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RESEARCH ARTICLE

The Clinical Spectrum of Mutations in *CAP2*

Aviva Levitas[1], Hanna Krymko[1], Leonel Slanovic[1], Ruti Parvari*[,2,3]

[1] Department of Pediatric Cardiology, Soroka University Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel
[2] The Shraga Segal Department of Microbiology, Immunology and Genetics, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel
[3] The National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer Sheva, Israel

*ruthi@bgu.ac.il

ABSTRACT

Background: Dilated cardiomyopathy leads to contractile dysfunction, progressive heart failure, and excessive risk of sudden cardiac death. We reported a homozygous damaging variation in *CAP2* causing dilated cardiomyopathy and supraventricular tachycardia in two cousins of one family. Additional homozygous mutations in *CAP2* with clinical presentations were reported.

Aim: To present the different *CAP2* mutations described in patients of various populations with a spectrum of clinical descriptions and possibly correlate the mutations to the clinical findings. This is important for the diagnosing and prognosis of patients with mutations in this gene.

Methods: Clinical evaluation of an additional patient of the family we previously reported. Literature searches of clinical studies of patients affected by mutations in CAP2, animal models for the gene, and the role of CAP2 in the assembly of actin in the thin filaments of the sarcomere.

Results: All patients had dilated cardiomyopathy necessitating heart transplants at a very young age. Two patients with one loss of function mutation presented additionally with structural heart abnormalities. Another loss of function mutation in one patient associated with nemaline myopathy, mild hypotonia, atrophic, and widened scarring. One report did not detail the patient's mutation and presented tricuspid and pulmonary atresia. Animal models of mice and sheep had additional defects not reported in human patients. The pathology is caused by the loss of the function of CAP2 in actin polymerization and in the "α-actin switch" occurring during differentiation and required for the sarcomere structure and function.

Conclusions: The homozygous mutations in *CAP2* cause severe Dilated cardiomyopathy. Additional phenotypes may not be seen in all individuals, and the severity of the mutation and disease do not correlate.

Key words: Dilated cardiomyopathy; CAP2; Loss of function versus reduced function mutation, clinical characteristics

Introduction

Dilated cardiomyopathy (DCM), a leading cause of heart failure, is the predominant type of cardiomyopathy affecting approximately 1 in 400 people [1] and is frequently diagnosed in young adults [2,3]. DCM is associated with high morbidity and mortality rates and is the most frequent indication for cardiac transplantation [3]. It has many known causes, including infectious agents, drugs or toxins, peripartum stress, nutritional deficiencies, and autoimmune disorders [4]. Approximately half of all cases are idiopathic, and the estimated familial DCM is 30%–50% [5]. Autosomal dominant inheritance is the most common inherited form [6], usually presenting in the second or third decade of life [5,7]. Mutations in titin, lamin A/C, and myosin heavy chain (MHC7) account for over 30% of genetic DCM [4]. The other known mutated genes contribute to the structural and force-generating mechanism of the heart, while others affect calcium handling, mitochondrial function, and the nuclear membrane structure [4,8,9]. Autosomal-recessive mutations leading to DCM are far less common. Among the few existing cases are genes encoding cardiac structural proteins, proteins important for properly transmitting the generated force, cardiolipin metabolism, and the production of ATP [10,8].

In cardiac and skeletal muscle, actin is the prominent component of thin filaments within the smallest contractile unit of muscle, the sarcomere. Muscle cells can intricately fine-tune their thin filament lengths to maintain uniformity [11]. Unlike nonmuscle cells, thin filament length regulation predominantly occurs at the pointed ends, which are associated with dynamic protein complexes that contribute to actin filament assembly and overall sarcomere structure. The pointed-end complex contains leiomodin (Lmod) and tropomodulin (Tmod). Lmod and Tmod are essential to muscle function as mutations in these proteins lead to debilitating human skeletal and cardiac diseases [e.g., nemaline myopathy [12] and dilated cardiomyopathy (DCM)[13].respectively]. Cyclase-associated protein 2 (CAP2) was also identified as a component of the pointed-end complex [14]. CAP2 was recognized as a crucial contributor to actin polymerization, accelerating F-actin disassembly in the presence of actin depolymerization factor (ADF)/cofilin [15] and through its interaction with profilin, catalyzing nucleotide exchange of ADP actin monomers, recharging them for another round of polymerization [15,16]. Recent studies have also highlighted its role in cardiac and striated muscle development, and suggested that it is essential for muscle maturation [14,17].

