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Research paper

Whole exome sequencing identifies a novel mutation (c.333 + 2T > C) of *TNNI3K* in a Chinese family with dilated cardiomyopathy and cardiac conduction disease

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ABSTRACT

Dilated Cardiomyopathy (DCM) and cardiac conduction disease (CCD) are two kinds if diseases that can induce heart failure, syncope and even sudden cardiac death (SCD). DCM patients can experience CCD at the same time. In recent research, some disease-causing genes and variants have been identified in patients with DCM and CCD, such as *Alpha-Actinin-2* and *TNNI3 Interacting Kinase* (*TNNI3K*). In this study, we employed whole-exome sequencing (WES) to explore the potential causative genes in a Chinese family with DCM and CCD. A novel splice site mutation (c.333 + 2T > C) of *TNNI3K* was identified and co-segregated with the affected family members. This novel mutation was also absent in 200 healthy local controls and predicted to be disease-causing by Mutationtaster. The splice site mutation (c.333 + 2T > C) may result in a premature stop codon in exon 4 of the *TNNI3K* gene and can induce nonsense-mediated mRNA decay. Real-time qPCR also confirmed that the level of *TNNI3K* mRNA expression was decreased significantly compared with the controls, which may lead to myocardial structural disorder and arrhythmia. In this study we reported the third novel mutation of *TNNI3K* in DCM and CCD patients which further supported the important role of TNNI3K in heart development and expanded the spectrum of *TNNI3K* mutations. The results may contribute to the genetic diagnosis and counseling of families with DCM and CCD.

1. Introduction

Dilated Cardiomyopathy (DCM), accounting for 30–40% of all heart failure cases in large clinical trials, is the leading cause of sudden cardiac death (SCD) and heart failure (McNally et al., 2013; Haas et al., 2015). The typical characters of DCM are cardiac dilation and systolic dysfunction and especially left ventricular enlargement. The estimated prevalence of DCM ranges from 0.05% to 0.4% (Hershberger et al., 2013). Cardiac conduction disease (CCD) is a serious and potentially life threatening disorder of the heart (Smits et al., 2005). The clinical spectrum of CCD includes asymptomatic patients with incidental electrocardiographic abnormalities, as well as patients presenting with syncope and cardiac arrest (Bezzina et al., 2015). Previous studies have demonstrated that mutations in sarcomere and myofilament related genes may result in DCM (Perez-Serra et al., 2016; Lonati et al., 2017), such as *MYH7*, *TTN*, and *MYBPC3*. And ion channel related genes variants may be involved in CCD (Miles and Behr, 2016), for example, mutations of *KCNH1* may lead to Long QT syndrome. However, DCM patients can experience CCD at the same time. Some genes have been identified to cause both DCM and CCD, such as *SCN5A*, *DES* and so on (McNair et al., 2004; Arbustini et al., 2006).

The *TNNI3K* gene encodes the protein TNNI3 interacting kinase which was located at the sarcomere Z disc (Tang et al., 2013). It may play a crucial role in cardiac physiology. Previous studies have found that TNNI3K can phosphorylate cardiac tropnin I (cTnI), which play an important role in the regulation of cardiomyocyte contractility (Theis et al., 2014). Overexpression of *TNNI3K* can induce a reduction of sarcomere length, change the composition of titin isoform and promote cardiac dysfunction (Vagnozzi et al., 2013; Lal et al., 2014). Furthermore, TNNI3K also affects the cardiomyocyte proliferation after injury (Theis et al., 2014). Until now only two mutations of TNNI3K have been detected in patients with DCM and CCD (Theis et al., 2014; Xi et al.,

Abbreviations: DCM, Dilated Cardiomyopathy; SCD, Sudden cardiac death; CCD, Cardiac conduction disease; TNNI3K, *TNNI3 Interacting Kinase*; cTnI, cardiac tropnin I; ECG, 12-lead echocardiogram; WES, Whole exome sequencing; PCR, Polymerase chain reaction; LV, Left ventricular; LA, Left atrial

