



Clinical exome sequencing revealed that *FLNC* variants contribute to the early diagnosis of cardiomyopathies in infant patients

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Background: *FLNC* encodes actin-binding protein and is mainly concentrated in skeletal and cardiac muscle. Mutations in *FLNC* were found in cardiomyopathies. To date, studies on *FLNC*-cardiomyopathies have mainly been reported in adults. There are limited studies that have investigated *FLNC* variants in pediatric patients with cardiomyopathies.

Methods: We summarized the patients who carried rare variants of *FLNC* from May 2016 to May 2019 in the Center for Molecular Medicine, Children's Hospital of Fudan University, from clinical exome sequencing data.

Results: A total of 5 patients with *FLNC* rare variants were included. Of them, 3 were male and 2 were female. The median age was 3 months (range from 19 days to 30 months). A1186V was a known pathogenic variant reported in pediatric patients with cardiomyopathy (PMID: 29858533), and the other four variants were novel. In the four novel variants, there are one splicing (c.2265+4del) and three missense (p.R441I, p.C1639Y, and p.A2648S). Two patients (patients 1 and 3) were diagnosed with restrictive cardiomyopathy, two patients (patients 2 and 5) were diagnosed with dilated cardiomyopathy, and one patient (patient 4) was diagnosed with arrhythmia.

Conclusions: All five patients have survived to date. In summary, *FLNC* rare variants identified by clinical exome sequencing provide genetic evidence to make early diagnosis of cardiomyopathy in infant patients.

Keywords: *FLNC*; pediatrics; cardiomyopathy

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Introduction

FLNC is the coding gene of filamin C, and is an actin-binding protein, mainly concentrated in skeletal and cardiac muscle (1). *FLNC* is distributed in the Z disk and the sarcomere in the formation of muscle, suggesting that the gene is involved in sarcomere formation (2). The *FLNC* gene is located in chr7q32-q35, comprises 47 exons, and belongs to the filamin family. This family, including Filamin A, Filamin B and Filamin C, are large molecule proteins mainly distributed in the cytoplasm, where they maintain the three-dimensional structure of cortical actin cross-linking protein and play an important role in the adhesion between cells and cell and interstitial and intracellular signaling pathways (3). *FLNA*, *FLNB* and *FLNC* genes are highly conserved and widely expressed in the development process. *FLNC* mutation was first found in patients with filamin associated myofibrillar myopathy (4).

In 2014, Valdes-Mas *et al.* found by exome sequencing that A1539T in *FLNC* in a family segregates with hereditary cardiomyopathy. Since then, approximately 90 mutations in *FLNC* have been found in cardiomyopathies, including restrictive cardiomyopathy (RCM) (5), hypertrophic cardiomyopathy (HCM) (6), and dilated cardiomyopathy (DCM) (7). These mutations can be divided into the following types: missense, splicing, small deletions, small insertions, small indels, gross deletions, and gross insertions. To date, the genetic roles of *FLNC* mutations have mainly been reported in adults. There are limited studies investigating the relationship between *FLNC* variants and cardiac phenotypes in pediatric patients.

In the present study, we summarized the variants of *FLNC* in pediatric patients who have the phenotype of cardiomyopathy from a large cohort of clinical exome sequencing (CES) data to identify *FLNC* variants in patients with cardiomyopathy. We sought to provide new variants and more evidence for *FLNC* variants in pediatric patients.

Methods

Ethical compliance

The criteria for genetic testing were approved by the ethics committee of Children's Hospital of Fudan University (2016-235). Pretest counseling was performed by physicians, and appropriate informed consent was signed by the patient's parents in clinics. The data we analyzed were from pediatric patients with cardiomyopathy who underwent CES from May 2016 to May 2019 in the Center for Molecular Medicine,

Children's Hospital of Fudan University and the Maternal & Child Health Hospital of Guangxi Zhuang Autonomous Region. The clinical manifestations and phenotype data were collected from medical record, and family members' blood was collected for parental Sanger confirmation.

