Next-Generation Sequencing Screening Reveals Novel Genetic Variants for Dilated Cardiomyopathy in Pediatric Chinese Patients

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Abstract

Dilated cardiomyopathy (DCM) is a myocardial disease characterized by bilateral or left ventricular cardiac dilation and systolic dysfunction leading to heart failure and sudden cardiac death in children. Most studies focus on the genetic alterations in DCM-related genes in adult populations; however, it remains enigmatic about the mutational landscape in pediatric DCM patients, especially in the Chinese population. We exploited the next-generation sequencing (NGS) technologies to genetically analyze 46 pediatric patients and to decipher the genotype-phenotype correlation in these patients' clinical outcomes. Our results indicated DCM-associated pathogenic mutations in 10 genes related to the structure or function of the sarcomere, desmosomal and cytoskeletal proteins. We also identified 6 pathogenic mutations (5 novel) in the titin (*TTN*) gene leading to the formation of truncated *TTN* protein variants in 6 (13%) out of 46 patients each. Furthermore, we investigated the correlations between *TTN* gene mutation and clinical outcomes in these patients.

Conclusion: Our data suggest that one-third of cases of pediatric DCM are caused by genetic mutations. The role of *TTN* variants should not be underestimated in pediatric DCM and age-dependent pathogenic penetrance of these genetic mutations needs to be considered in the case of familial DCM. Thus, NGS analysis can be applied to decode the yet unknown DCM etiological genetic factors in pediatric as well as adult patients.

Introduction

Dilated cardiomyopathy (DCM) is the predominant type of primitive myocardial disease, characterized by left or bilateral cardiac dilatation and systolic dysfunction in the absence of any other comorbid condition [1]. It has an estimated prevalence of 1:2500 in the general adult population, but its prevalence among children is relatively less, e.g., 1 in 170,000 in the USA and 1 in 140,000 in Australia [2-4]. Since DCM is a type of inherited heart disease, it can lead to high mortality and arrhythmias, heart failure, and sudden cardiac death [5] . About 30-50% of DCM cases are of familial origin, and sporadic DCM is more commonly observed in pediatric patients [6]. Male individuals are found to be 3 times more susceptible to DCM pathogenesis than females [7, 8]. DCM is a genetically heterogeneous disease. Notably, children may develop familial DCM along with advanced heart failure despite the absence of such symptoms in their adult parents [9, 10].

Previous studies have shown the association of more than 60 genes related to the structural or functional components of the cytoskeleton, desmosome, nuclear lamina, sarcomere, mitochondria, and calcium-binding proteins, with DCM pathogenesis in a Mendelian autosomal dominant pattern [6, 8, 11, 12]. These genetic defects exhibit variable expressivity and variable penetrance in DCM patho-mechanism [13]. Identifying disease-causing genetic variants in probands may help asymptomatic family members to assess their risk of developing cardiomyopathy [14]. Next-generation sequencing (NGS) technology has been extensively utilized to decode the correlation between genetic mutation and phenotypic presentation in several human diseases [15]. Previous studies have primarily focused on the relationship between genetic mutation and clinical phenotype in adult DCM patients. But, the genetic landscape of pediatric DCM remains enigmatic. Therefore, in this study, we assembled a cohort of pediatric patients with DCM and presented their clinical phenotypes along with NGS analysis, including whole-exome sequencing and targeted gene panels in combination with cardiomyopathy-related gene-filtering on 46 DCM pediatric patients, identifying the underlying pathogenic mutation. For the first time, we demonstrated the linkage between genetic cause, the phenotypic presentation and clinical outcomes in pediatric patients with DCM in Shandong province in China. Furthermore, the study provides the first comprehensive landscape of genetic variation in pediatric patients with DCM by applying stringent ACMG criteria for classification, supporting genetic testing, and counseling of patients. Moreover, our investigation highlights the yet unknown clinical-pathological correlation between truncating gene mutations in titin (*TTN*) gene, a crucial component of muscle fiber, and clinical outcomes in pediatric DCM patients with Chinese genetic background.

Materials And Methods

Subjects and Clinical Evaluation

Patients diagnosed with DCM were recruited at the pediatric department of Shandong Provincial Hospital between 2014 and 2020. Patients with specific etiologies such as congenital heart disease, myocarditis, rheumatic heart disease, systemic hypertension, cardiotoxicity, ischemic heart disease, metabolic and syndromic diseases were excluded from this study. Finally, the study cohort consisted of 46 patients (n=46; mean age 6.5 months (range: 1-156). All parents of the study subjects gave their informed consent for the study according to the Declaration of Helsinki. Echocardiographic measurements were indexed to age and body surface area (BSA), and the corresponding Z-scores were derived whenever applicable. Clinical diagnosis of DCM was performed when the Z-score of the left ventricular end-diastolic (LVEDD) and/or end-systolic volume (LVESD) was recorded above 2 standard deviations (SD) of BSA (based on Detroit data) [16], with left ventricular ejection fraction (LVEF)≤45% or fractional shortening <20% in the absence of any comorbidities [17].

Probands ≤13 years and available family members were evaluated by medical history, physical examination, 12-lead electrocardiography, and transthoracic echocardiography. We collected the clinical parameters such as age on the onset of DCM, gender, family history of DCM or sudden cardiac death, LVEF and LVEDD at the discovery set. Positive family history was defined as cardiomyopathy or sudden cardiac death (SCD) reported to clinical geneticist at the time of evaluation. Whenever possible, the positive family history was confirmed by obtaining clinical records. We set the DCM recovery parameter as LVEF≥55%. All patients were followed up in the outpatient clinic or by telephone interview until 31st December 2020 and the mean follow-up time was 12.5 months (range: 1-84). The latest echocardiographic data and outcomes were subsequently recorded. For the survival analysis, event-free survival was calculated from the date of onset to the date of heart failure-related death.

Next-Generation Sequencing and Bioinformatics

Genomic DNA was extracted from peripheral blood samples using QIAamp Blood Midi Kit (QIAGEN, Germany) according to the manufacturer's instructions. Out of 46 patients, two target panels were designed for NGS-based genetic analysis- 25 patients were analyzed by Sinopath genetic technology (Beijing,

China), including 241 cardiac genes and 16 patients were analyzed by Novocardio genetic technology Co. Ltd (Beijing, China), including 101 cardiomyopathy-related genes. The remaining 5 patients' genomic DNA was analyzed by the whole-exome sequencing (trio WES) on the Illumina platform.

