator mutation, or a terminator mutation to a non-terminator;
5. Mutation is predicted to be harmful mutation by functional prediction software.

Sanger Sequencing Validation

Candidate disease-causing variants were validated by sanger sequencing. DNA was amplified in a 25μL volume of PCR reaction system with 12.5μL 2×TSINGKE Master Mix, 0.5μL forward primer (5'-GGCACCTACATCATCAACATCAA -3'), 0.5μL reverse primer (5'-CCATCAGTTAAACCTCCTCCTCTG-3'), 2μL DNA and 9.5μL double steaming water with ABI 9700 PCR Amplifier (Applied Biosystem, America). The amplified family DNA fragments were commissioned for sanger sequencing by Tsingke Biotechnology Co., Ltd. Chromas 2 was used to analyze the sequencing results and find genetic mutations by comparing them with normal sequences.

Function Prediction

Variants were also checked for their frequency in databases including Ensembl (http://www.ensembl.org) and the Exome Aggregation Consortium (ExAC: http://exac.broadinstitute.org). We also searched the Human Gene Mutation Database (HGMD)[12] for known (published) associations between variants and human inherited disease. The potential effect of variants were predicted using software such as SIFT (http://sift.jcvi.org/), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) and Mutation Taster (http://www.mutationtaster.org/). The conservation was tested using multiple sequence alignments by ClustalX (http://www.clustal.org/). The 3D structure of filamin-C was built by Swiss-model (https://swissmodel.expasy.org/repository/uniprot/Q14315).[13]

Clinical Interpretation

The American Society for Medical Genetics and Genomics (ACMG) and the Society for Molecular Pathology (AMP) have publicized standards and guidelines for the interpretation of gene variants.[14] For each sequence variation, the guidelines integrate existing research evidence to classify the variation. When high-throughput sequencing is used to detect genes responsible for Mendelian genetic diseases, mutations in the sequence are divided into five categories: "pathogenic", "possibly pathogenic", "unclear", "possibly benign", and "benign". The publication of this standard greatly assists clinicians and geneticists in the assessment of identified variants. At present, this guideline is an important standard for genetic testing institutions to issue genetic testing reports. We used this guideline for clinical interpretation of the mutations identified in our study.

Results

Clinical Data

The proband was a 20 years old boy with severe HF symptoms including dyspnea, massive ascites, edema of both lower limbs and chest congestion. Echocardiography showed significant biatrial enlargement, normal left ventricular wall thickness and preserved systolic function of both ventricles (Table 1). Medical history showed the patient experienced persistent cardiac symptoms for years before admission to our hospital, and the cardiac transplantation was recommended when he was 19 years old because of the diagnose of RCM with severe HF, while rejected by the patient and his families (Table 2). Other tests were conducted to demonstrate the cause of RCM. Based on the results of examinations of complete blood count, immune fixation electrophoresis, quantitative blood and urine light chain, quantitative blood immunoglobulin, autoantibody and serum AFP level, we made the diagnosis by exclusion of the eosinophilia, amyloidosis, autoimmune diseases and neoplastic diseases (Table 3). Hypothesis was proposed that it may be genetic factors that contribute to the disease, though no other family members were affected. ECG and echocardiography showed his parents were normal. To test our hypothesis and find the cause of the disease in our patients, we performed genetic screening.

Table 1 Cardiac Ultrasound Results of Proband and Family Members

Measuring Part	Measured Value (cm)		Normal Reference Value (cm)	
	III-4	II-6	Children	Adult
Internal diameter of ascending aorta (AAO)	2.4	2.8	1.8–2.2	2.5–3.3
Left atrial diameter (LA)	5.5	3.3	2.0–2.4	2.7–3.5
Left ventricular diameter (LV)	4.6	4.2	3.0–4.0	3.5–5.3
Interventricular septal thickness (IVS)	0.9	0.9	0.5–0.8	0.8–1.1
Right atrial diameter (RA)	11.7	3.2	2.4–3.0	3.2–4.5
Right ventricular diameter (RV)	5.4	3.6	2.4–3.2	3.2–4.4
Main pulmonary artery diameter (PA)	2.0	2.3	1.4–1.9	2.4–2.8
Shortening fraction (FS)	32%	33%	>25%	>25%
Ejection fraction (EF)	60%	62%	50–70%	50–70%

