

Striking Cardiac Phenotypic Variability in A Chinese Family With A MYH7 Splice Site Mutation

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Abstract

Background: Left ventricular non-compaction cardiomyopathy (LVNC) is a rare congenital heart defect (CHD), genetics defects have been found in patients with LVNC and their family members; and MYH7 is the most common genetic associated with LVNC.

Methods: A trio (fetus and the parents) whole-exome sequencing (WES) was performed when the fetus was found with Ebstein's anomaly (EA), heart dilatation, perimembranous ventricular septal defects (VSD), mild seroperitoneum and single umbilical artery (SUA).

Results: Whole-exome sequencing identified a maternal inherited heterozygous splice site mutation in MYH7 (NM_000257.3:c.732+1G>A). Subsequent Sanger sequencing confirmed that the mutation was heterozygous in the fetus, the old sister, the grandmother, and the mother. QPCR experiment using RNA from blood lymphocytes but were unable to amplify any product.

Conclusion: This familial case underlines that the striking cardiac phenotypic of MYH7 mutation (the c.732+1G>A splice site variant) may be highly variable. The mechanistic studies which could uncover candidate genes modulating cardiac phenotype associated with LVNC/EA should be proceed.

Introduction

LVNC is characterized by excessive trabeculations of the left ventricular (LV) resulting from the development disorder of endocardial tissue. It was first described by Grant [1] in 1926, more recent population studies have reported that the clinic symptoms were range from severe prenatal manifestations to asymptomatic cardiomyopathy presenting in adults [2]. Genetics play a conspicuous part in the long-term outcome in patients with LVNC and their family, and the LV systolic dysfunction were related to genetics. Some patients with EA associated with LVNC were cause by mutations in MYH7, which is the most common genetic. Consequently, due to the autosomal dominant inheritance with variable penetrance [3], the genetic test and timely screening are not restricted to at-risk relatives, and for patient without a family history is also necessary.

We describe a fetus of LVNC combined with EA, perimembranous VSD, mild seroperitoneum and SUA. MYH7 specific splice site mutation segregated with cardiac abnormality and was observed in the fetus' at-risk first degree relatives.

Case Report

A 33-years-old gravida 3 para 1 pregnant woman who diagnosed with fetal anomaly in their local hospital were referred to our hospital at 24⁺² weeks gestation. The woman was healthy and did not take any medication during her pregnancy.

A second trimester ultrasound and echocardiography showed several malformations: EA (the displacement of the septal tricuspid leaflet from the mitral valve annulus was 0.58 cm) with severe tricuspid regurgitation, heart dilatation, perimembranous VSD (Figure 1). The Fractional shortening (FS) was reduced to 11.3%. Mild seroperitoneum, SUA, Slight pericardial effusion were observed. After detailed counseling, the couple decided to terminate the pregnancy with genetic tests and have an autopsy.

On pathologic examination, the gross pathological appearance is the myocardial wall of the left ventricle is thick and loose, elongated anterior tricuspid leaflet, and mild displacement of posterior and septal tricuspid. Histologically, there is focal necrosis of the heart muscle and pathological pigmentation.

When the genetic abnormal MYH7 was identified in the fetal, the at-risk first degree relatives of the pregnant were recommended to undergo screening. Her daughter has normal growth and development, since she is clinically asymptomatic who had never got any cardiac evaluation ever before. The mother of the pregnant and family members had no symptoms to arrhythmias and major events such as heart failure, thromboembolism, and sudden cardiac death.

Transthoracic echocardiography was taken to assess the left ventricular size, thickness, and systolic and diastolic function, and look for any associated congenital heart defects. Echocardiography performed on the pregnant indicated normal cardiac anatomy (Video 1), but we found the old daughter (III-1) and mother (II-1) of the pregnant were LVNC which characterised by preserved cardiac function and left ventricular prominent trabeculations and sinusoids communicating with its cavity on apical four-chamber view, especially in their apex. It was also show normal left ventricular size and with preserved systolic and diastolic function. The end-systolic ratio of noncompacted to compacted (NC/C) myocardium was >2.0 at the end of diastole, which meeting the Jenni echocardiography criteria [4] (Figure 2). Both of them also noted mild mitral and tricuspid regurgitation. There were no intracardiac thrombi and the right ventricle (RV) had normal size and systolic function. The electrocardiogram of the grandmother was abnormal which showed a T-wave inversion. Considering the LVNC is isolated and asymptomatic, the 7 years girl and her grandmather don't obtained the cardiovascular magnetic resonance imaging (CMR).