After filtering out low-quality reads, Burrows-Wheeler Aligner (BWA-MEM v0.7.12) was used to align the clean reads to the reference genome (UCSC Genome Browser hg19) for sorting and duplicate marking. Insertion and/or deletion (InDel) sequence determination and base quality score calibration were carried out by local realignment using Genome Analysis Toolkit software (GATK v3.2) [18]. Single-nucleotide polymorphisms (SNPs) and InDels calling were performed by GATK's Haplotype caller [19]. All the determined variants were annotated by ANNOVAR and associated with multiple databases, such as 1000 genome, ESP6500, dbSNP, EXAC, HGMD (Human Gene Mutation Database), and were predicted by SIFT, PolyPhen-2(PP2), MutationTaster (MT), GERP++.

The pathogenicities of all variants were assessed in accordance with the American College of Medical Genetics and Genomics guideline (ACMG)[20], then Sanger sequencing was performed to confirm the presence of pathogenic or likely pathogenic variants and their parental origins.

Statistical analysis

Statistical analysis was performed with SPSS (version 26.0) software. All data were expressed as mean ± SD or non-parametric as median and lower and upper quartiles. The difference in continuous variables was assessed by the Student's t-test and the Mann–Whitney U test was used when the distributions were asymmetrical. Characteristics of different groups such as patients with or without disease-causing mutations were compared using the Chi-square test for categorical variables if appropriate; otherwise, Fisher's exact test was used. The Kaplan–Meier method was used to calculate survival free from death, and the log-rank test was used to compare survival curves between different patient groups. All statistical tests were two-sided, and P values 0.05 were statistically significant.

Results

Clinical Characteristics

The clinical characteristics of 46 (female-26 and male-20) pediatric patients with DCM onset are presented in Table 1 and Table 2. Almost all the patients were presented with a common respiratory syndrome like shortness of breath and cough. Notably, heart rhythm disturbances (1 atrial tachycardia, 2 premature ventricular contractions with low voltage, 1 transient junctional rhythm, 7 premature ventricular contractions, 2 left anterior fascicular blocks, 1 left bundle branch block, 3 atrial premature beats) were observed in 17 patients. It is important to note that 67.4% of patients (31/46) manifested the disease before 1 year of age with a median age of diagnosis of 6.5 months, and 84.8% (39/46) cases were diagnosed before the child's third birthday. At presentation, the mean LVEF was 28.5%, and the mean LVEDD Z-score was 7.02. The mean serum N-terminal pro-brain natriuretic peptide (NT-pro BNP) level was 14406pg/ml, much higher than the reference range (Table 3). During follow-up of median 12.5 months, 54.3% of them (25/46) were recovered from DCM and their LVEF was rescued to normal range. Because of the severity of DCM, 10 out of 46 patients died, and the average death timing range was 1-36 months (median 7 months) after the initial diagnosis. Importantly, 90% of deaths occurred within the first year after diagnosis, and it was found to be a critical time period reflected by the significant morbidity and mortality. Genetic analysis of DCM showed dramatically high heterogeneity in these patients, while only 3 patients had a familial history of DCM. No significant sex difference was observed in LVEF, LVEDD Z-scores, age of onset, serum NT-proBNP level at diagnosis, and in follow-up time, the outcome of death or recovery (all P>0.05).

Genetic Characteristics of Mutation-Positive Group

Based on the sequencing results, the cohort was divided into two groups: the mutation-positive (MP) group (16/46) and the mutation-negative (MN) group (30/46), as shown in Table 3. Considering the stringent selection criteria and reclassification of DCM according to the ACMG guidelines, the genetic analysis suggested that these 16 patients could be classified as either 'pathogenic' or 'likely pathogenic' mutant carriers. All carriers were identified with only one pathogenic or likely pathogenic mutation in DCM-associated genes (Figure 1). We identified 10 genes with disease causing heterozygous mutations, including those with integral sarcomere functions like titin (*TTN*) [(OMIM *604145), (n=6, 37.5%)], myosin heavy chain 7 (*MYH7*) [(OMIM *613426) (n=1, 6.25%)], troponin T2 (*TNNT2*) [(OMIM *601494) (n=1, 6.25%)], Nexilin (*NEXN*) [(OMIM* 613122) (n=1, 6.25%)], troponin I3 (*TNNI3*) [(OMIM*613286) (n=1, 6.25%)], cytoskeletal structure-related genes like filamin-C (*FLNC*) [(OMIM*617047) (n=1, 6.25%)], vinculin (*VCL*) [(OMIM*611407) (n=1, 6.25%)], and other genes like RNA binding motif Protein 20 (*RBM20*) [(OMIM*613172) (n=2, 12.5%)], NK2 homeobox 5 (*NKX2-5*) [(OMIM:108900) (n=1, 6.25%)] and PR domain containing 16 (*PRDM16*) [(OMIM*615373) (n=1, 6.25%)]. Table 4 summarizes the main clinical features and the details of identified variants in DCM patients. Excluding 3 patients with familial mutations in *TTN*, *MYH7* and *NEXN* genes, 5 de novo variants were identified in *NKX2-5*, *TNNI3*, *PRDM16,* and *RBM20* (n=2) genes. Among the identified 16 gene mutations (missense-5, non-sense-5, frameshift-4 and splice site-2), 2 same mutations were identified in a pair of monozygotic (MZ) twin patients, and 10 (62.5%) other mutations were novel.,

In total, we identified 6 different (5 novels) *TTN* truncation variants, including 3 nonsense, 1 frameshift, and 2 splice-site variants, in 13% of patients (Table 4). Furthermore, we mapped the identified variants to protein domains of *TTN* gene (Figure 3a), which showed consistent results with previous reports. Notably, 4 variants were in I-band, and the remaining 2 in A-band and M-band regions, respectively.

Genotype and Clinical Phenotype Analyses

The phenotypic severity of these patients at presentation was measured by LVEDD Z-score, LVEF, and serum proBNP level. There were no significant differences in distribution based on sex (P=0.202), age (P=0.23), LVEF (P=0.935), LVEDD Z-score (P=0.42), and serum proBNP level (P=0.115) between MP and MN groups. The MP group probands exhibited lower phenotypic severity (echocardiographic parameters) than MN group DCM probands, with the same LVEF of 28.5% and LVEDD Z-score of 6.4 versus 7.08, respectively. Also, we observed a trend that the onset age (median 9.5 months) in MP

group was slightly higher than that in the MN group (median 6 months). Nine MP patients developed DCM before 12 months of age, and the age distribution was between 1 month and 13 years.