Table 2 Medical History for the Proband

Date	Case History	Treatment
2010	Palpitations and shortness of breath after activity without inducement, relieved after rest	Untreated
2014.03	Color ultrasound indicated pericardium thickening in the left and right atrioventricular groove, and constrictive pericarditis was considered	Regular follow-up, no special treatment
2015.04	Discomfort increased, color ultrasound indicated that the right heart was larger than the right atrium, and the right heart function was reduced, accompanied by atrial fibrillation	Oral warfarin, spironolactone, furosemide
2015.12	Bloating discomfort starts to appear and gradually increases	-
2016.02	Ultrasound suggests restrictive cardiomyopathy	Suggested heart transplant, rejected. Outpatient drug therapy
2016.05	Abdominal distention discomfort, abdominal bulge	Symptomatic treatment, discharge after taking anticoagulation, diuretic drugs
2016.11	Palpitation and shortness of breath suddenly worsened, and the diagnosis was atrial fibrillation with rapid ventricular rate, severe heart failure, and abdominal effusion	After symptomatic treatment, a heart transplant was recommended, but rejected. He was discharged after his symptoms improved
2017.03	Chest tightness and abdominal distension aggravated again, symptoms did not ease after the local hospital, transferred to the Union Hospital, with "restrictive cardiomyopathy" into the cardiology department	Suggested heart transplant, rejected. He was discharged after his symptoms improved

Table 3 Clinical Examination for the Proband

Clinical Characteristics	Clinical Diagnose
Clinical symptoms	Abdominal distension, stomachache, chest distress, breathing difficulties, massive ascites, edema of both lower limbs
Vital signs	Body temperature: 36.6°C
	Heart rate: 100 beats/min (arrhythmia)
	Respiratory rate: 20 times/min
	Blood pressure: 90/60mmHg
	Sanity
Echocardiography examination	The right atrium was significantly enlarged, Right heart dysfunction
	Tricuspid valve function is significantly limited, extremely severe insufficiency. Mild mitral valve insufficiency
	Pericardial effusion and arrhythmia
	Comprehensive clinical consideration is RCM
Other Specs	
Full autoimmune test	Negative, exclude autoimmune disease
Full ANCA test	Negative, exclude autoimmune disease
Immunofixation electrophoresis	Normal, exclude amyloidosis
Urine Bence-Jones protein test	Negative, exclude amyloidosis
Blood cell count	Normal, exclude eosinophilia
AFP, CEA test	Normal, exclude tumor

Genetic Screening and Validation

The WES result of the patient shown a mass of variants. We filter the exonic variants with a minimum depth of 20×, after those variants with a frequency lower than 1% were selected, and finally, we chose nonsynonymous single nucleotide variant (SNV), stop-loss or stop-gain SNV, which were more likely to affect the structure and function of genes, for further analysis. Expression and functional profiles of associated genes were investigated, and we found that gene *FLNC* encoding filamin-C was the most likely candidate gene. A missense heterozygous mutation in *FLNC* (c.6451G>A, p. G2151S) was discovered in patient by WES and verified by sanger sequencing (Figure 2). We screened the proband's