A trio (fetus and the parents) whole-exome sequencing was performed as described previously [5]. Briefly, genomic DNA was extracted, hybridized and enriched for whole-exome sequencing. The captured libraries were sequenced using Illumina NovaSeq 6000 (Illumina, Inc., San Diego, CA, USA). Then, the sequencing data were aligned to the human reference genome (hg19/GRCh37) using BWA (<http://bio-bwa.sourceforge.net/>) and PCR duplicates were removed by using Picard v1.57 (<http://picard.sourceforge.net/>). GATK (<https://software.broadinstitute.org/gatk/>) was employed for variant calling. ANNOVAR (<http://wannovar.wglab.org/>) was used for variant annotation and interpretation. Variants were filtered out if their minor allele frequency among East Asians in the GnomAD database: $\geq 0.1\%$ for variants associated with autosomal dominant disorders and $\geq 1\%$ for variants associated with autosomal recessive disorders, respectively. We then evaluated each variant considering a careful review of the literature and in silico prediction tools (SIFT, Polyphen2, and Mutation Taster for missense variants and MaxEntScan, GeneSplicer and Human Splicing Finder for splicing variants). For

known CHD or cardiomyopathy genes, pathogenicity of variants was determined according to the American College of Medical Genetics and Genomics guidelines that recommend classifying variants into five categories: pathogenic, likely pathogenic, uncertain significance, likely benign, or benign[6].

As a result, Whole-exome sequencing identified a maternal inherited heterozygous splice site mutation in MYH7 (NM_000257.3:c.732+1G>A). The mutation has been reported previously in several individuals with LVNC and one individual with isolated EA [7-10]. In contrast, it is present in only one individual (allele frequency: 3.98e-6) in the gnomAD database (<https://gnomad.broadinstitute.org>). This variant is reported as Pathogenic in ClinVar. This variant showed a deleterious effect by multiple in silico algorithms. In summary, the variant is classified as likely pathogenic according to the American College of Medical Genetics and Genomics guidelines [6]. Subsequent Sanger sequencing confirmed that the mutation was heterozygous in the fetus, the grandmother, the mother and the old sister (Figure 3). In the family, the MYH7 mutation segregated with cardiac abnormality and was observed in 3/3 affected individuals, where a blood sample was available, and in one apparently healthy individual. To further assess the effect of this variant at the mRNA level, we attempted to perform the qPCR experiment using RNA from blood lymphocytes (other tissue was not available) but were unable to amplify any product (data not shown).

Discussion

This familial case underlines the striking cardiac phenotypic variability associated to the c.732 + 1G > A splice site variant. Our index case presented as an Ebstein anomaly, its old sister and grandmother showed an isolated asymptomatic LVNC, while its mother had no clinical manifestations. Our report also emphasizes the importance of genetic and clinical screening of the relatives of the proband.

MYH7 splice site variants have generally been considered as non-pathogenic and not associated with cardiomyopathy. However, the c.732 + 1G > A splice donor variant has previously been reported to occur in multiple unrelated patients with LVNC and one patient with EA, and to co-segregate with the cardiac phenotype in one family, indicating that c.732 + 1 is a mutational hot spot that has mutated recurrently in LVNC. It is significantly enriched over the general population rate in gnomAD (1/251490). These data suggest that this mutation, although located at the splice site, is associated with LVNC/EA, unlike other non-pathogenic MYH7 splice site mutations. Further investigation is needed to determine why this particular splice site variant is specifically associated with LVNC.