Clinical exome sequencing

Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. High-throughput sequencing was performed using standard protocols in the Clinical Laboratory Improvement Amendments compliant sequencing laboratory in Wuxi NEXTCODE (288 Fute Zhong Road, Waigaoqiao Free Trade Zone Shanghai 200131, China CLIA ID 99D2064856). The Agilent ClearSeq Inherited Disease Kit Illumina Cluster and SBS kit were used for clinical exome sequencing. Sequencing was performed using next-generation sequencing on an Illumina HiSeq 2000/2500 platform. It can cover a minimum of 98% of the genome with 20X coverage or higher. Clean reads were aligned to the reference human genome (UCSC hg19), and variant calling was performed using the Genome Analysis Tool Kit, (GATK) v.2.3-9 by applying base quality score recalibration, indel realignment and performing SNP and INDEL discovery and genotyping. The three major databases for known pathogenic and likely pathogenic mutations were used including ClinVar, Online Mendelian Inheritance in Man (OMIM), and the Human Gene Mutation Database (HGMD, professional version). Numerous missense functional predictors, noncoding regulatory region annotations, and others were included. To filter out variants with high frequency, the allelic frequencies were annotated from the public datasets from the 1000 Genomes, the Exome Sequencing Project (EVS6500) and Exome Aggregation Consortium (ExAC), Kyoto, and Dutch allelic frequency databases. Data interpretation followed our previous work (8). All aligned reads, variants, and annotations were stored according to the genomic position in the WuXi NextCODE Genomically Ordered Relational Database (GORdb) to facilitate more rapid access by the Clinical Sequence Analyzer (CSA) user interface, Sequence Miner, and the WuXi NextCODE Genome Browser.

Sanger sequencing

Variants of patient and parents were validated by Sanger

sequencing using an automated sequencer (3500XL Genetic Analyzer, Applied Biosystems, USA). Primers were designed for polymerase chain reaction amplification using Primer Premier 5.0 software. Sequence analysis was performed using MutationSurveyor (SoftGenetics®, State College, PA, USA).

Protein structure analysis

The structures of FLNC in wild type and mutants were analyzed by UniProt (<http://www.uniprot.org/>), Mutalyzer (<https://www.mutalyzer.nl/>), and SWISS-MODEL (<https://www.swissmodel.expasy.org>). The FASTA sequences of FLNC from UniProt were downloaded. We selected 3 templates, including 6ew1.1 (9), 2j3s.1 (10), and 4m9p.1 (11), as the protein structure template. We used a computer program MODELLER (12) for automated modeling. Protein structures were visualized by PyMOL 2.3.

Results

Patient cohort

The present study enrolled 122 patients with cardiomyopathy for whom CES was conducted. Five patients were identified with FLNC variants, accounting for 4.1% (5/122) of all the enrolled patients.

Clinical characteristics

Five cases (3 males and 2 females) with cardiomyopathy characterized by similar phenotypes of severe malformations in the cardiac phenotypes were summarized. The median age was 3 months (range: 19 days to 30 months). All patients had no family histories. The clinical characteristics of each patient are shown in *Table 1*. Patient 1 and patient 3 were diagnosed with RCM. Patient 2 and patient 5 were diagnosed with DCM. Patient 4 was diagnosed with arrhythmia. All of them had an abnormal echocardiography (ECHO) result. All patients survived up to the last follow-up.

Patient 1 was a 2-year-6-month-old boy who was delivered after a full-term pregnancy and had no history of suffocation and rescue at birth. The patient presented cough and gasping for 1 week. ECHO showed that he had right atrial enlargement, left atrial enlargement, mitral regurgitation, tricuspid regurgitation, cardiac insufficiency, and pulmonary hypertension. Serum biochemistry tests showed high levels of pro-brain natriuretic peptide (Pro-BNP; 24,655 pg/mL; normal range <125 pg/mL) and LDH

(403 mg/L; normal range: 110–290 IU/L).

Patient 2 was a 3-month-3-day-old boy. His delivery is normal and had no history of suffocation and rescue at birth. He presented to our hospital for severe shortness of breath at 3 months. ECHO showed that he had left atrial enlargement, left ventricle enlargement, mitral regurgitation, patent foramen ovale (PFO), and cardiac insufficiency. Serum biochemistry tests showed high levels of Pro-BNP (15,017 pg/mL).