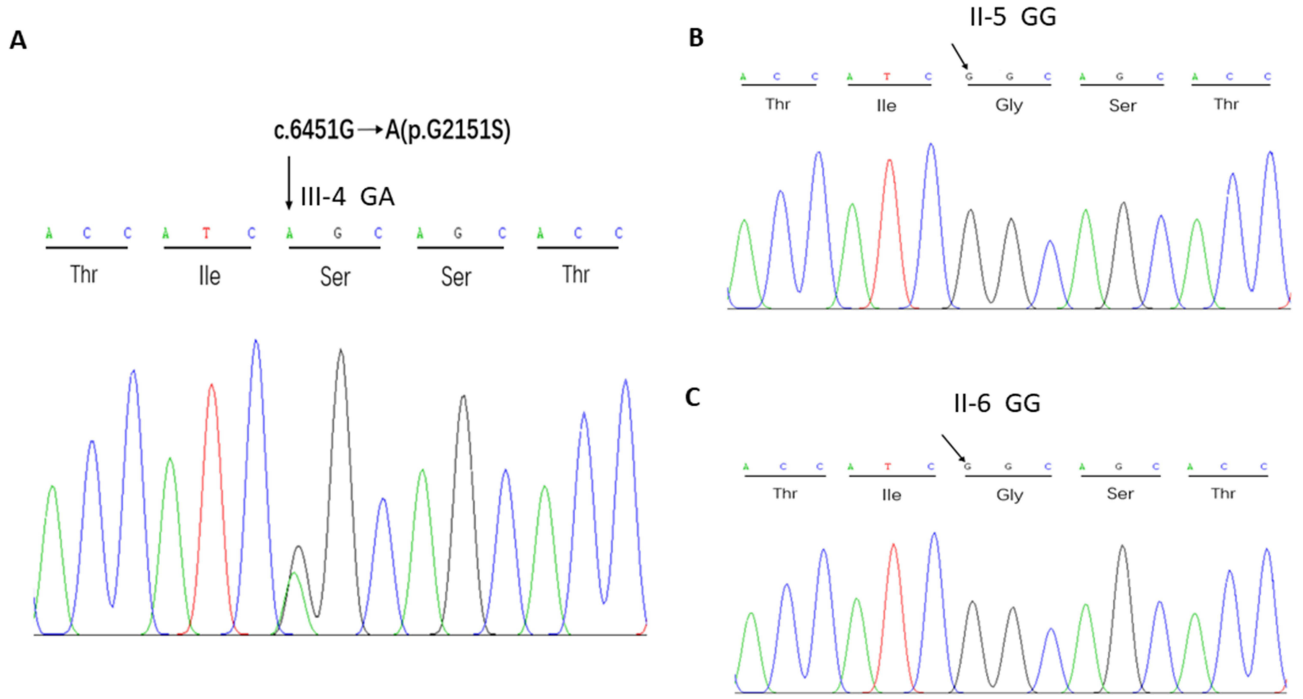

Figure 2 Sanger sequencing for RCM family. (**A**) Mutation of the *FLNC* (c.6451G>A, p.G2151S) identified in proband; (**B**) Partial sequence electropherogram of the *FLNC* gene with the nucleotide substitution identified in proband's father; (**C**) Partial sequence electropherogram of the *FLNC* gene with the nucleotide substitution identified in proband's mother. Arrow represents the position of the mutation of the *FLNC* (c.6451G>A).

family members for this mutation through sanger sequencing and found that his parents did not carry this mutation (Figure 2). The mutation was found to be co-isolated with the disease phenotype by family co-isolation analysis. We speculate that this mutation may be the cause of the disease in the patients. As the same mutation not be found in parents, we thought the *FLNC* mutation (c.6451G>A, p.G2151S) may be a de-novo mutation.

Functional Analysis of Pathogenic Mutation

To further test the hypothesis that this mutation is the cause of the disease in patients, we performed a comprehensive analysis of *FLNC* mutation (c.6451G>A, p.G2151S). Databases such as 1000 Genome Project and ExAC were examined, and no record was found about the mutation, while same mutation were found in HGMD, which was associated with HCM.[11] After forecast, all prediction algorithms, including SIFT, Polyphen-2 and Mutation Taster, showed a deleterious effect of *FLNC* mutation (c.6451G>A, p.G2151S), suggesting it is a deleterious mutation (Figure 3).

In addition, we found that the position of mutation and the near region showed high evolutionary conservation by using multiple sequence alignments (Figure 3). Generally, the conserved structure plays key roles in maintaining structural stability. When the conserved region is mutated, the stability of the structure will be destroyed, and the normal function of the protein will be affected. In terms of protein secondary structure, this mutation is located in the rod domain Ig-like domain of filamin-C, which is the main structure of filamin-C (Figure 4). There are many confirmed pathogenic mutations near this mutation (Figure 4). *FLNC* mutation (c.6451G>A, p.G2151S) may be located in the hot spot mutation region, further confirming the significance of this mutation. From the perspective of the tertiary structure of the protein, the filamin-C protein is encoded by 2725 amino acids. *FLNC* mutation (c.6451G>A, p.G2151S) caused the protein to change from Glycine to Serine at position 2151 (Figure 5). Glycine is a non-polar amino acid, while Serine is a polar amino acid. This important shift will have an impact on the structure of the filamin-C protein. We hypothesized that the mutation might affect the structure of the filamin-C protein and its normal function, then contributed to the disease.