Four papers report CAP2 mutations in humans [18-21], mice knockout models were reported [17,22-24], and a dominant polymorphism in sheep [25]. With the clinical evaluation of an additional patient in our large family, we aimed to present the different *CAP2* mutations described in patients of various populations with a spectrum of clinical descriptions and possibly correlate the mutations to the clinical findings. This is important for diagnosing and prognosis of patients with mutations in this gene.

Methods

Cardiac evaluation, including echocardiography and electrocardiography (ECG) of the additional patient from the large family was done as detailed previously [26]. In detail: For echocardiography, we used GE Vivid 9 and Vivid E95 with transducers 6S and 5S. Cardiac morphology, evaluated by two-dimensional (2D) and Doppler echocardiography (TTE) in the apical four-chamber and parasternal short-axis images, at the level of the ventricles, showed dilatation of left ventricle with global severe depression of LV function. In addition, 4–5 trabeculae and intertrabecular recesses in inferior and lateral walls of the LV were noted with normal origins of the coronary arteries. In these lacunar regions, the compacted versus noncompacted myocardium ratio was 1:2.5.

The pathogenic variation at the donor splice consensus of exon 7 c.636+1G>A of CAP2 was identied as detailed [18].

The literature search was done using the keyword CAP2 in PubMed.

Results

Study design: To present the spectrum of clinical descriptions of patients with *CAP2* mutations and possibly correlate the mutations to the clinical findings, we have added the clinical evaluation of an additional patient of the family we previously reported. Additionally, we have carried out literature searches of clinical studies of patients affected by mutations in CAP2, animal models for the gene, and the role of CAP2 in the assembly of actin in the thin filaments of the sarcomere.

Clinical evaluation and treatment of the patient: The patient's mother presented irregular fetal heartbeats at the 32nd week of pregnancy. The fetal echocardiography showed normal anatomy and function with a fetal heart rate of 146

beats/min with atrial premature beats (APBs). After birth, at the age of one month, the baby grew up nicely. Physical examination and echocardiography were normal, as well as the ECG which demonstrated a sinus rhythm with few APBS. At three months, the baby girl continued to gain weight, but the echocardiography showed worse results: mild left ventricular (LV) dilatation and dysfunction with noncompaction LV (NCLV). The 24h Holter monitor cached 13% of APB's with runs of supraventricular tachycardia (SVT).

The baby girl was treated with ACE and Propanolol. Despite the medical treatment, at five months, she was repeatedly hospitalized due to respiratory distress. Due to frequent SVT runs observed during hospitalization, furosemide and flecainide have been added to the regular treatment. At seven months, she was hospitalized in pediatric intensive care due to congestive heart failure and was treated with intravenous inotropes and amiodarone. Regardless of the intensive treatment, one month later, she died while waiting for a heart transplant. (Table 1)

Because the pathogenic variation at the donor splice consensus of exon 7 c.636+1G>A of CAP2, causing deletion of 64 highly conserved amino acids, was known for the family patients, she was verified and ascertained to be homozygous for the variation.

Table 1: Clinical evaluation and treatment of the patient

Age at presenting Echo	**Echo presentation**	**ECG Holter**	**Treatment**	**Clinical symptoms**
32wks	Normal anatomy and function	Multiples APBS		
first month	Normal anatomy and function	ECG Sinus rhythm with few APBS		
Three months	Mild LV dilatation LVEDD-30mm (Z-score-+3.18) EF- 48% NCLV	Holter: Sinus rhythm with multiple APBS 13% Multiple runs of SVT 240	Ace inhibitor propranolol	Mild tachypnea
Five months	Dilatation of LV 36mmLVEDD (Z-score- 4.45) EF-35% with LVNV Severe LV dysfunction	Holter: Sinus rhythm with multiple APBS and VPBS 9% Multiple runs of SVT	Ace inhibitor Furosemide Spirinolactone Propranolol Flecanide	Dyspnea CHF
Seven months	Severe LV dilatation and Dysfunction LVEDD-37mm (Z-score- +4.78) EF-24%		IV Inotrops + amiodarone	Severe CHF Hospital admission in PICU at age eight months, she died while waiting for a heart transplantation