Patient 3 was a 2-year-old girl. She was born by cesarean section. At 15 months of age, the ECHO showed that she had pulmonary hypertension. The girl breaths faster after slight activities and presents with perioral cyanosis when crying. She was transferred to our hospital for precision treatment. The results of ECHO in our hospital showed that she had atrial enlargement, mitral regurgitation, tricuspid regurgitation, and pulmonary hypertension.

Patient 4 was a 19-day-old boy who was born via cesarean section. He had no history of suffocation and rescue at birth. The electrocardiogram showed that the boy had arrhythmia in routine medical examinations. His heart rate was 282 beats/min. ECHO results showed that he had patent ductus arteriosus (PDA), PFO, pulmonary hypertension, mitral regurgitation, and tricuspid regurgitation. Electrocardiogram (EKG) showed that he had supraventricular tachycardia.

Patient 5 was a 2-month-7-day-old girl who was delivered after a full-term pregnancy and had no history of suffocation and rescue at birth. The girl presented cough for 2 days and moaned for 7 hours. ECHO showed that she had left atrial enlargement, left ventricle enlargement, and cardiac insufficiency.

Genetic testing results

The CES data were analyzed following the clinical genetic analysis pipeline developed by our teams (8,13). Five patients were diagnosed with *FLNC* mutations. One known pathogenic variant (c.3557C>T, A1186V) and four novel variants (c.2265+4del, p.R441I, p.C1639Y, and p.A2648S) were identified in this study (*Table 1*, *Figure 1*). A1186V found in patient 1 was reported in the previous study (14), and in three patients with RCM, and their ages were 1.4 years, 3 years, and 15 years old. A1186V was recorded as a pathogenic variant in ClinVar and the Human Gene Mutation Database (HGMD). One splicing variant (c.2265+4del) was identified in patient 2 and three novel missense variants were identified in three individuals

Table 1 Clinical characteristics of five patients

Clinical characteristics	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age	30 months	3 months	24 months	19 days	73 days
Gender	Male	Male	Female	Male	Female
Age of father/mother (years)	29/29	29/27	35/32	28/28	26/20
Family history	None	None	None	None	None
Medical history	The boy presented cough, gasp for a week and edema for 3 days. His face and lip presented cyanosis when he did not inhale oxygen	The boy presented cough for 5 days, and severe shortness of breath for 3 days	The girl had abnormal ECHO for 9 months. Her face and lip presented cyanosis when he crying	The boy was detected arrhythmia for 9 hours	The girl presented cough for 2 days, and moan for 7 hours. Her face and lip presented cyanosis. She had poor response
Gestational week	Full-term	Na	Full-term	Full-term	Full-term
Birth weight	3,250 g	3,600 g	2,950 g	3,280 g	3,050 g
Heart rate (beats/min)	130	121	120	282	136
Breath	48	35	36	43	40
Blood pressure (mmHg)	82/54	84/31	85/50	78/50	88/58
Growth and development	Normal	Normal	Normal	Normal	Normal
Muscle tone	Normal	Normal	Normal	Normal	Normal
Evidence of Peripheral Nerve/Eye Involvement	None	None	None	None	None
Other features	Milk allergy; facial edema	Chlamydia infection, pneumonia	Torticollis, bronchitis	Infection	Anemia
Examination					
CK-MB	33	67	20	26	20
CRP	<8	<8	<8	<8	<8
LDH	403 (IU/L, 110–290)	409 (IU/L, 180–430)	349 (IU/L, 110–290)	284 (IU/L, 114–240)	383 (IU/L, 110–290)
Pro BNP	24665	15017	NA	NA	NA
LVEF (%)	68	39	71	65	41
SV (mL)	35	25	20	11	17

Table 1 (continued)

Table 1 (continued)