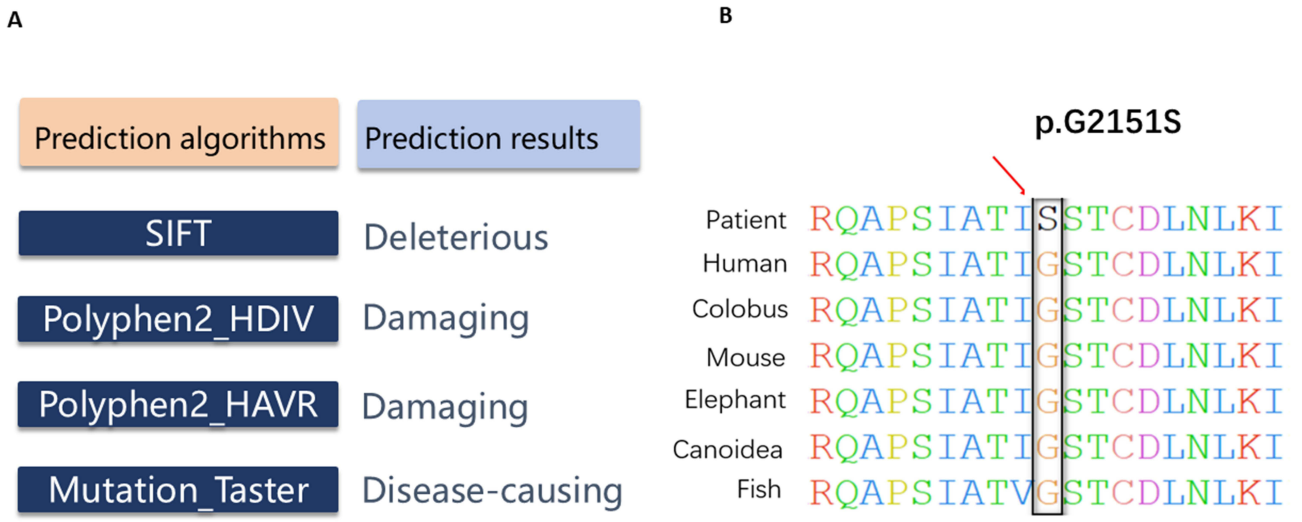

Figure 3 Prediction of mutation harmfulness and conservatism. (**A**) Prediction algorithms including SIFT, Polyphen-2 and Mutation Taster showed a deleterious effect of *FLNC* mutation (c.6451G>A, p.G2151S); (**B**) Alignment of this region of the cardiac *FLNC* amino acid sequence from multiple species demonstrating the conservation of the p.2151. Red arrow represents the position of the mutation of the *FLNC* (p.G2151S); Black box represents the amino acid of p.2151 in *FLNC*.

