Pediatric restrictive cardiomyopathy due to a heterozygous mutation of the *TNNI3* gene

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Abstract

Pediatric restrictive cardiomyopathy is rare and most commonly idiopathic in origin. Here, we applied a candidate gene approach and identified a missense mutation in the cardiac *troponin I* gene in a 12-year-old Chinese girl with restrictive cardiomyopathy. This study indicates that mutation in sarcomere protein genes may play an important role in idiopathic pediatric restrictive cardiomyopathy.

Keywords: cardiomyopathy, restrictive, sarcomere protein gene, *troponin I*

INTRODUCTION

Restrictive cardiomyopathy (RCM) is very rare in children and it is characterized by dilated atria, elevated end-ventricular diastolic pressure, and severe diastolic dysfunction resulting from increased stiffness of the myocardium^[1,2]. RCM carries a poor prognosis with a low survival rate and ultimately requires heart transplantation^[3-6]. Pediatric RCM is most commonly idiopathic and its molecular basis is still unclear. Recently, mutations in the sarcomeric protein genes (cardiac troponin I, *TNNI3*; cardiac troponin T, *TNNT2*; α -cardiac actin, *ACTC*; β -myosin heavy chain, *MYH7*) have been identified in pediatric RCM, which suggests that sar-

comeric protein mutations may be important causes of RCM^[7-13]. Here, we performed genetic investigations of candidate genes that have been reported in RCM and identified a missense mutation in the *TNNI3* gene in a 12-year-old girl with RCM.

SUBJECTS AND METHODS

Patient and clinical evaluation

The patient and her family were recruited at Nanjing Children's Hospital into an ongoing research protocol approved by the institution's ethics committee. All participants gave informed consents and were evaluated by family history, physical examina-

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tion, electrocardiogram and echocardiogram. In addition, the patient underwent cardiac magnetic resonance imaging.

DNA extraction and sequencing

Genomic DNA was extracted from peripheral blood samples using a PUREGENE DNA purification kit (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Genetic analysis was performed for 4 candidate genes (TNNT3, TNNT2, ACTC, MYH7) known to associate with RCM by bidirectional sequencing of all the coding exons. Sequence variants were then tested in the family as well as 100 healthy control.

RESULTS

Clinical characteristics of the index case and her family

The patient was a 12-year-old Chinese girl and experienced her first episode of dyspnea on exertion. She had normal growth parameters. An echocardiogram disclosed that the girl had a structurally normal heart with normal biventricular dimensions, normal biventricular wall thickness and normal systolic functions, but with massive biatrial enlargement (**Fig 1**), markedly left ventricular (LV) diastolic dysfunction, which is consistent with restrictive LV filling pattern: a decreased e-wave deceleration time (102 ms, normal > 150 ms), an abnormal E/A wave ratio of 2.29 (normal < 2), and a decreased isovolumic relaxation time (30 ms, normal > 70 ms). Electrocardiogram (ECG) showed sinus rhythm, and biatrial enlargement, and diffuse T-wave

Fig. 1 Characteristic of echocardiography and amino acid sequence alignment in the *TNNT3* gene across multiple species. A 2-dimensional echocardiographic image (apical 4-chamber view) showing a markedly enlarged LA and RA with normal biventricular size. RA: right atrium. LA: left atrium. RV: right ventricle. LV: left ventricle.

changes (**Fig 2**). The case had a mild short PR interval, but showed no evidence of neuromuscular diseases and metabolic diseases. Magnetic resonance imaging (MRI) revealed no delayed gadolinium enhancement and demonstrated enlargement in both atria. There was no evidence of ventricular hypertrophy and pericardial abnormality. Etiologic investigations revealed normal karyotype, normal plasma amino acids and urine organic acids, and normal plasma autoantibody. The results of the major laboratory tests were within normal range except for a marked elevation in plasma pro-BNP level (1023 ng/L) (the upper limit of normal range, 115 ng/L). She was subsequently diagnosed as restrictive cardiomyopathy. Endomyocardial biopsy was not performed in this case due to refusal by the parents.

Fig. 2 ECG of the patient. ECG showing sinus rhythm, incomplete right bundle branch block, ST segment depression in the inferior leads, and marked biatrial enlargement.

Fig. 3 The *TNNI3* R192H mutation is located in a highly conserved domain, as illustrated across multiple species including humans, mouse, rat, chicken and xenopus. AA: amino acid position. The R190H and R204 mutations have previously been reported to be associated with RCM.