Clinical characteristics	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
FS (%)	37	18	39	33	19
LAD (mm)	31	17	28	11	20
LVEDD (mm)	34	38	27	22	32
LVESD (mm)	21	31	16	15	26
B-ultrasound	Liver is 6 cm below the rib and 7 cm below the xiphoid. Spleen is 1 cm below the rib	Liver is 3 cm below the rib	Liver is 3.5 cm below the rib 5 cm below the xiphoid	Normal	Liver is 2.5 cm below the rib
X-ray	Bronchial pneumonia; heart shadow increased	Pneumonia	Heart shadow increased	Normal	Heart shadow increased
ECHO	Right atrial enlargement; left atrial enlargement; mitral regurgitation; tricuspid regurgitation; cardiac insufficiency; pulmonary hypertension	Left atrial enlargement; left ventricle enlargement; mitral regurgitation; patent foramen ovale; cardiac insufficiency	Right atrial enlargement; enlargement; mitral regurgitation; tricuspid regurgitation; pulmonary hypertension	Patent ductus arteriosus; patent foramen ovale; pulmonary hypertension; mitral regurgitation; tricuspid regurgitation	Left atrial enlargement; left ventricle enlargement; cardiac insufficiency
Electrocardiogram	Sinus rhythm; right atrial enlargement; left atrial enlargement	NA	NA	Supraventricular tachycardia	Sinus rhythm; atrial enlargement
CT	Right atrial enlargement; left atrial enlargement; pulmonary hypertension	NA	Abdominal dropsy	NA	NA
Genotypes features					
Chromosome location (hg19)	chr7:128485076	chr7:128482431	chr7:128489025	chr7:128498223	chr7:128478768
FLNC exon	exon21	exon14	exon28	exon47	exon8
c.variants	c.3557C>T	c.2265+4del	c.4916G>A	c.7942G>T	c.1322G>T
p.variants	p.A1186V	/	p.C1639Y	p.A2648S	p.R441I
Mutation type	Missense	Splicing	Missense	Missense	Missense
Zygote	Het	Het	Het	Het	Het

Table 1 (continued)

Table 1 (continued)

Clinical characteristics	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Public AF (GNOMAD; ExAC; 1000Genome)	0 0;0 0;0 0	0 0;0 0;0 0	0 0;0 0;0 0	0 0;0 0;0 0	0 0;0 0;0 0
Internal database (35000)	0 0	0 0	0 0	0 0	0 0
Prediction (SIFT, PolyPhen, MutationTaster)	D P D; 0/0.8/1	–	D D D; 0/1/1	D/D/D; 0.03/1/1	T B D; 0.1/0/1
HGMD	PMID: 29858533	No	No	No	No
Inherited source	De novo	NA	De novo	NA	Paternal
Clinical outcome					
Clinical diagnosis	Restrictive cardiomyopathy	Dilated cardiomyopathy	Restrictive cardiomyopathy	Arrhythmia	Dilated cardiomyopathy
Prognosis	Survival	Survival	Survival	Survival	Survival
Age of last follow-up	40 months	15 months	51 months	48 days	32 months

Het, heterozygous; AD, autosomal dominant; NA, not available; ECHO, echocardiogram; LVEF, left ventricular ejection fraction; SV, stroke volume; FS, fractional shortening; LAD, left atrial diameter; LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter. SIFT, "D" meaning deleterious, score less than 0.05, "T" meaning tolerated, score greater than or equal to 0.05; PolyPhen2, "D" meaning probably damaging, 0.957≤ score ≤1, "P" meaning likely damaging, 0.453≤ score ≤0.956, "B" meaning benign, 0≤ score ≤0.452; MutationTaster, "D" represents as disease-causing meaning likely deleterious. Normal value: CK-MB: <25 IU/L; CRP: <8 mg/L; Pro BNP: 0–125 pg/mL. Normal value in parentheses. 0|0: het|hom/hemi.