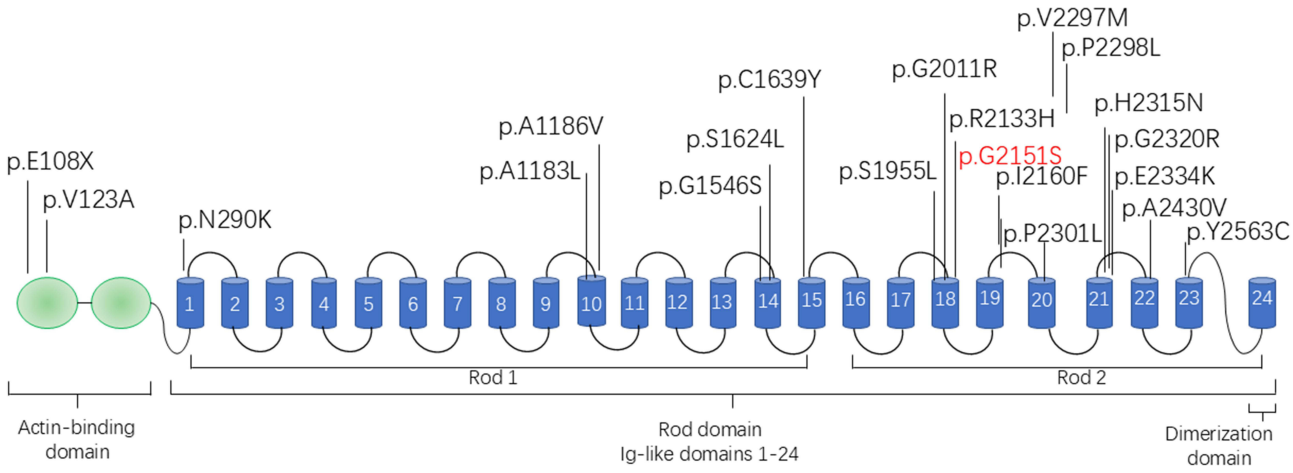

Figure 4 Structural diagram of the filamin-C and the positions of cardiomyopathy-associated mutations. Red text represents the position of the mutation of the *FLNC* (p.G2151S).

Clinical Interpretation of Pathogenic Mutation

The *FLNC* mutation (c.6451G>A, p.G2151S) was classed as "pathogenic" according to the 2015 ACMG-AMP Guidelines, which provided clinical interpretation of genetic variants. *FLNC* mutation (c.6451G>A, p.G2151S) is a de-novo mutation, and no record was found in databases such as 1000 Genome Project and ExAC, what's more, a variety of methods predict that this mutation will cause harmful effects on genes or gene products. This interpretation is supported by other evidence, which is summarized in Table 4.

Discussion

Using the WES and sanger sequencing, we identified a de-novo missense mutation (*FLNC*, c.6451G>A, p.G2151S) from an RCM family. Through multiple functional predictions, we found that it is a deleterious mutation, and the mutation might impact the structure and normal function of filamin-C protein, then contributed to the RCM.

RCM is a rare form of cardiomyopathy characterized by increased myocardial stiffness, which leads to diastolic dysfunction, biatrial enlargement and arrhythmias and conduction disturbances. RCM is a kind of restrictive diastolic

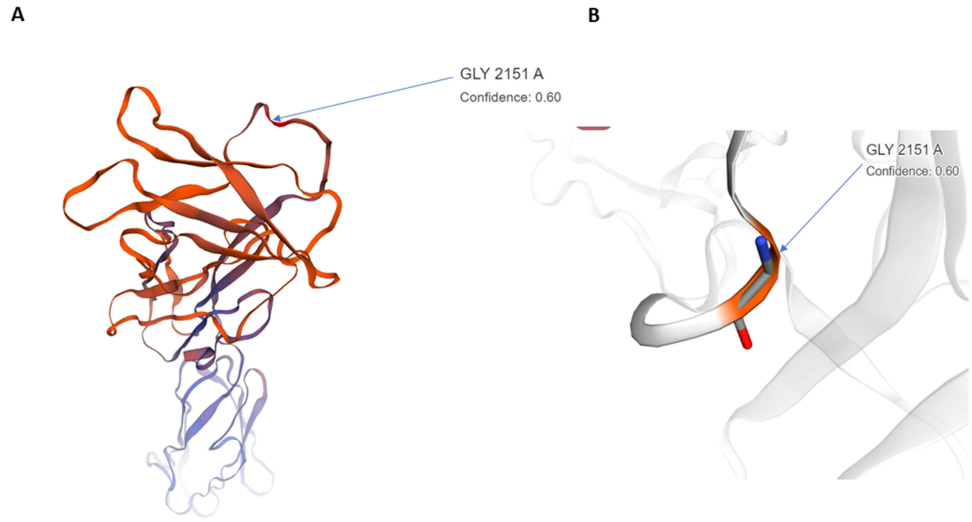

Figure 5 Part of the 3D structure of filamin-C protein using Swiss-model. (**A**) 3D structure of filamin-C protein from amino acid 2039 to 2302; (**B**) 3D structure of amino acid 2151 for filamin-C. Blue arrow represents the position of the mutation of the *FLNC* (p.G2151S).

dysfunction as the main characteristics of cardiomyopathy, its diagnosis currently lacks a recognized standard, need comprehensive diagnosis combined with clinical manifestation and imaging examination, echocardiography and cardiac magnetic resonance imaging (MRI) is an important auxiliary examination. RCM is a disease with poor prognosis and no effective drug treatment, it is beneficial to diagnose RCM at the early stage because of its poor prognosis so that early interference strategies can be initiated.