Genetic analysis was performed for 4 candidate genes (*TNNI3*, *TNNT2*, *ACTC*, and *MYH7*) known to associate with restrictive cardiomyopathy by bidirectional sequencing of all the coding exons. A heterozygous 575G > A mutation in exon 8 of *TNNI3* was identified in the patient. The mutation resulted in the substitution of histidine for arginine at amino acid 192 (R192H). Amino acid 192 (arginine) is highly conserved across species and this missense mutation has previously been described to be associated with both restrictive and hypertrophic cardiomyopathy^[7,14] (**Fig. 3**). This mutation was absent in her parents and 100 healthy subjects screened at our institution. No mutation at *TNNT2*, *MYH7*, and *ACTC* was detected in the case.

DISCUSSION

Over the past decade, molecular genetic analyses have revealed dozens of mutations in sarcomeric protein genes, which encode the contractile unit of cardiac muscles, in both DCM and HCM. These findings have shown that mutations in specific functional regions of sarcomeric protein genes result in different phenotypes. An interesting feature of cardiomyopathies is that RCM may overlap with HCM, especially in many familial cases. Up to now, more than 900 mutations have been confirmed to be associated with HCM. Since the first sarcomere gene mutation was identified in the *TNNI3* gene in RCM in 2003^[7], several heterozygous mutations in *TNNI3*, *TNNT2*, *ACTC*, and *MYH7* have been reported to be associated with RCM^[7-13], indicating that RCM may also be caused by single heterozygous mutations in the genes encoding sarcomeric proteins.

In this report, we identified a known heterozygous missense mutation in exon 8 of *TNNI3* (R192H) in a 12-year-old Chinese girl. According to our knowledge, this is the first reported genetic study of Chinese RCM patient. The R192H cTnI mutation was firstly

reported by Mogensen in 2003^[7] in a 19-year-old RCM patient, who died of heart failure. This mutation was also found in a RCM family with hypertrophic physiology, in which all the three persons affected had a early onset of RCM from the first to third decades^[14]. All those reports suggest that this mutation may have a good genotype-phenotype correlation with early age of disease onset. RCM and HCM may occur in one family with the same sarcomere gene mutation, which shows phenotypic heterogeneity in cardiomyopathies^[7,14]. The R192H mutation is located in the highly conserved C-terminal region in *TNNI3* and in vitro studies showed that this mutation disrupts interactions within the troponin complex^[15]. Gomes et al.^[16] and Parvatiyar et al.^[17] found that the R192H mutation increased the Ca^{2+} sensitivity of force development in skinned fibers. Furthermore, Du et al.^[18] showed that the transgenic R193H mice, similar to human R192H, demonstrated RCM characteristics.

Cardiomyocyte contraction is regulated primarily by the interactions between the intracellular calcium concentration and its major sensor-the troponin complex. *TNNI3* is the inhibitory component of the troponin complex and it can bind to actin-tropomyosin and prevent muscle contraction by inhibition of actin-tropomyosin-activated myosin ATPase activity^[17,19]. Studies have identified different functional domains of *TNNI3*^[17,19]. The occurrence of *TNNI3* mutations in RCM was first reported by Mogensen, who found 6 novel mutations located in the conserved and functional important regions of the gene^[7]. To date, at least eight *TNNI3* mutations including seven substitutions and one deletion were identified in RCM that occupied several critical functional domains: the inhibitory region, the switch region and the C-terminus^[7,20,21]. Studies found that the C-terminus of cTnI plays an important role in maintaining the diastolic parameters of the heart^[22]. Three of the eight mutations are located in the

conserved C-terminal region of *TNNI3* protein. *TNNI3* C-terminus is required for normal inhibitory function and studies using transgenic mice demonstrated that mutations existing in this region had serious consequences for cardiac function^[23]. In vitro studies also have revealed RCM-linked *TNNI3* C-terminus mutations sensitized the myofilaments to Ca^{2+} , slowed relaxation and Ca^{2+} transient decay rate^[24]. However, most identified HCM linked *TNNI3* mutations clustered within the same functional domains shared by RCM^[25], and the molecular mechanism whereby mutations in the same functional region of *TNNI3* result in diverse phenotypic expression needs further studies.

In summary, we report a *TNNI3* missense mutation (R192H) in idiopathic RCM in a 12-year-old Chinese girl. This case further improves our knowledge of the causes of cardiomyopathic disease and shows that the spectrum of sarcomeric gene mutations may be involved in pediatric RCM.

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