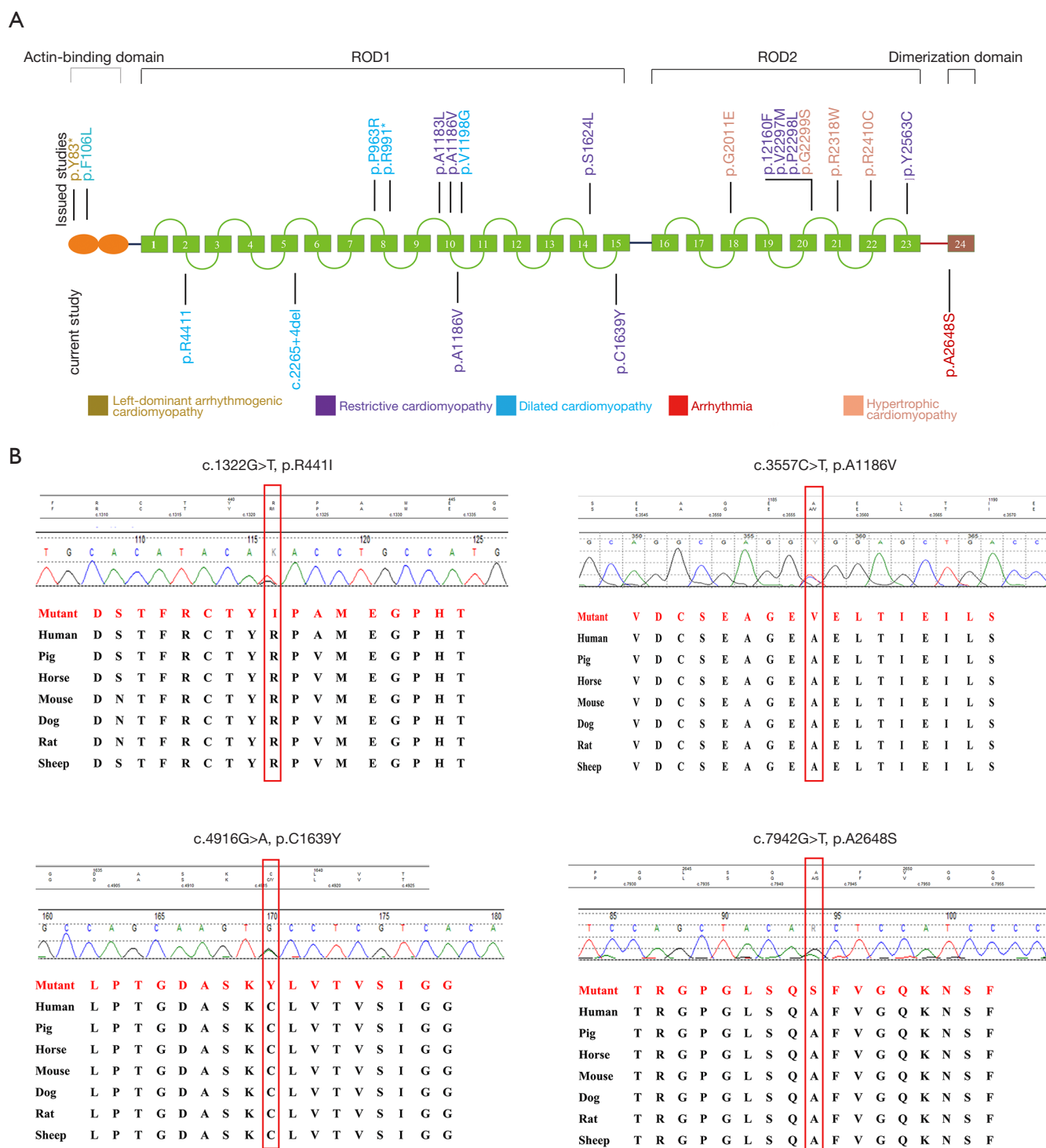


Figure 1 Summary of pathogenic FLNC variants in children and results of genetic tests in our patient. (A) Mutation plot and domain organization of the *FLNC* protein sequence performed using the UniProt database. The actin-binding domain is marked with orange; the ROD1 and ROD2 domains are numbered and marked with green; the dimerization domain is marked with brown. Summary of *FLNC* variants identified in our study (below) and identified in previous studies (above); (B) All missense variants were detected by CES and confirmed by Sanger sequencing. All missense variants were located in conserved regions among humans, pigs, horses, mice, dogs, rats, and sheep.