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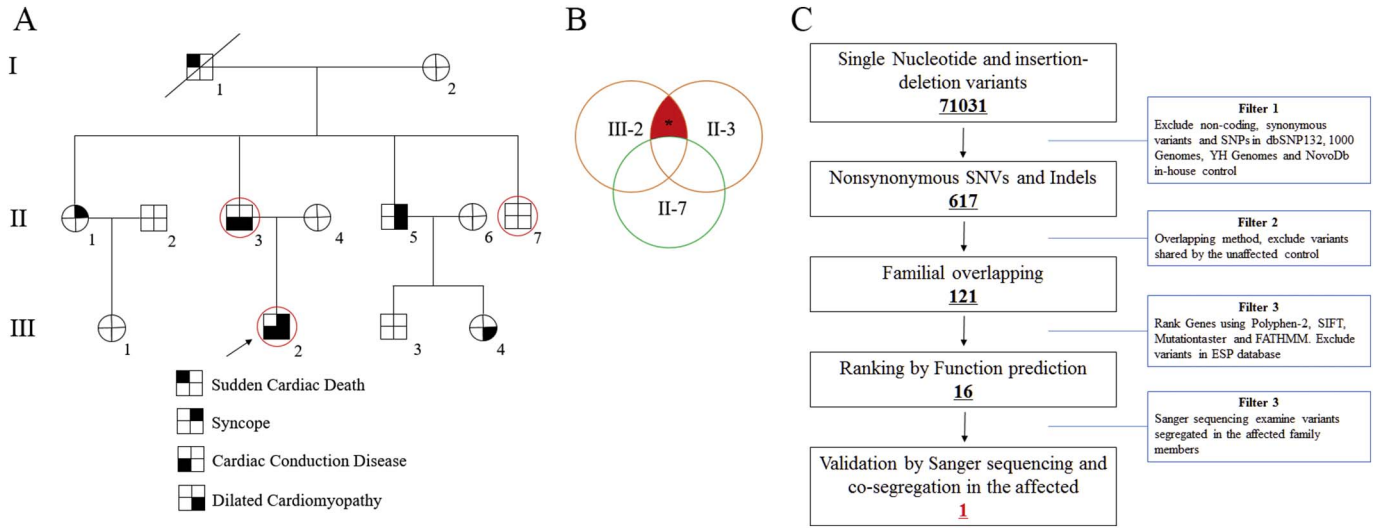

Fig. 1. The pedigree and whole-exome sequencing analysis strategies of this family. (A) Pedigree of the family. Black circles/squares are affected, white are unaffected. Arrow indicates the proband. Three large circles in red represent the three individuals underwent whole exome sequencing. (B) Overlapping filter strategy. Asterisks denotes remaining variants for further analysis that are present in two affected members (II-3 and III-2) but not in the normal control (II-7). (C) Schematic representation of the filter strategies employed in our study.

2015).

2. Materials and methods

2.1. Subjects

The study protocol was approved by the Review Board of the Second Xiangya Hospital of the Central South University in China, and the study participants gave informed consent. All members of this family were enrolled in this study (Fig. 1A). Blood was obtained from the affected probands and family members. Subjects were reviewed by medical records including by B ultrasonic and 12-lead echocardiogram (ECG).

2.2. DNA extraction

Genomic DNA was extracted from peripheral blood lymphocytes of all the family members. Genomic DNA was prepared using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) as we have described previously (Liu et al., 2017).

2.3. Whole exome sequencing

The main part of WES was provided by the Novogene Bioinformatics Institute (Beijing, China). The exomes were captured using Agilent SureSelect Human All Exon V6 kits, and the platform of high-throughput sequencing was performed in Illumina HiSeq X-10. The basic bioinformatics analysis including Reads, Mapping, Variant detection, Filtering, and Annotation was also endowed by Novogene Bioinformatics Institute. The strategies of data filtering refer to Fig. 1B,C.

2.4. Mutation validation and co-segregation analysis

All the filtered mutations and co-segregation analysis among all family members were validated by Sanger sequencing. The primer pairs were designed by Primer 5 (the sequences of primers will be provided upon request), and sequences of the polymerase chain reaction (PCR) products were determined using the ABI 3100 Genetic Analyzer (ABI, Foster City, CA).

2.5. Real-time qPCR analysis

Total RNA was extracted by the PureLink® RNA Mini (Thermo Fisher Scientific, #12183025) from mononuclear cells in the patient and healthy controls. cDNA was synthesized from a total of 1 μg of RNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, #K1621) with oligo (dT) primers. Real-time qPCR reactions were carried out in Fast 7500 Real-Time PCR Systems (Applied Biosystems) using Maxima SYBR Green/ROX qPCR Master Mix (2×) (Thermo Fisher Scientific, #K0221). And $2^{(-\triangle\triangle Ct)}$ was used to analyze the comparative *TNNI3K* mRNA expression levels between mutation group and healthy group. Each assay was performed in five independent tests. The data were analyzed by unpaired two-tailed tests using Graph Pad Prism V.5 software (V.5.0). And the sequences of PCR primers will be provided upon request.

3. Results

3.1. Clinic data

We enrolled a family with DCM and CCD (Fig. 1A). The proband (III-2), a twenty-six-year-old male from Hunan province of central south China, was admitted to our hospital due to syncope during athletic sports. The ECG records showed a sinus bradycardia and cardiac dilation (Fig. 2A,B). B ultrasonic testing further confirmed the enlargement of left ventricular (LV) and left atrium (LA) (62 and 47 mm, respectively). The final diagnose of the proband was DCM. Case history investigation revealed that this patient had a syncope experience approximately three years ago. Family history survey showed that his grandfather (I-1) died at forty-two years old during sleep for an unknown reason. His father (II-3) also presented a cardiac dilatation (LV = 69 mm and LA = 50 mm) and ventricular premature beat (Fig. 2C,D). His aunt (II-1) had a syncope experience but refused to accept testing. His uncle (II-5) also had a syncope history when he was thirty years old and showed a cardiac dilatation phenotypes (LV = 61 mm and LA = 44 mm) (Fig. 2E), but no anomalies in ECG records. His cousin (III-4), a four years old girls, refused to accept ECG testing and cried loudly.

3.2. Genetic analysis

Whole-exome sequencing yielded 10.11 Gb data with 99.8%