RCM is divided into primary and secondary, secondary RCM, which includes some specific myocardial diseases, that is, part of a multisystem disease. This is despite increased understanding of the genetic causes of cardiomyopathy over the past decade, and genetic studies on RCM are rare and complex. It is known that RCM can be the heart expression of sarcomeric protein associated diseases, such as mutations in *TNNI3*,[15] *TNNT2*,[16] *DES*,[17,18] *MYH7*,[19] *TTN*[20] and *BAG3*.[21] The same genes in different mutations can cause different types of cardiomyopathies, on the contrary, the phenotypic differences of the same gene mutation in different family members in the same family are also very large, sometimes the phenotype may overlap.[7,22] Menon et al[23] reported a family of autosomal dominant genetic cardiomyopathy with a disease-causing variant (*TNNT2*, p.I79N) in 2008, the proband was diagnosed with RCM at the age of 53, and family screening found that the clinical phenotypes of the affected family members were diverse, which could be expressed as RCM, HCM and DCM. With the development of NGS, it is a good choice to detect genetic causes of sporadic or familial RCM by WES especially when there is no other manifestation except for cardiac symptoms.

Here, we found an *FLNC* missense mutation (c.6451G>A, p.G2151S) that may be the disease-causing variant of a sporadic RCM with severe HF. Filamin-C protein (encoded by *FLNC*) plays key roles in sarcomere assembly and membrane proteins anchoring of actin cytoskeleton.[9,24,25] Filamin-C is essential for skeletal muscle as *Flnc* knockout mouse always dies of respiratory failure described by Dalkilic et al.[26] Although there are no signs of cardiac phenotypes

Table 4 Clinical Interpretation of the Mutation by ACMG/AMP 2015 Guideline

Mutation	Mutation type	Mutation Classification	Criteria	Strength of Criteria
c.6451G>A, p.G2151S	Missense mutation	Pathogenic	PS2	Strong
			PM2	Moderate
			PM5	Moderate
			PP1	Support
			PP2	Support
			PP3	Support
			PP4	Support

in model mouse,[26] some cases were reported that mutations in *FLNC* were associated with cardiomyopathies including HCM,[11] DCM[27] and RCM.[28] Filamin-C is located at the Z-disc periphery, costameres, and intercalated discs in cardiomyocytes. It involved in the maintenance of sarcomere stability. *FLNC* mutations resulted in the abnormalities in the Z-discs and sarcomere structures in patients with myopathy. Filamin-C is under constant and regular mechanical stress and experiences periodic unfolding and refolding, which inevitably leads to irreversible unfolding and functional loss. Ig-like repeat 18–21 domains are a mutational hotspot region, which interacts with Z-disc proteins, muscle development and contraction-related proteins, as well as a crucial point for protein phosphorylation.[25] Heat shock protein HSPB1 can interact with the compact structure formed by Ig-like repeat 18–21 domains of filamin-C to attenuate its extension in the stretching process.[29] In addition, the heat shock protein HSPB7 binds dimerization domain of filamin-C to enhance its resistance to mechanical stress-induced conformational changes.[30] *FLNC* mutation (c.6451G>A, p.G2151S) located at intradomain between Ig-like repeat domains 19 and 20, belonging to the ROD2 subdomain, which is key to interactions with several structural and signaling proteins. ROD2 subdomain is essential for *FLNC* dimerization and secondary protein structure acquisition.[31] Mutation in the ROD2 subdomain may precipitate a misfolded protein and impaired crosslinking, leading to sarcomere disarray and mechanotransduction impairment.[32] Different mutation types of *FLNC* may affect the development of RCM through different mechanisms.[9] *FLNC* mutations, of which most are non-truncating, are predicted to result in protein misfolding and aggregation, while for truncating mutations of *FLNC*, no aggregation of filamin-C protein was found in patients with RCM. These truncating *FLNC* mutations may cause filamin-C protein haploinsufficiency, which leads to a range of phenotypes. Non-truncating mutations such as p.V123A, p.R2133H, p.A2430V, p.S1624L, p.I2160F, p.Y2563C and p.P2298L have been preliminarily confirmed to affect filamin-C protein aggregation, which is usually concentrated around the nucleus.[9] Overexpressing p.A1183L and p.A1186V also resulted in filamin-C aggregation and Z-discs break.[9] However, not all non-truncating mutations of *FLNC* induce protein aggregation, for example, mutation pV2297M resulted in diminished sarcomeric localization while without protein aggregate formation.[33] The latest study also found that the mutations p.P2301L and p.E2334K do not cause protein aggregation.[31] The lack of an abnormal or "loss of function" of filamin-C protein may explain those phenomena. What's more, there are still some mutations associated with RCM that we do not know exactly how they work. More research is needed in the future to further explore the specific mechanism.