Abbreviations: Echo: echocardiogram, LV: left ventricle, LVEDD: left ventricular end diastolic diameter, EF: ejection fraction, NCLV: non compaction left ventricle, SVT: supraventricular tachycardia, APBS: atrial premature beats, VPBS: ventricular premature beats, PICU: pediatric intensive care unit. IV: intravenous, CHF: congestive heart failure, WKS: weeks

Comparison of the three patients of the family carrying the c.636+1G>A mutation: All patients are cousins of one large family. We have previously described two children patients- a 12-year boy and a 5-year girl [18]. Both children had normal growth for age and normal milestone development. Their intelligence score was standard, and they were educated in regular schools. Their physical examination focused particularly on cardiac and neuromuscular findings. The cardiac evaluation included echocardiography and ECG. The patients underwent a comprehensive eye examination by an ophthalmologist, assessing vision and ability to focus, visual acuity, pupil function, extraocular muscle motility, visual fields, intraocular pressure and ophthalmoscopy through a dilated pupil. All results were normal in both patients.

At age five, the girl presented with severe respiratory distress and ECG demonstrating SVT. Her pulse rate was 150-170 beats/min. She did not

respond to intravenous adenosine treatment, followed by intravenous amiodarone and inotropes administration. The echocardiography showed severe dilatation and dysfunction of the left ventricle with myocardial noncompaction of the left ventricle. The origin of the coronary arteries was normal. She died at the age of nine years from severe congestive heart failure while waiting for a heart transplant.

The boy presented at the age of 12 years with a pulse rate of 200 beats/min and an ECG showing SVT with severe respiratory distress. Like the girl, he failed to respond to intravenous adenosine and was treated with inotopes such as furosemide and intravenous amiodarone while mechanical ventilation was applied. His echocardiography showed a picture identical to that observed in the girl, with severe dilatation and dysfunction of the left ventricle, myocardial noncompaction of the left ventricle, and the normal origin of the coronary arteries. Two days later, the boy died from severe congestive heart failure.

A comparison of the presenting symptoms of the baby girl with the above-described girl and boy, disclosed the baby girl presented early in her fetal life at 32 weeks with APBs but with normal cardiac function until three months. At that time, the echocardiography showed decreased cardiac function, but her growth was not impaired (her growth parameters are 95th percentile in height and weight) and normal milestone development. Despite the medical treatment, the clinical deterioration was rapid, and she needed a heart transplant at the age of 7 months. Summary of the clinical details for all pateints for whom they were available is presented in Table 2.

Table 2. Clinical **evaluation of patients.** The annotation of the patients is according to the reference.

Patient (Ref)	Prenatal Fetal echo	Age at onset	Presenting symptoms at onset	Echo at onset	Age at follow-up and present situation	Echo at follow -up	Outcome	CAP2 variation
III1 (16)		5Y	SVT With CHF	LVESD 50 mm(z-score+8) LVEDD 58 mm(z-score+4.73) EF-25-30%	9 y Severe CHF Hospital admission In PICU	LVESD 55 mm(z-score+8.6) LVEDD 62 mm(z-score+5.2) EF-30%	exitus	c.636+1G >A
III5 (16)		12Y	SVT with severe CHF	LVESD 68 mm(z-score+13) LVEDD 72 mm(z-score+8.3) EF-5%	2 days later died in PICU with severe CHF		exitus	c.636+1G >A
This study	32WKS Normal anatomy and function with Multiples APBS	3month	Mild tachypnea Holter: Sinus rhythm APBS13% Multiples runs of svt 240	3month mild LV Dilatation NCLV of LVEDD-30mm (Z-score +3.18.) EF-35% Severe LV dysfunction	7 months Severe CHF Hospital admission In PICU	Severe LV dilatation, and dysfunction LVEDD-37mm Z-score-+4.78 EF-24%	exitus	c.636+1G >A
(17)	28 WKS Normal anatomy and function	At birth	Severe CHF acidosis ECMO Sinus rhythm	Mild RV hypertrophy severe LV+RV systolic dysfunction EF-34%	5 months Poor growth mild hypotonia nemaline myopathies	LV dilatation and dysfunction LVEDD-(Z-score-+5)EF-19% NCLV	Heart transplantation	c.1288 delT
(18)	24wks Normal cardiac anatomy and function 30wks Functional tricuspid and pulmonary atresia RV dilatation and dysfunction LV preserved function	At birth	Severe respiratory disress +pleural effusions Sinus rhythm, Intubated+ IV inotropic drugs +prostaglandin Drainage of pleural effusion	At birth severe LV+RV systolic dysfunction with Functional tricuspid and pulmonary atresia Pleural effusions	Age of 3 weeks Discontinued of prostaglandin	Decreased LV dysfunction He was discharged home with oral medication	?	Homozygous, undefined