Despite the observation of better echocardiographic parameters and seemingly better phenotypes at presentation in MP group patients, there was no significant difference in the outcome (the number of deceased patients, P=0.145) and LVEF recovery patients, P=0.665) between the two groups during the follow-up of median time of 12.5 months (P=0.972). Survival curves free from death and recovery also showed similar results (Figure 3). 37.5% (6/16) of MP DCM patients eventually suffered from heart failure related death compared to only 13% (4/30) of the MN group. Although no significant differences in survival free of death (P= 0.093) were documented, however, there was a trend toward a worse prognosis in our patients at advanced ages (Figure 2a) when compared to the MN patients. The outcome of cardiac death occurred in patients with *TTN* truncating mutants (c.50065C>T, c.98421_98422insGG, c.37454-2A>T), and *NKX2-5* (c.242delA), *TNNT2* (c.422G>A), *TNNI3* (c.544G>A) mutations. Notably, 5 out of 6 disease causing mutations belong to sarcomeric genes (*TTN*=3, *MYH7,TNNT2*, *TNNI3*).

In this study, a significant proportion of the patients exhibited marked improvement and better prognostic outcomes in response to heart failure treatment. In both groups, almost 50% of pediatric patients could recover (LVEF above 50%). There was a trend toward better recovery in MN patients at advanced ages (Figure 2b).

TTN Truncation Variants in Pediatric DCM Patients

Here, we describe the clinical and genetic characteristics of 4 probands with 6 *TTN* mutations (as summarized in Table 4).

P2: A 12 years-old girl presented with dyspnea and fatigue. Her chest x-ray revealed enlarged heart size, and echocardiography showed LVEDD value 5.86cm and LVEF of 27%. Her brother died of severe congenital heart disease at <1 year age. Her LVEF was recovered to 58% after treatment. A novel 'likely pathogenic' nonsense heterozygous truncation mutation (c.43298T>G, p.Leu14433*) in *TTN* gene was confirmed in the proband and her mother by genetic analysis (Figure 4b). But her mother had no symptoms.

P4: A 12 years-old boy with DCM performed a cardiology outpatient clinical evaluation due to the sudden onset of shortened breath with abdominal pain, cough, chest tightness, and fatigue. The proband performed an electrocardiogram (ECG) showing the left anterior fascicular block. Also, he exhibited severely elevated plasmatic transaminases (AST = 275U/L, ALT = 881U/L). The abdominal CT elsewhere revealed ascites, pleural effusion. Remarkably, the plasmatic NT-proBNP level was also drastically high (960.4pg/ml) (normal values less than 125pg/ml at the proband's age). He also performed an electrocardiogram of the left anterior fascicular block and echocardiography showing LVEF of 20%, LVEDD of 7.29cm. After receiving the regimen of spironolactone 40mg/day, captopril 8.3mg/day, furosemide 40mg/day, digoxin 0.25mg/day, potassium chloride sustained release tablets 6000mg/day for 13 days, a repeated echocardiography showed a more severe dilated left ventricle (7.45cm) and a suspicion for left ventricular apex noncompaction. Three months after diagnosis of DCM, he was hospitalized again and died of ventricular tachycardia at a young age. NGS and Sanger sequencing confirmed the 'pathogenic' *TTN* (c.50065C>T, p.Arg16689*) variant in the proband and his mother (Figure 4b). His mother had no symptoms, and she refused to perform echocardiography. For the first time, a pathogenic variant in *TTN* was associated with the DCM phenotype.

P8: A 10 years-old girl, who suffered from DCM for about 3 years and showed no response to heart failure medication, died of heart failure and pneumonia. At the onset, she had an increased serum phosphocreatine kinase (up to 2900u/L) level, and her ECG showed premature ventricular contractions. We identified a novel 'likely pathogenic' frameshift mutation in *TTN* (c.98421_98422insGG, p.I30808Pfs * 7) by NGS analysis in the proband and her father. Her father didn't show any symptoms. Involvement of skeletal myopathy was suspected in this patient because of increased phosphocreatine kinase level. The skeletal myopathy was then identified by muscle biopsy (failing to record the pathologic result). This suggests that this variant can cause disease in both heart and skeletal muscles.

P13: A girl diagnosed with DCM at the age of 3 months had an autosomal dominant family history of DCM. Her maternal grandfather (diagnosed at 36 and died at 52 years), the brother of the grandfather (diagnosed at 42 and died at 46 years), and their sister (diagnosed at 44 and died at 52 years) died of DCM. The proband presented with shortness of breath and atrial tachycardia. She was given a treatment of dopamine cardiotonic, furosemide, spironolactone, amiodarone, and captopril. Unfortunately, 6 months later Her mother felt shortness of breath after activity and performed echocardiography elsewhere, exhibiting LVEF=29% and LVEDD=6.8cm. After 3 years of follow-up, the proband could recover her LVEF to 62% with no signs of arrhythmia. But her mother still receives drug treatment and has not been recovered yet. By trio WES method, we identified a novel truncating 'likely pathogenic' mutation in *TTN* gene (c.105541A> T, p.K35181X) in the proband and her mother, but not in her father and 8-years old brother (Figure 3), who both had normal echocardiography parameters.

RBM20 Gene Mutation in Pediatric DCM

P6 and **P7**, monozygotic (MZ) twin brothers (a kinship coefficient of 0.4901), were diagnosed with DCM with LVEF 29% and 31%, respectively at the age of 12 years (the interval time of onset was 9 months). They both felt nausea, chest tightness, and shortness of breath. Both ECG showed ventricular premature beats. The MZ twin was administered with standard heart failure medication. After seven years of follow-up, the LVEF of the two patients were 56% and 55%, respectively, and remain asymptomatic. By WES screening, a 'pathogenic' de novo mutation c.2746G>A (p.E916K) in *RBM20* gene was identified. The missense mutation was absent from controls (1000G, ESP, and ExAC-ALL), and was predicted to be 'pathogenic' by PolyPhen2, SIFT databases. The amino acid p.E916 is highly conserved across vertebrate species (Figure 5C). We confirmed this missense mutation by Sanger sequencing in the twin patients but not in their parents (Figure 5B).