When we overview the echocardiography for the fetal, we evaluated the tricuspid valve leaflets, the function of both ventricles, and the diagnosis of hydrops combined with cardiomegaly which caused by EA's severe tricuspid valve insufficiency. The hypertrophic dilated LVNC diagnosis was missed which mixed phenotype characterised by left ventricular thickening, dilation at presentation. This suggests that in the process of echocardiography, besides paying attention to the examination of structural deformity, we should also pay attention to the morphological and functional changes of myocardium and its causes.

Conclusion

Our report suggests that MYH7 specific splice site mutations, such as the c.732 + 1G > A mutation, are associated with cardiac phenotypes. Our findings also underlines that the clinical spectrum of MYH7 mutation may be highly variable. They incite to drive mechanistic studies that could uncover candidate genes modulating cardiac phenotype and why this specific splice site mutation is particularly associated with LVNC/EA.

Abbreviations

LVNC Left ventricular non-compaction cardiomyopathy

CHD congenital heart defect

WES whole-exome sequencing

EA Ebstein's anomaly

VSD ventricular septal defects

SUA single umbilical artery

LV left ventricular

FS Fractional shortening

RV right ventricle

CMR magnetic resonance imaging

Declarations

Ethical Approval and Consent to participate

This study was approved by the Ethics Committee of Beijing Anzhen Hospital, Capital Medical University.

Consent for publication

Written consent for publication has been obtained.

Availability of supporting data

The data that support the findings of this study are available on request from the corresponding author.

Competing interests

None.

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Author Contributions

Suzhen Ran, Hairui Sun and Yihua He designed the study. Qian Ran and Xiaohang Zhang collected the clinical data and samples from the family. Hairui Sun and Peng Tu analyzed and interpreted the data. Hairui Sun and Peng Tu wrote the manuscript. All authors read and approved the final manuscript.