Abbreviations: Ref: Reference, LVESD: left ventricular end systolic diameter, LVEDD: left ventricular end diastolic diameter, EF: ejection fraction NCLV; non compaction LV. SVT: supraventricular tachycardia. APBS: atrial premature beats. PICU: pediatric intensive care unit. IV: intravenous. CHF: congestive heart failure .RV: right ventricular. ECMO: extracorporeal membrane oxygenation. WKS: weeks

Reports of patients homozygous for other pathological variations in *CAP2*: We searched the literature for additional reports on patients carrying pathological variations in *CAP2*. Recently, Gurunathan et al. [19] reported a c.1288 delT (homozygous pathogenic variant) in *CAP2*, yielding a CAP2 protein with a p.C430fs. This pathogenic variant causes the deletion of the last 45 amino acids of CAP2 protein. Both parents were heterozygous for the same variant but had no history of heart or muscle disease. Analysis of the patient's derived fibroblasts, and cardiomyocytes derived from induced pluripotent stem cells confirmed that the p.C430fs pathogenic variant appears to cause loss of both CAP2 protein and mRNA. The patient boy presented at birth with severe dilated cardiomyopathy, biventricular dysfunction and left ventricular noncompaction, and finally needed extracorporeal membrane oxygenation. At the age of 13 months, he underwent orthotopic heart transplantation. In addition, the patient underwent muscle biopsy demonstrating nemaline rods myopathy, which was also evident in the heart biopsy. He was noted to have many widened and atrophic scars in the areas of his prior ECMO cannulation and sternotomy, and his skin was soft and doughy. A failure to gain weight and an intellectual delay were noticed until the age of 5 years (Table 2). A second report on another loss of function pathogenic variation: a nonsense mutation at Y316 was reported in a third family with a history of DCM in three children, two of whom died. The mutation was identified in homozygosity by sequencing one child with DCM [21]. The clinical description was not detailed as the study reports on Genomic testing in 1019 individuals from 349 Pakistani Families. The information provided on the patients is atrial situs ambiguous, patent ductus arteriosus, patent foramen ovale, and congestive heart failure. The third report provides evidence of a homozygous variation in CAP2 in a patient, while both parents were heterozygous for the same variant. However, the variation was not provided. The patient was a neonate presenting with right ventricular cardiomyopathy born to a couple that had lost an older child with a similar condition [20] . The authors state that this case illustrates the fetal manifestation of cardiomyopathy due to *CAP2* mutation with functional atresia of the tricuspid and pulmonary valves that was documented to be normal earlier in gestation—prenatal diagnosis allowed for close monitoring in utero and timing of delivery. Postnatal management utilized prostaglandin and inotropic support in place of surgical intervention, which proved beneficial in this patient (Table 2). This is the only report of a mutation in *CAP2*, which did not end in cardiac transplant or death, however it is not clear how long the patient was followed up.

Animal models: Two groups studied a mouse model in which the *Cap2* gene was knocked out [22-24,27]. Both reported sudden cardiac death, arrhythmias, cardiac conduction abnormalities, a reduction in body size, and a more severe phenotype in males. Additional phenotypes in mice are ocular findings, including microphthalmia and cataracts, short stature, delayed muscle development, and abnormal wound healing associated with reduced infiltration of macrophages [17,22-24]. A recent paper described that mice with knockout *Cap2* present myopathy, and their myofibrils remained in an undifferentiated stage at the onset of excessive voluntary muscle contractions, predisposing them to form ring fibers that compromise skeletal muscle function [17].

In sheep, a *CAP2* polymorphism was associated with growth and thus suggested to be used as a genetic marker for improving growth traits in Hu sheep farming [25].

The role of CAP2 in actin dynamics in cardiac muscle: CAP2 was identified as a crucial contributor to actin polymerization, striated muscle development, and severe muscle disease when mutated [14,28]. CAP accelerates F-actin disassembly in the presence of actin depolymerization factor (ADF)/cofilin [15] and through its interaction with profilin, catalyzes nucleotide exchange of ADP actin monomers, recharging them for another round of polymerization [15,16].