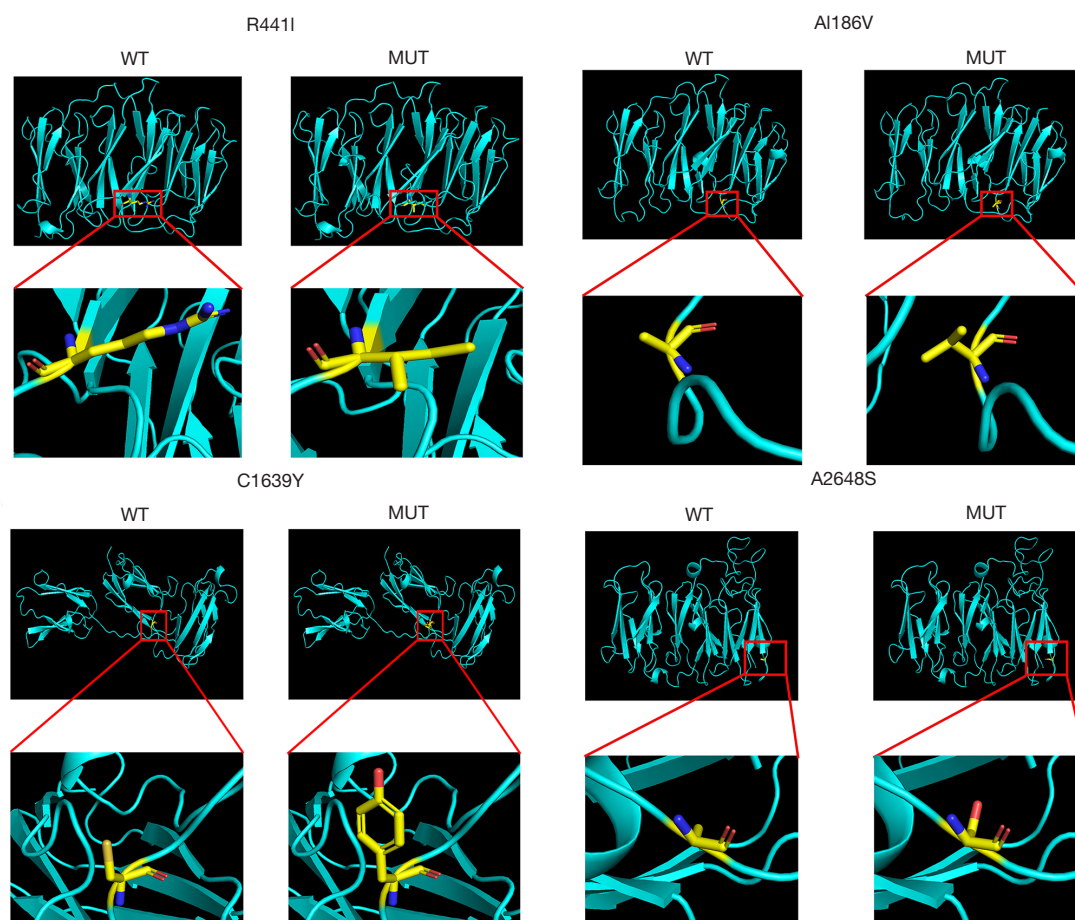


Figure 2 SWISS-MODEL-predicted structures of *FLNC* variants. The structures of the proteins are shown as cartoons. Different elements in variants are marked by different colors (carbon atom: yellow; hydrogen atom: gray; nitrogen atom: blue; oxygen: red; and sulfur atom: orange).

(c.4916G>A, p.C1639Y in patient 3; c.7942G>T, p.A2648S in patient 4; c.1322G>T, p.R441I in patient 5).

The four novel variants were not found in known public databases (GnomAD, ExAC, and 1000Genome) and internal database (more than 30,000 CES samples). Except for A2648S in the dimerization domain, others are located in the Ig domain. All missense variants are highly conserved in different species. The prediction results of SIFT, PolyPhen, and MutationTaster were harmful. Detailed information is shown in *Table 1*, and the predicted structures are shown in *Figure 2*.

Discussion

In 2005, a disease-related mutation within the *FLNC* gene

was first described in myofibrillar myopathy by Vorgerd *et al.* (4), and more reports considered *FLNC* to be related to myofibrillar myopathies (15–17). In 2014, Valdes-Mas *et al.* reported *FLNC* in the HCM family (p.A1539T) (6). Since then, ten more studies have described *FLNC* mutations associated with cardiomyopathy in different races, including Caucasian and Chinese populations (5,18,19). Recently, the Mayo Clinic reported that a novel truncate variant in *FLNC* may serve as a proarrhythmic genetic substrate for arrhythmogenic bileaflet mitral valve prolapse syndrome in a 51-year-old man and his relatives with similar symptoms (20). The author regarded that this genetic substrate may underlie, at least in part, some cases of arrhythmogenic mitral valve prolapse syndrome. In addition, *FLNC* mutations are associated with high-risk

dilated and arrhythmogenic cardiomyopathies, and cause sudden cardiac death (7,21). However, only 24 patients with 16 types of mutations in the FLNC-associated cardiac phenotype have been reported, which disappears when leaving most of them become adults (5,19,21,22).