Discussion

DCM is a common type of pediatric heart disease leading to poor clinical outcomes and heart transplantation [29, 30]. In this cohort, with a presentation of mean LVEF of 28.5% and the mean LVEDD Z-score of 7.02, DCM presented a severe disease phenotype. 67.4% of patients (31/46) manifested DCM before 1 year of age. Genetic factors play an important role in DCM pathogenesis. However, genetic discovery and prognostic understanding are still a challenge for DCM therapy. Hence, we sought to discover the landscape of genetic variations in pediatric patients with DCM. In this study, among 46 pediatric patients with DCM, we found that 16 (34.8%) patients carried at least one pathogenic or likely pathogenic mutation in a disease-causing gene. Consistent with previous studies, mutations in sarcomere genes were detected most frequently in all these DCM patients [31]. The prevalence of pathogenic mutations seemed to be similar as in recently published cohorts [32, 33]. There was no difference in clinical presentations and prognosis, no matter whether there were gene mutations or not. Mutations were identified frequently in childhood-onset of DCM, especially in sarcomeric genes. Importantly, *TTN*-related mutations were found in 10 out of 16 MP patients. *TTN* truncating mutations account for 12%-27% of all adult DCM cases, suggesting the importance of diagnostic sequencing [34-38]. However, the *TTN* truncating mutations have been rarely identified in pediatric patients previously [39-41]. Also, recent studies in pediatric DCM have shown similar results [14]. Notably, a genetic analysis in a cohort of 66 severe childhood cardiomyopathy, including 37 DCM cases, could not identify any *TTN* truncating mutations in these pediatric patients [22]. Likewise, another study involving 30 Chinese pediatric subjects with sporadic DCM pathology also could not find any pathogenic *TTN* gene mutations [37]. However, in another study only one pathogenic *TTN* truncating variant has been identified in a 16 years-old boy among 36 pediatric DCM patients [38]. Obvious differently from low prevalence of *TTN* truncating mutants, we identified 6 different *TTN* truncating variants in 6 (13%) of 46 pediatric patients.

The prognosis of DCM cases with *TTN* truncating mutations has been found to be different and conflicting in adult studies from moderate to severe outcomes. Notably, DCM patients with *TTN* mutations have shown a good response to treatment [39]. A cohort of 70 patients with end-stage DCM has shown recovery after left ventricle assist device implantation [40]. While another study has shown *TTN* mutation-positive patients frequently present severe cardiomyopathy and a worse 5-year prognosis [4].

The different clinical outcomes in *TTN* MP patients were also observed in our findings. Three patients with *TTN* mutations (c.18230-1G>A, c.43298T>G, c.105541A>T) recovered after medical treatment with no further symptom, while 3 other patients harboring *TTN* mutations (c.50065C>T, c.98421_98422insGG, c.37454-2A>T) showed no response to treatment and died from heart failure, suggesting clinical heterogeneity. The conflicting prognosis prompts that apart from the identified genetic factors, post-transcriptional, environmental, hormonal, and other factors could also modify the rate of disease progression. Therefore, it might be difficult to predict the clinical outcomes in pediatric DCM cases based on *TTN* truncating mutations alone.

It has been found that the penetrance of *TTN* truncating mutations can reach up to 100% by the age of 70 years [42], which leads to discordant segregation with phenotype, and the same variant has also been detected in the unaffected relatives. In some pedigrees of unaffected relatives, especially in familial DCM, a clinical follow-up with aging should be carried up. The age onset of girl **P13** (3 months) with *TTN* truncating mutation (c.105541A>T, p.K35181X) was obviously different from her mother (23 years) and her grandfather (36 years), suggesting the possible involvement of some other factors influencing DCM pathogenesis.

In addition, we identified a pathogenic RBM20 variant (p.E916K) in the twin patients (**P6** and **P7**) with pediatric DCM. RBM20 mutations have been reportedly associated with cardiomyopathy [43]. Notably, the variant c.2746G>A (p.E916K) of RBM20 mutant identified in this study was located on exon11, a known hot spot for cardiomyopathy-associated mutations (Figure 6). However, the mechanistic relation of RBM20 mutations with DCM onset is still unclear.

A de novo mutation in PRDM16 gene in an 8-months girl (**P9**) was identified to be associated with pediatric DCM. Previously known mutants of this gene are mostly missense mutations; however, a nonsense mutation leading to functional loss of PRDM16 was detected in this case. The role of *PRDM16* gene mutations could be very important, warranting further studies to identify its pathogenic mechanism.

Limitations

The study suffers from the following limitations: 1) it was a single-centered, retrospective, and a small cohort study; 2) follow-up time was not long enough to estimate the long-term outcomes; 3) clinical assessment was highly recommended for family members of the probands, but only a minority of the relatives were willing to participate in this evaluation, so gene mutation carriers of a family could be underestimated in our cohort; 4) bioinformatic prediction could give us some useful information about the pathogenicity of mutants associated with DCM, however, it cannot reflect the real pathobiology of the mutants in the cardiac myocytes.

Conclusion

DCM is a genetically heterogeneous disease in children and adults. Using NGS analysis, we could detect that more than one-third of cases of DCM were caused by genetic mutations in genes related to the structure or function of sarcomeric, desmosomal and cytoskeletal proteins as well as decipher their genotype-phenotype correlations in pediatric DCM patients. Here, we discovered 6 *TTN* truncating mutations (5 novel) correlated with severe disease phenotypes. Furthermore, we found 16 mutations in 10 genes in 16 patients that were likely to be associated with DCM pathogenesis. Most pediatric patients were diagnosed with DCM before 1 year of age. Also, most deaths occurred within the first year of life after diagnosis. The cardiac death occurred in patients harboring mutations in *TTN* (3), *NKX2-5*(1), *TNNT2*(1) genes. Hence, this study advances the genetic understanding of pediatric DCM and highlights certain genetic defects with severe clinical courses. To the best of our knowledge, this is the first study reporting that the presence of *TTN* truncating mutations could be associated with the onset and development of DCM in pediatric patients. Further studies are needed to define the mechanisms by which pathogenic *TTN* variants affect outcomes in pediatric and adult patients with DCM.

Declarations

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Availability of data and material

The data and materials are available upon request.

Code availability: N/A.

Authors' Contributions

All authors contributed to the study conception and design. YW and BH collected patient data and prepared the manuscript. YF, XY, JL, and JW contributed to the clinical understanding of the case and revision of the manuscript. YY, HY, LZ, and JZ analyzed and interpreted the genetic data and surveyed the literature relevant to the mutations. All authors reviewed the results and approved the final version of the manuscript.

Ethics approval

Our study received ethics approval (NSFC□NO.2018-115) from the ethic committee of Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University.

Informed consent was obtained from all individual participants included in the study.

Consent to participate: N/A.

Consent for publication

All authors and study participants declare their unconditional consent toward publication of this manuscript and its associated data in the peer-reviewed journal.

Abbreviations

ACMG, American College of Medical Genetics and Genomics. ALT, Alanine transaminase. AST, Aspartate transaminase. CT, Computed Tomography. DCM, dilated cardiomyopathy. *FLNC,* filamin C. HGMD, Human Gene Mutation Database. LVEDD left ventricular end-diastolic diameter. LVEF, left ventricular ejection fraction. LVNC, left ventricular noncompaction. *MYH7,* ß-Myosin Heavy Chain gene, MP, mutation positive. MN, mutation negative. *NEXN,* Nexilin F-Actin Binding Protein gene. *NKX2-5,* NK2 homeobox5. NGS, Next Generation Sequencing. NT-proBNP, N-terminal pro-brain-natriuretic peptide. *PRDM16,* positive regulatory domain16. *RBM20,* RNA-binding protein20. *TNNI3,* troponin I. *TNNT2,* troponin T. *TTN,* titin. *VCL,* vinculin. WES, Whole-Exome Sequencing.