The N-terminal region of CAP consists of a coiled-coil and a helical-folded domain (HFD)[29,30], whereas the C-terminal region contains two poly-proline-rich domains, a Wiskott–Aldrich-homology 2 (WH2) domain, and a CAP retinitis pigmentosa (CARP) domain [29]. The coiled-coil region of human CAP1 and CAP2 allows tetramers of HFDs to form, increasing cofilin-dependent actin depolymerization [31]. Mammalian CAPs interact with filamentous actin (F-actin) via the HFD [31] and with globular actin (G-actin) using the WH2 and CARP domains [29]. CAP2 is the muscle and cardiac-specific isoform. The splicing mutation affecting the patients of the large family causes deletion of the 64 amino acids of HFD, resulting in its destruction and possibly reducing the quantity of CAP2 protein in patients' cells [18]. The study of fibroblast cells from a patient with the splice site mutation showed that the patients' cells are more sensitive to the disruption of the actin filaments by cytochalasin B, which disrupts the actin filaments [32]. Additionally, the kinetics of

reformation of actin filaments after removing the drug was slower [18]. The mutation reported by Gurunathan et al. [19] yielding a CAP2 protein with a p.C430fs, is predicted to cause the deletion of the last 45 amino acids of CAP2 protein, which are in the carboxy region of the CARP domain. However, such a protein was not detected; instead, both CAP2 protein and mRNA were lost. The nonsense mutation at Y316 [21] will eliminate all the CARP domain, but no details were provided on the expression of this *CAP2* mutation.

In cardiac and skeletal muscle, actin is the prominent component of thin filaments within the sarcomere. For cells to execute proper contraction, striated and cardiac muscles require precise overlap between actin-thin filaments and myosin-thick filaments. Muscle cells intricately fine-tune their thin filament lengths to maintain uniformity by dynamic protein complexes contributing to actin filament assembly and overall sarcomere structure. The proteins composing the complex contain leiomodin (Lmod), tropomodulin (Tmod), and CAP2. All are essential for muscle function as mutations in any of them lead to debilitating human skeletal and cardiac diseases [28]. Surprisingly, unlike Lmod2 and Tmod1, CAP2 had a minor effect on thin filament length regulation [28]. Mutations in *Lmod3* have associated Nemaline myopathy [12], and mutations in *LMOD2*, a member of the Tmod family, with dilated cardiomyopathy (DCM) [13]. The mutations in *CAP2* were associated with the clinical presentations described above. Two recent papers have recognized CAP2 as a unique component of the thin filament pointed end protein complex, whose primary function is to regulate thin filament architecture, actin polymerization, and cardiac and striated muscle development [14,17]. A crucial step during myofibril differentiation is the sequential exchange of α-actin isoforms from smooth muscle (α-SMA) to cardiac (α-CAA) in the heart and to skeletal muscle α-actin (α-SKA) that, in mice, occurs during early postnatal life. This "α-actin switch" requires the coordinated activity of actin regulators because it is vital that sarcomere structure and function are maintained during differentiation. Colpan et al., [14] suggest that since CAP2 depolymerizes and inhibits actin incorporation into thin filaments, it plays an essential role in cardiomyocyte maturation by regulating α-actin composition in mature thin filaments. Cap2-KO cardiomyocytes demonstrate an impaired α-actin isoform switch, exhibiting high levels of α-SMA and α-SKA incorporated into thin filaments. Cap2-KO cardiomyocytes display morphological changes, rounder, less-rod shaped cells, and disorganized actin filaments, suggesting perturbed maturation of pre-myofibrils. Kepser et al [17] report that the α-actin switch in skeletal muscle of Cap2-KO mice is delayed during myofibril differentiation but takes place successfully in adult mice. The myofibrils of Cap2-KO mice remained in an undifferentiated stage at the onset of the excessive voluntary movements in postnatal mice. The delay in the α-actin switch coincided with the start of motor function deficits and histopathological changes, including a high frequency of type IIB ring fibers and additional skeletal muscle abnormalities such as internalized nuclei, ring fibers, and disturbed mitochondrial distribution, all leading to significant motor function deficits. However, Colpan et al. found that this switch never occurs in Cap2-KO hearts, even in adulthood [14]. These findings are consistent with the observation that lethality of Cap2-KO mice is attributed to cardiac, but not skeletal deficits. Therefore, they propose that CAP2 is more influential and indispensable for α-actin exchange and sarcomeric remodeling in cardiac muscle compared with skeletal muscle. Iwanaski et al. suggest a model for CAP2 function in thin filaments in the heart: Since CAP2 functions to facilitate the 'α-actin isoform switch', it regulates the exchange of α-SMA to α-SKA and α-CAA early in cardiac development [14]. Furthermore, based on evidence from cardiac and skeletal muscle [14,17], the role of CAP2 in the α-actin isoform switch is possibly accompanied by cofilin 2 acting in synergy to accelerate actin depolymerization and facilitate the α-actin isoform switch. After the assembly of thin filaments with other myofibrillar proteins (e.g., tropomyosin, titin, myosin, and α-actinin), Tmod1 aids in maintaining and stabilizing α-CAA thin filaments through its capping function. Later in heart development, when Lmod2 expression increases and the heart has started to beat [33], Tmod1 is displaced by Lmod2 [34], leading to the elongation and development of mature thin filaments. At this point,Tmod1 and Lmod2 act as competing factors, working together to maintain proper thin filament lengths and efficient muscle contraction by cycling through elongation (Lmod2) and capping (Tmod1) at the pointed end of thin filaments. Thus, these proteins contribute to cardiac and skeletal muscle structure, revealing that they are necessary for life [28].