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Figures

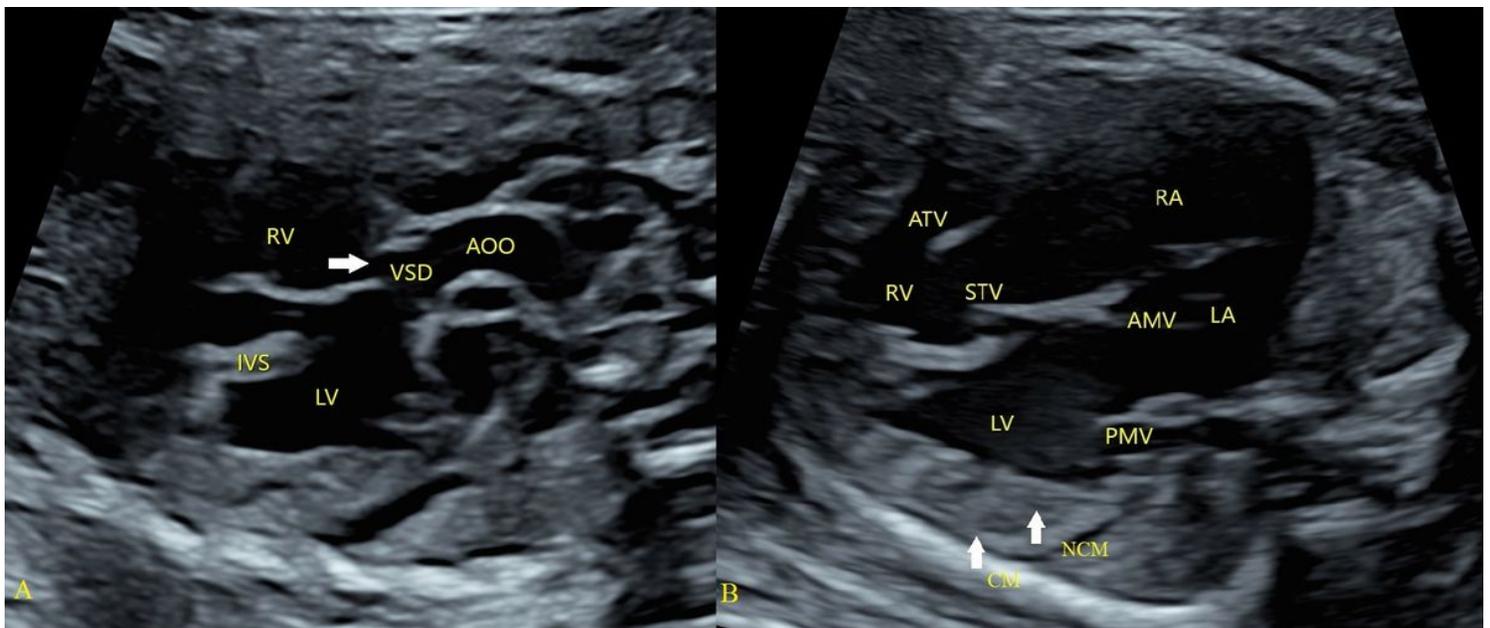


Figure 1

Phenotype of the fetus: perimembranous ventricular septal defect (A); Ebstein's anomaly, left ventricular noncompaction (B). A. The ventricular septal defect (A, white arrow) was located below the aortic valve. B. The displacement of the septal tricuspid leaflet from the mitral valve annulus was 0.58 cm; left ventricular noncompaction and heart dilatation. AAO: ascending aorta, LV: left ventricle, LA: left atrium, RV: right ventricle, RA: right atrium, CM: compact myocardium, NCM: Non-compact myocardium, IVS: interventricular septum, STV: septum tricuspid valve, ATV: anterior tricuspid valve, AMV: anterior mitral valve, PMV: posterior mitral valve.