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Tables

Table 1 Age Distribution of Pediatric Patients with DCM Onset.

Age group years	N		Death (n) during follow-up
	females	males	
<1	17	14	5
1-3	5	3	2
3-6	1	0	0
6-10	1	0	1
10-13	2	3	2
Total	26	20	10

Table 2 Clinical Characterization of Pediatric DCM Patients Between Female and Male Onset and Latest Outcomes.

	All patients, n=46		P-value
	Females, n=26	Males, n=20	
Age, months (range)	7(1-144)	6(1-156)	0.657
LVEF% (range)	30(20-42)	27.5(17-35)	0.355
LVEDD Z-score (range)	7.46(2.36-13.65)	6.75(3.01-12.87)	0.807
NT-proBNP pg/ml (range)	15466(84.55-35000)	6485(960-35000)	0.594
Deceased during study, n (%)	6(23.1)	4(20)	0.059
Recover during study, n (%)	15(60)	10(50)	0.608

Abbreviations: LVEF, left ventricular ejection fraction; LVEDD, left ventricular end diastolic diameter (Z-score, normal reference range between -2 standard deviation [SD] and +2 SD); NT-proBNP, N-terminal fragment of pro-b-natriuretic peptide.

Table 3 Clinical Characteristics of Mutation-Positive and Mutation-Negative Groups.

clinical characteristics	All (n=46)	Mutation positive (n=16)	Mutation negative (n=30)	P-value
Sex				0.202
Male (n%)	20(43.5)	9(56,25)	11(36.7)	
Female (n%)	26(56.5)	7(43.75)	19(63.3)	
Age onset				
months (range)	6.5(1-156)	9.5(1-156)	6(1.5-108)	0.23
12 months (n%)	31(67.4)	9(56.2)	22(73.3)	
12-156 months(n%)	15(32.6)	7(43.8)	8(26.7)	
LVEF% (range)	28.5(17-42)	28.5(20-33)	28.5(17-42)	0.935
LVEDD Z-score (range)	7.02(2.36-13.65)	6.4(3.6-13.65)	7.08(2.36-12.87)	0.42
NT-proBNP pg/ml(range)	14406(54.5-35000)	6276(960.4-35000)	16305(84.5-35000)	0.115
Follow-up (months) (range)	12.5(1-84)	12(1-84)	12.5(1-69)	0.972
Deceased during study, n (%)	10(21.7)	6(37.5)	4(13.3)	0.06
Recover during study, n (%)	25(54.3)	8(50)	17(56.7)	0.665

Abbreviations: LVEF, left ventricular ejection fraction; LVEDD, left ventricular end diastolic diameter (Z-score, normal reference range between -2 standard deviation [SD] and +2 SD); NT-proBNP, N-terminal pro-brain natriuretic peptide.

Table 4 Clinical and Genetic Characteristics of Patients Identified in Mutation-Positive Group.

Patient#	Sex	Age of Onset	Family History	Time to Death after diagnosis	Modern age	LVEF recovery (above50%)	Arrhythmia	Gene	Varia (cDN
P1	Male	12months	No	-	3years	Yes	sinus rhythm	*TTN*	c.182 splici
P2	Female	12years	No	-	14years	Yes	premature ventricular contractions with low voltage	*TTN*	c.432 p.Leu
P3	Female	5months	No	-	1.9years	Yes	transient junctional rhythm	*FLNC*	c.424 p.Y14
P4	Male	12years	No	3months	-	No	left anterior fascicular block	*TTN*	c.500 p.Arg
P5	Male	10months	No	4months	-	No	sinus rhythm	*NKX2-5*	c.242 p.K81
P6	Male	13years	No	-	21years	Yes	Low voltage, premature ventricular contractions	*RBM20*	c.274 p.E91
P7	Male	13years	No	-	21years	Yes	Low voltage	*RBM20*	c.274 p.E91
P8	Female	10years	No	36months	-	No	premature ventricular contractions	*TTN*	c.984 p.I328
P9	Female	8months	No	-	4years	Yes	premature ventricular contractions	*PRDM16*	c.764 p.S25
P10	Male	1month	Brother, DCM	-	7months	No	atrial premature beats	*MYH7*	c.271 p.C90
P11	Female	9months	No	-	4years	No	premature ventricular contractions	*VCL*	c.142 p.Q48
P12	Female	1.5months	No	1month	-	No	sinus rhythm	*TTN*	c.374 splici
P13	Female	3months	Mother and grandfather, DCM	-	4years	Yes	atrial tachycardia	*TTN*	c.105 p.K35
P14	Male	3months	Brother, heart enlarge and death	-	6.5years	Yes	atrial premature beats	*NEXN*	c.113 p.E38
P15	Male	18months	No	1month	-	No	sinus rhythm	*TNNT2*	c.422 p.R14
P16	Male	3.5months	No	2monthss	-	No	premature ventricular contractions	*TNNI3*	c.544 p.E18

Continued

Patient#	Mutation type	De Novo Mutation	GnomAD	SIFT And score	MT and score	PP2 and score	GERP++ and score	REVEL	ACMG Variant Classification
P1	Splicing	Unknown	-	-	D (1)	-	C (5.95)	-	Likely pathogenic PVS1; PM2
P2	Nonsense	No	-	-	A (1)	-	C (5.56)	-	Likely pathogenic PVS1; PM2
P3	Frameshift	No	-	-	-	-	-	-	Likely pathogenic PVS1; PM2
P4	Nonsense	No	-	-	A (1)	-	C (5.08)	-	pathogenic PVS1; PM3_Strong; PM2
P5	Frameshift	Yes	-	-	-	-	-	-	Likely pathogenic PVS1; PM2
P6	Missense	Yes	-	D (0.024)	N (0.849)	P (0.874)	C (4.71)	B (0.244)	Likely pathogenic PS4; PM2; BP4
P7	Missense	Yes	-	D (0.024)	N (0.849)	P (0.874)	C (4.71)	B (0.244)	Likely pathogenic PS4; PM2; BP4
P8	Frameshift	No	-	-	-	-	-	-	Likely pathogenic PVS1; PM2
P9	Nonsense	Yes	-	-	A (1)	-	C (3.39)	-	Likely pathogenic PVS1; PM2
P10	Missense	No	-	D (0)	D (1)	P (0.998)	C (5.55)	D (0.892)	Pathogenic PM3_Strong; PM1, PM2, PM5, PP3
P11	Nonsense	No	-	-	A (1)	-	C (4.41)	-	Likely pathogenic PVS1; PM2
P12	Splicing	Unknown	-	-	D (1)	-	C (4.34)	-	Likely pathogenic PVS1; PM2
P13	Nonsense	No	-	-	A (1)	-	C (4.78)	-	Likely pathogenic PVS1; PM2
P14	Frameshift	No	-	-	-	-	-	-	Likely pathogenic PVS1; PM2
P15	Missense	No	0.000008	D (0)	D (1)	P (0.999)	C (4.28)	D (0.501)	Likely pathogenic PS1; PM1; PP3
P16	Missense	Yes	-	T(0.364)	D (1)	B (0.005)	C (4.72)	B (0.319)	Likely pathogenic PM3_Strong, PM2, BP4