Valdes-Mas et al[11] reported a same *FLNC* mutation (c.6451G>A, p.G2151S) in a HCM patient, and filamin-C protein aggregates in the HCM patient were confirmed by Immunohistochemical staining. Our study complementarily demonstrated the deleterious effect of the *FLNC* mutation (c.6451G>A, p.G2151S) on cardiac phenotype and indicated intricate Phenotype–Genotype Correlations of cardiomyopathy with *FLNC* gene mutations. More comprehensive approaches should be attempted to elucidated mechanisms of RCM as well as other types of cardiomyopathy, in which many variants may contribute to the phenotype through an interaction way, or the disease process is affected by multi-factors as described by Ware and Cook.[34]

Due to the complex phenotype of RCM, there is no very accurate diagnosis and effective treatment. Compared with traditional single-gene disease research methods, WES has the advantages of short time, high throughput and high sensitivity. It has become the crucial means to search for pathogenic genes of single-gene disease and is of great significance for the pathogenesis research, diagnosis, treatment, prevention and prognosis of single-gene disease. If a genetic cause is clinically suspected, WES should be taken into account, which may be advantageous. Now, there are new treatments that target sarcomere. An allosteric inhibitor of myocardial specific myosin adenosine triphosphatase (MYK-461), which showed improvement in symptoms in a Phase 3 trial of HCM, may also be suitable for patients with RCM and fibroid mutations that lead to excessive actin cross-bridging.[35] In addition, small-molecule drugs, such as omecamtiv mecarbil and danicamtiv, that increase contractility may be particularly efficacious for patients with fibroid mutations and DCM.[36,37] The application of these new technologies and the development of new drugs will greatly help the diagnosis and treatment of cardiomyopathy, especially RCM.

Our findings broaden the genetic mutation map of cardiomyopathy, especially RCM, which has important clinical significance. A comprehensive analysis of the genetic and molecular causes of diseases helps to obtain an accurate diagnosis, especially for diseases where the clinical outcome is unclear or the pathology overlaps with other diseases. This mutation can be included as a potential target for genetic detection of cardiomyopathy. Genetic testing of

cardiomyopathy in patients with suspected hereditary cardiomyopathy can help to clarify the diagnosis and genetic etiology of patients. Moreover, through genetic screening, it is possible to exclude the disease of some family members in the family, reduce the economic burden and psychological burden of the family, and early detection and early intervention can be achieved for asymptomatic carriers.

Although *FLNC* mutation (C.6451G >A, p.G2151S) was found to be associated with hereditary RCM in our study, we could not detect the changes of filamin-C protein due to the lack of myocardial biopsy samples from patients. Moreover, how the *FLNC* mutation affects the filamin-C protein, leading to the RCM phenotype, is unclear. This will be our focus in the future.

Conclusion

Here, we reported a missense mutation of *FLNC* gene (c.6451G>A, p.G2151S), known to be pathogenic in HCM, was associated with RCM, indicating the genetic overlap among cardiomyopathies. The finding broadens the knowledge of genetic etiology of RCM and extends our knowledge of Phenotype–Genotype Correlations of RCM with *FLNC* gene mutations.

Ethics Approval and Consent to Participate

This study followed the guidelines set forth by the Declaration of Helsinki and passed the review of the Ethics Committee of Wuhan Union Hospital (UHCT-IEC-SOP-016-02-01). All participants signed a written informed consent form.

Consent to Publish

Consent for publication was obtained from all participants in this study.

Acknowledgments

We appreciate all participations and supports of this study.

Disclosure

No conflict interests exist in this work.

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