Discussion

CAP2 gene should be recognized as a new clinical entity for neonatal and infantile-onset progressive DCM. Four papers present the clinical evaluation of 8 patients with 4 differet mutations in

the gene [18-21], and here we offer the clinical assessment of an additional patient. There is no apparent correlation between the severity of the disease or the associated symptoms with the causative mutation. Complete absence of expression of *CAP2* associated with a male infant presenting, within the first few hours of life, with severe dilated cardiomyopathy, biventricular dysfunction, and left ventricular noncompaction. A muscle biopsy of the patient and cardiac biopsy also identified nemaline rods [19]. This is the only report demonstrating nemalin rods associated with a *CAP2* loss of function mutation. However, this finding is not surprising but in agreement with the mouse knockout model of CAP2, which presented histopathological changes, including a high frequency of type IIB ring fibers and additional skeletal muscle abnormalities leading to significant motor function deficits [17]. Additionally, mutations in *Lmod3,* which has a role in the polymerization of the thin actin filament, were associated Nemaline myopathy [12]. DCM and atrial situs ambiguous, patent ductus arteriosus, and patent foramen ovale were reported in 3 patients with the nonsense Y316 mutation, but no other details were provided neither on the clinical presentation, nor on the expression of *CAP2* [21]. The structural abnormalities described in the patients were not documented in the other patients and cannot be readily correlated with the known function of *CAP2*. The report on the neonate patient presenting with right ventricular cardiomyopathy with functional atresia of the tricuspid and pulmonary valves did not provide the *CAP2* mutation [20]. The structural defects of atresia could result from right ventricular cardiomyopathy. This is the only report of a mutation in *CAP2* which did not end in cardiac transplant or death; however, the length of follow- up of the patient was not provided. Finally, we add a description of a new neonatal patient severely affected by the splicing mutation documented in two patients of a large family [18]. The two other patients presented a later onset and milder phenotype of DCM with the splicing mutation in comparison to the neonatal patients in the other studies. They may have suggested that the mutation is less pathogenic. However, the description of the new patient determines that all the mutations observed thus far in *CAP2* cause severe, very early DCM resulting in death if a heart transplant is not an available option. This clinical presentation is in line with the role of *CAP2* in actin polymerization and the "α-actin switch" occurring during differentiation which is required for the sarcomere structure and function.

Conclusions

All the mutations in CAP2, which are presently reported, lead to an early and fatal DCM. This can now be appreciated not only by the role of CAP2 in actin polymerization but also by its role in facilitating the switch of the α-actin isoform and maturation of the pre-myofibrils in cardiomyocytes. In its absence, not only the contraction but also the structure of cardiomyocytes is perturbed, displaing morphological changes, rounder, less-rod shaped cells, and disorganized actin filaments, suggesting perturbed maturation of pre-myofibrils.

Competing Interests

The authors declare no competing interests.

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