ACMG: American College of Medical Genetics and Genomics; CADD: combined annotation-dependent depletion scores; DCM: dilated cardiomyopathy; PM: pathogenic moderate; PP: pathogenic supporting; PS: pathogenic strong; BP: supporting benign; PVS: pathogenic very strong; SIFT: Sorting Intolerant from Tolerant tool; WES: whole-exome sequencing; panel 1: Sinopath genetic technology including 113 cardiac genes; panel 2: Novocardio genetic technology Co. Ltd (Beijing, China), including 101 cardiomyopathy-related genes. P6 and P7 were twin brothers. SIFT Sorts intolerant from tolerant (D, damaging; T, tolerant), PP2, polymorphism phenotyping v2 (D, damaging; P, possible damaging; B, benign), MT mutation taster (D, disease causing; A, disease causing automatic; N, polymorphism; P, polymorphism_automatic) GERP++ genomic evolutionary rate profiling (C, conserved; N, non-conserved), Revel (D, damaging; B, benign).

Figures

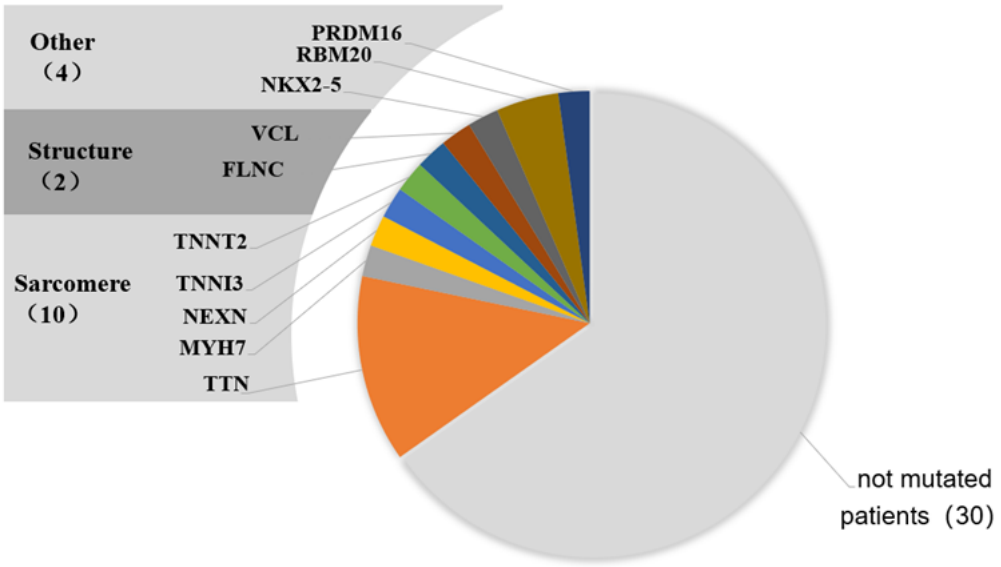

Figure 1

Distribution of Pathogenic or Likely Pathogenic Variants in the DCM Cohort Illustrates the distribution of pediatric DCM patient populations based on the mutations in genes related to either sarcomere or cytoskeletal structure or other cellular functions. Results show that 30 out of 46 patients had no underlying genetic mutation related to DCM pathogenesis. While the majority of the mutation-positive patients exhibit pathogenic or likely pathogenic mutations in the sarcomeric genes.

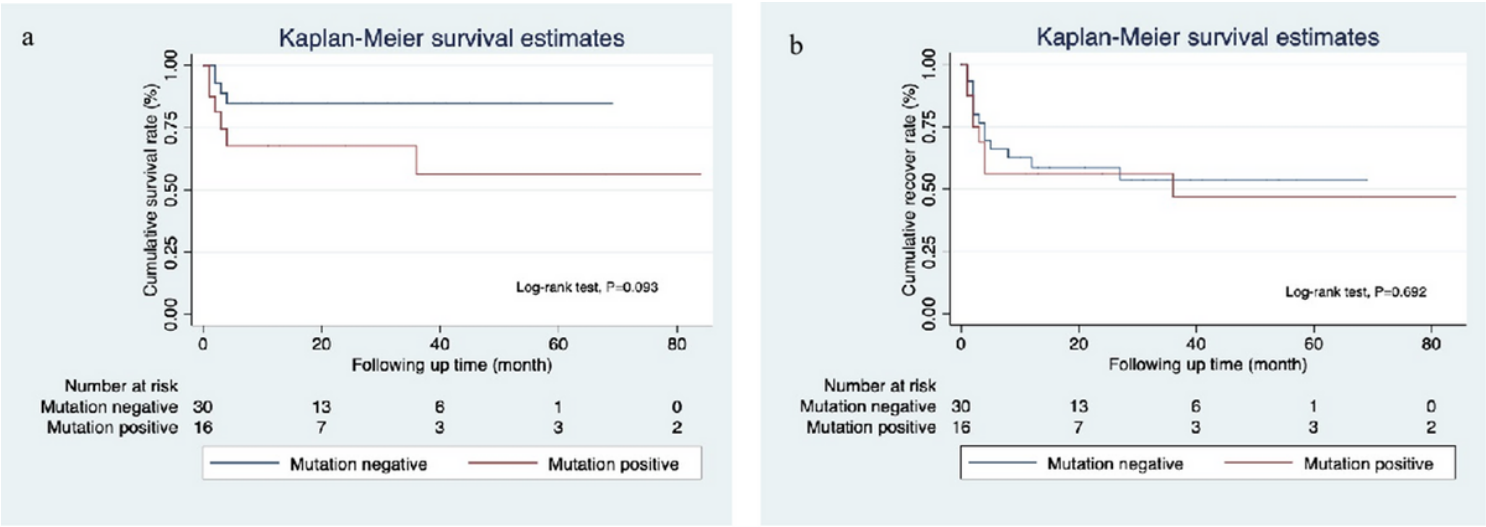

Figure 2

Survival Curves Free from Death(a) and Recovery (EF above 50%) a) Kaplan-Meier survival estimates of progression free from death of mutation-positive and mutation-negative DCM patients according to the genetic and clinical phenotype analyses. b) Kaplan-Meier survival estimates of progression of recovery of mutation-positive and mutation-negative groups after receiving drug treatments for heart failure and arrhythmia.