In this study, we included 5 pediatric cases displaying a FLNC-associated cardiac phenotype. The spectrum of FLNC-associated phenotypes includes RCM, arrhythmia, and DCM. The missense variant (R441I) in patient 5 with DCM is paternally inherited. When followed up by telephone, the patient's father (26 years old) did not currently complain of heart problems. A previous study showed that *FLNC* variants such as E637G, R1150Q, and T2099N can be found in patients with HCM or in healthy individuals (18), indicating incomplete penetrance. Valdes-Mas *et al.* (6) reported that, with 14 of 16 mutation carriers over 40 years of age exhibiting symptoms of HCM, the penetrance is more than 87%. The father of patient 5 carrying R441I maybe be due to incompletely penetrance or later onset cardiomyopathy.

A search of the PubMed database revealed a total of 17 publications reporting *FLNC* variants related to 294 patients with cardiac phenotypes. Of these, 7 family studies reported 16 mutations in 24 pediatric patients with DCM, RCM, HCM, or left-dominant arrhythmogenic cardiomyopathy (5,14,19,21-24). Of these, 10 were male and 14 were female. The diagnosed age ranged from 3 months to 17 years old (Table 2). Among all the reported *FLNC* induced cardiomyopathies, there are only four patients diagnosed with RCM accompanied by a CHD phenotype (5,14). Thus, CHD may be an early onset phenotype accompanied by cardiomyopathy. In this study, a rare novel *FLNC* variant (A2648S) was found in patient 4. Notably, arrhythmia, a symptom of cardiomyopathy (25), was identified by EKG in this patient. Moreover, ECHO observed that he had PDA and PFO, but not cardiomyopathy due to young age (19 days). We think this patient may progress to cardiomyopathy with age. Therefore, our early diagnosis can provide an opportunity for early comprehensive intervention that delays other cardiac phenotypes disease progression.

Recently, several studies have focused on the relationship between the *FLNC* genotype and imaging phenotype. Ader *et al.* (24) found no particular association between

the rhythm disturbance and the position of *FLNC* variants in 28 patients with cardiomyopathies. However, Sveinbjornsson *et al.* (7) indicated that the *FLNC* variant in DCM patients similarly affects many ECG traits, with the most notable association with the R-wave amplitude. In addition, the HCM patients with and without *FLNC* mutations had similar left ventricular ejection fraction features (18). Interestingly, a similar result was reported by Augusto *et al.* (26). Moreover, they conducted a comprehensive genotype-imaging phenotype study and found that *DSP/FLNC* genotypes cause more regionality in left ventricular impairment in DCM patients. Thus, *FLNC* variations may account for different imaging phenotypes compared with other gene mutations. In the current study, we did not conduct genotype-imaging phenotype analysis because only 5 patients were included. We will explore the relationship between genotype and imaging phenotype in future studies.

More interestingly, the variant (p.A2648S) found in this patient is located within the dimerization domain which results in the inability to dimerize properly and renders the protein more susceptible to proteolysis (4,27). This phenomenon may be related to myocardial development and may cause cardiomyopathy. However, further experiments with more patient samples are needed to confirm this hypothesis. The remaining four variants are located within ROD1, which can lead to filamin aggregates (15). In addition, based on protein structure analysis, we found that one variant made the amino acid more hydrophobic (A1186V), one variant changed the hydrophilic amino acid into a hydrophobic amino acid (R441I), and two variants (C1639Y and A2648S) changed the hydrophobic amino acids into hydrophilic amino acids (Figure 2). Combined with the prediction of the variants by *in silico* tools (MutationTaster, PolyPhen, and SIFT), the prediction results may illustrate pathogenesis. Further functional studies are needed in the future.

Conclusions

In summary, the *FLNC* variant was identified as an early diagnosis genetic alteration associated with cardiomyopathy occurrence in infant patients. This research expanded the mutation spectrum of the *FLNC* gene.