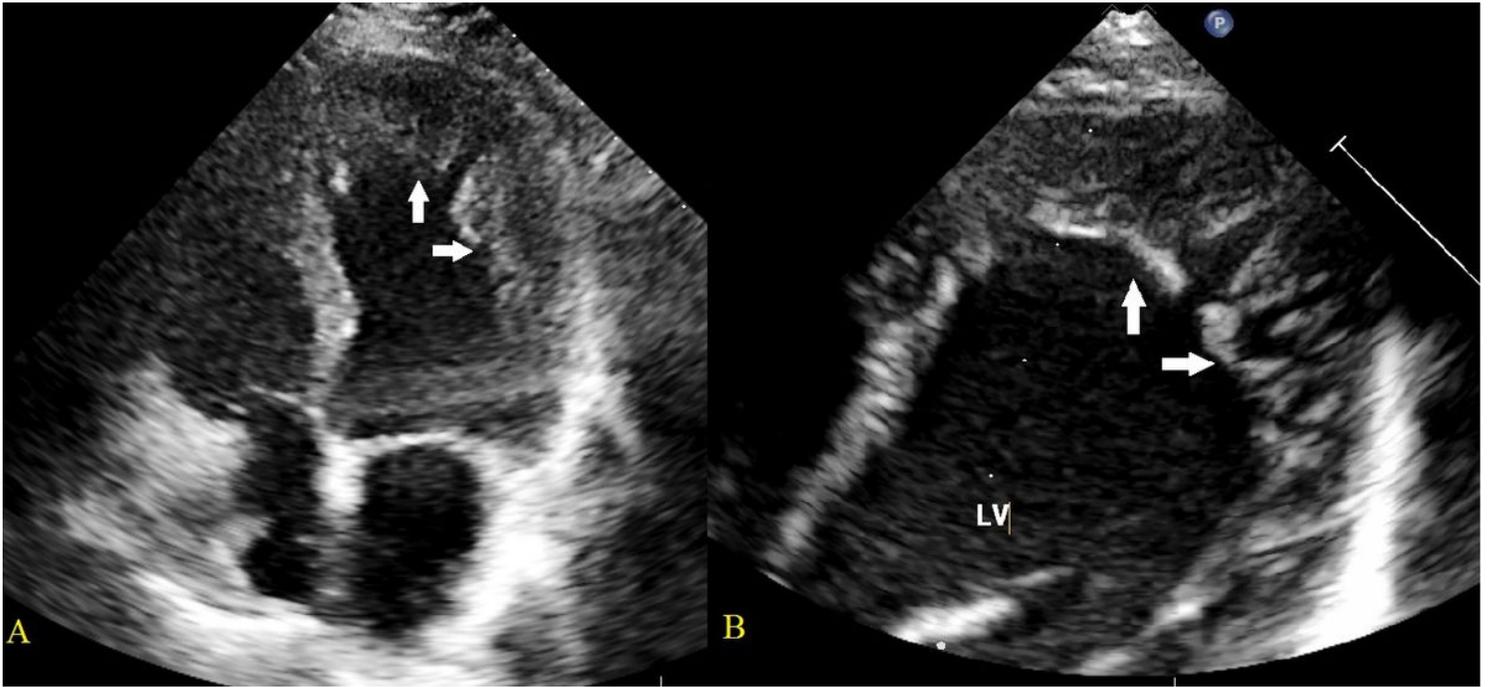


Figure 2

Transthoracic echocardiographic images. Apical 4-chamber views demonstrate excessive trabeculations in the old daughter and grandmother. (A, B white arrow).

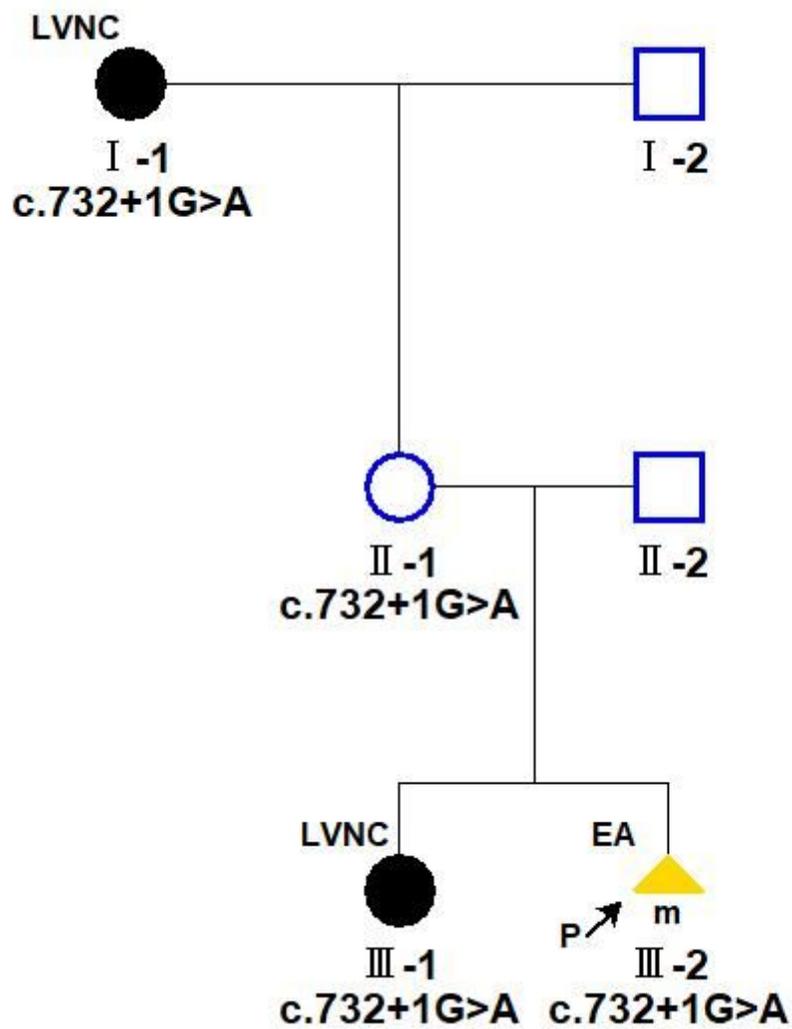


Figure 3

Black round symbols indicate individuals with LVNC; Yellow triangle symbols, individual with the presence of EA.

Supplementary Files

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