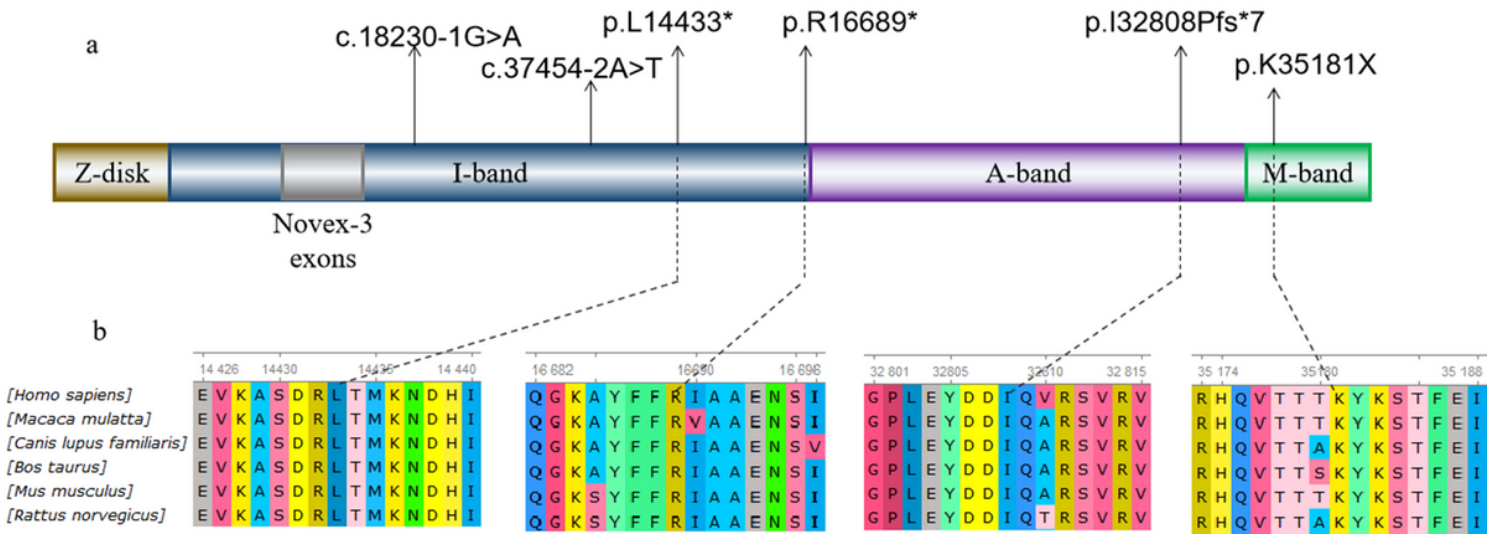

Figure 3

Illustration of Domain Mapping of TTN and Conservation of TTN Protein Amino Acid Sequences Across Vertebrates a) Functional domains of TTN protein showing DCM-linked pathogenic or likely pathogenic mutations mostly in the I-band domain, and other mutations scattered in the A-band and M-band. b) Alignment of highly conserved TTN protein sequences for different vertebrates indicating the corresponding location of that mutation in TTN protein.