Table 2 Clinical characteristics of reported pediatric patients with FLNC mutations

ID	Variants	Gender	Age	Cardiac phenotypes	Race(s)	Myopathy	Other-cardiac findings	Others	Outcomes	Reference (PMID)
Family 132: III-1	p.S1624L	Male	15 years old	RCM	Caucasian	None	None	None	NA	26666891
Family 132: III-3	p.S1624L	Female	13 years old	RCM	Caucasian	None	Congestive heart failure	RA-thrombus, lymphangiectasia	Heart transplantation at age 14 years	
Family 132: IV-1	p.S1624L	Female	2 years old	RCM	Caucasian	None	Patent foramen ovale	None	Heart transplantation at age 2 years	
Family 291: III-5	p.I2160F	Female	15 years old	RCM	Caucasian	None	None	None	NA	
III-1	p.F106L	Male	2 years old	DCM	Ashkenazi Jewish	None	No	Thin and translucent skin, low-set ears, microcephaly, and micrognathia	Heart transplantation at age 2 years	27601210
III-2	p.R991*	Male	2 years old	DCM	Ashkenazi Jewish	None	Arrhythmias	None	Dying at age 6 years	
Family 26958: II-1	p.Y83*	Male	17 years old	Left-dominant arrhythmogenic cardiomyopathy	NA	NA	NA	Left ventricle subepicardial fibrosis	Sudden death after a soccer match	27908349
Family 29544: III-2	p.P963R	Female	3 months old	DCM	NA	NA	NA	Left ventricle myocardial fibrosis and endomyocardial fibroelastosis	Heart transplantation	
Patient 25	p.A1186V	Male	1.4 years old	RCM	NA	Yes	Atrial septal defect	Cox dystopia, umbilical hernia, kyphoscoliosis, hyperopia	Dying at age 2 years	29858533
Patient 16	p.A1186V	Female	3 years old	RCM	NA	Yes	Atrial septal defect	Cox dystopia, white line hernia, microsomia	Alive at age 7 years	
Patient 22	p.A1186V	Female	15 years old	RCM	NA	Yes	None	Scoliosis, Retentio testis	Heart transplantation at 19 years	
Patient 15	p.A1183L	Female	6 years old	RCM	NA	Yes	Atrial septal defect	Cox dystopia, white line hernia, microsomia	Listed for heart transplantation at age 3 years	

Table 2 (continued)

Table 2 (continued)

ID	Variants	Gender	Age	Cardiac phenotypes	Race(s)	Myopathy	Other-cardiac findings	Others	Outcomes	Reference (PMID)
Family 1: IV-3	p.P2298L	Female	4 years old	RCM	African American	None	None	None	Listed for heart transplantation at age 7 years	30260051
Family 1: IV-2	p.P2298L	Male	8 years old	RCM	African American	None	None	None	Transplant at age 11 years	
Family 1: III-1	p.P2298L	Female	3 years old	RCM	African American	None	None	None	NA	
Family 1: III-2	p.P2298L	Female	10 years old	RCM	African American	None	None	None	NA	
Family 2: IV-7	p.Y2563C	Female	1 year old	RCM	Caucasian	None	None	Unspecified developmental delay, hypotonia, dysmorphic facial features, and clasped thumbs	Heart transplant	
Family 2: IV-6	p.Y2563C	Female	1 year old	RCM	Caucasian	None	None	Unspecified developmental delay, hypotonia, dysmorphic facial features, and clasped thumbs	Heart transplant	
18	p.V1198Gfs*64	Female	0.5-year-old	DCM	NA	None	NYHA II, Syncop	NA	NA	31245841
19	p.G2299S	Male	16 years old	HCM	NA	NA	None	NA	NA	
20	p.G2011E	Male	17 years old	HCM	NA	None	atrial fibrillation, acute heart failure	Dyspnea	NA	
21	p.R2410C	Male	9 years old	HCM	NA	None	NA	NA	NA	
22	p.R2318W	Male	14 years old	HCM	NA	None	NYHA II	Acute heart failure	NA	
IV-3	p.V2297M	Female	14 years old	RCM	NA	NA	NA	NA	NA	29212899

RCM, restrictive cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; NA, not available.

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Footnote

Conflict of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The criteria of genetic testing were got approved of the ethics committee of Children's Hospital of Fudan University (2016-235). Pre-test counseling was performed by physicians, appropriate informed consent was signed by the patient's parents in clinics.

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