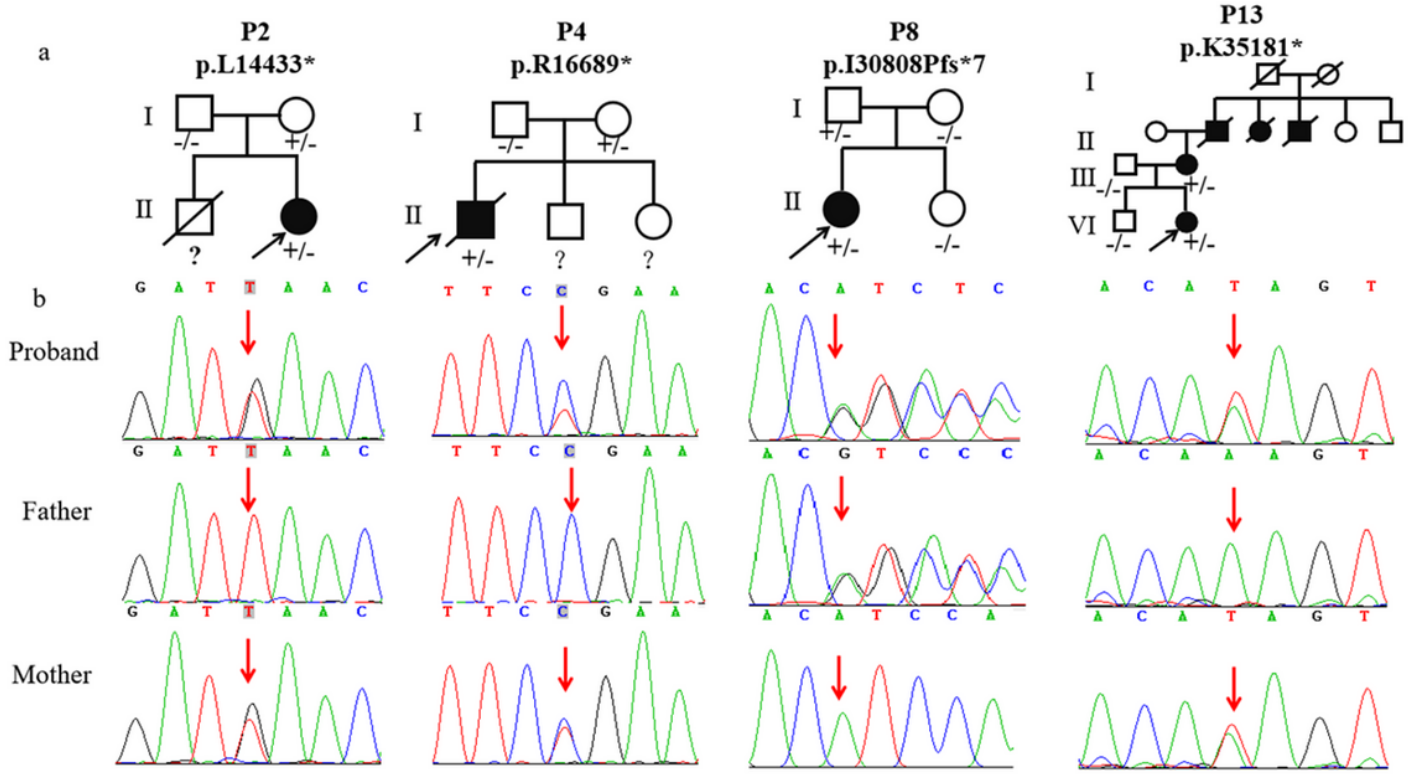

Figure 4

Pedigree Tree of 4 Patient Families harboring TTN truncating gene mutations a) Pedigree tree of 4 families with TTN pathogenic or likely pathogenic variants for which genetic segregation was possible. All family members with available DNA samples were included. Squares indicate males, circles indicate females, slashes indicate deceased individuals, black shading indicates phenotype. The arrows indicate the proband. Heterozygous carriers (+/−) and non-carriers (−) of a TTN variant are indicated. All family members with dilated cardiomyopathy carried TTN truncating mutation. Family segregation analysis was performed in 4 families with truncating TTN mutations, and it revealed positive segregation with the disease in all cases. b) Sequencing results of the TTN mutations.

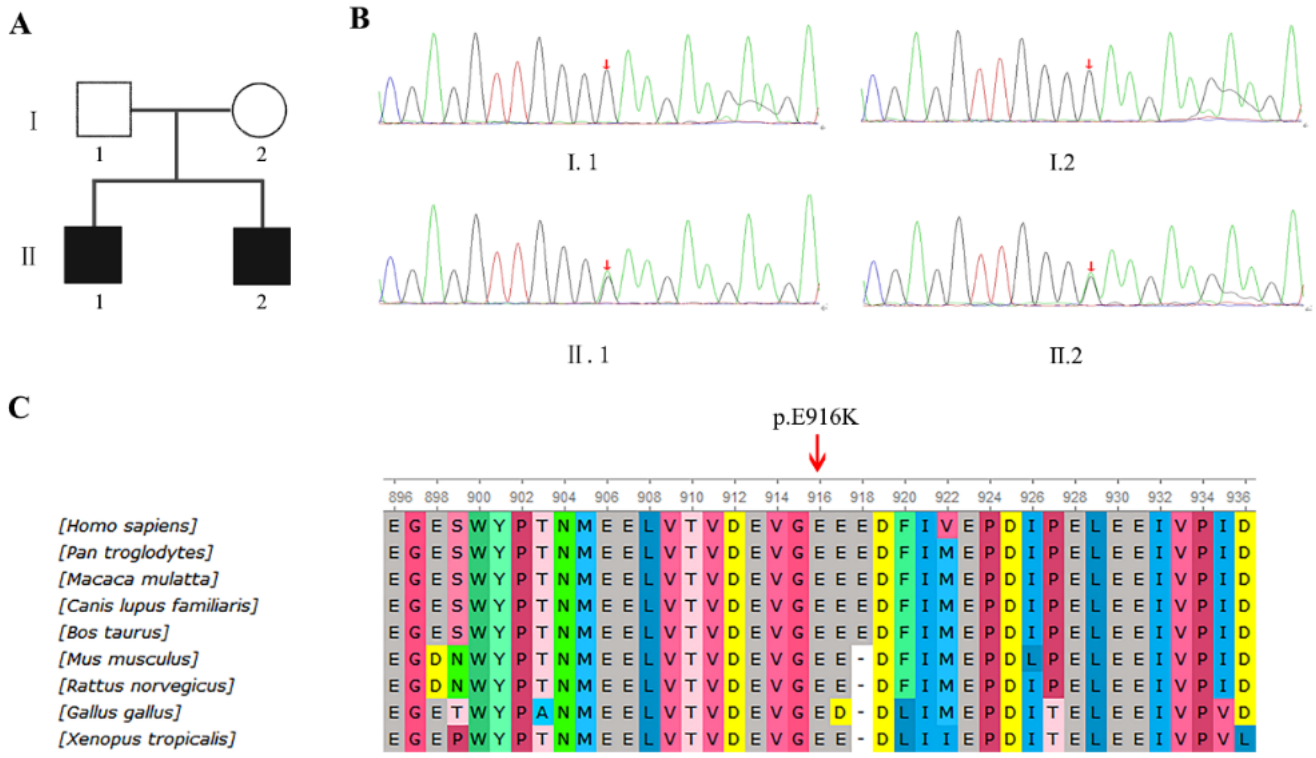

Figure 5

Family Pedigree of Patients P6 and P7 a) Pedigree analysis of probands P6 and P7 harboring DCM-associated RBM20 gene mutation. b) Sequence analysis of monozygotic twin probands and their parents. c) Alignment of RBM20 protein sequences across different vertebrates indicating mutation of highly conserved E916 amino acid residue.

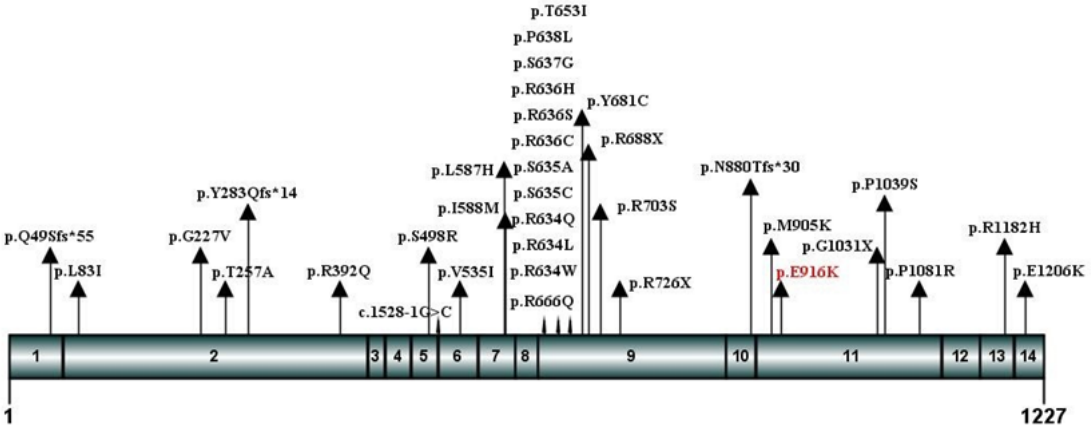

Figure 6

Mutations of RBM20 Gene in the HGMD database Illustration of DCM-linked mutations in RBM20 protein as documented in the Human Gene Mutation Database. The novel missense mutation p.E916K identified in our NGS analysis is indicated in red color.

Supplementary Files

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