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RESEARCH NOTE

A novel variant of *TNNC1* associated with severe dilated cardiomyopathy causing infant mortality and stillbirth: a case of germline mosaicism

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Abstract. Pediatric cardiomyopathies (CM) are rare and challenging to diagnose due to the complex and mixed phenotypes. With the advent of next-generation sequencing (NGS), variants in several genes associated with CM have been identified, such as Troponin C (TnC), encoded by the *TNNC1* gene. *De novo* variants in *TNNC1* have been associated with different types of CM, including dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM). The American College of Medical Genetics and Genomics recently added *TNNC1* to their recommended list of genes for reporting secondary findings. In this study, we report a *de novo* variant, c.100G > C (p.Gly34Arg) in the *TNNC1* gene identified in three siblings with a diagnosis of severe DCM causing infant death for one of the siblings and stillbirth in the other two pregnancies. The identification of the same *de novo* variant in all affected siblings is suggestive of germline mosaicism in this family.

Keywords. pediatric cardiomyopathy; *TNNC1* gene; germline mosaicism; infant mortality.

Introduction

Cardiomyopathy (CM) is a disorder of the heart muscle, usually associated with abnormally enlarged, thickened or stiffened heart muscles. Phenotypic presentation of cardiomyopathy includes dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM), arrhythmogenic right ventricular cardiomyopathy (ARCM), and left ventricular noncompaction cardiomyopathy (LVCM) or noncompaction cardiomyopathy (NCM). CM is not very common in the pediatric population, with one in every 100,000 children in the US being diagnosed, according to the pediatric cardiomyopathy registry (PCMR). A majority of the cardiomyopathies diagnosed in the pediatric population are under 12 month of age (Maron *et al.* 2006; Wilkinson *et al.* 2010), with the diagnosis of specific types of CM being complicated due to complex and mixed phenotypes (Lipshultz *et al.* 2003; Towbin *et al.* 2006; Webber *et al.* 2012). DCM and HCM are the most common types in infants with increased mortality rate or transplant requirement compared to older children, according to PCMR, followed by RCM, NCM and mixed CMs occurring infrequently, and ARCM being rare (Jefferies *et al.* 2015; Lee *et al.* 2017).

Variants in genes encoding sarcomeric/myofilament proteins are associated with several of these cardiomyopathies (Landstrom *et al.* 2008; Garfinkel *et al.* 2018; van der Velden and Stienen 2019; Yotti *et al.* 2019; Tadros *et al.* 2020). Actin, myosin, tropomyosin (Tm), and troponin complex (TnT, TnC, and TnI) constitute the main components of the

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sarcomere myofilament. The troponin complex regulates the interaction of actin and myosin in response to calcium, and troponin (TnC) is the calcium-binding protein that is expressed in cardiac and skeletal muscles. Troponin has two isoforms encoded by the genes *TNNC1* and *TNNC2*, which are expressed in both developing and adult hearts (Filatov *et al.* 1999). *TNNC1* has a relatively severe prognosis with high mortality and early onset and is known to be associated with both the DCM and HCM types of sarcomeric CM (Willott *et al.* 2010; Ingles *et al.* 2019; van der Velden and Stienen 2019; Mazzarotto *et al.* 2020). *De novo* mutations in *TNNC1* seem to be more frequent in pediatric CMs and are linked to severe disease development (Hassoun *et al.* 2021). The significant predisposition to DCM linked to (likely) pathogenic variants in *TNNC1* has resulted in this gene being added to the ACMG's list of genes recommended for reporting of secondary findings (Miller *et al.* 2022).

Here, we present a case report of pediatric cardiomyopathy in three siblings, one deceased at 3 months of age and the subsequent two pregnancies resulted in stillbirth. Dilated cardiomyopathy was present in all the affected siblings and genetic testing performed identified a *de novo* variant in *TNNC1* gene in all three affected siblings. These results are suggestive of germline mosaicism.

Methods

Case presentation

A 38-year-old woman with a history of uterine septum and transverse terminal upper limb defect with a history of 12 miscarriages, had a daughter who died suddenly at three months of age. The woman had a unilateral upper limb hypoplasia with transverse terminal defect as well as bicornuate uterus. Her sister was affected by severe facial paralysis. The mother and her sister were evaluated by a geneticist in childhood and considered to have variable manifestations of Moebius syndrome. No one else in the family has features of Moebius syndrome. Given the history of multiple miscarriages, karyotype analysis was done for both parents and found to be normal. Her issues with recurrent early miscarriages stopped after a hysteroscopic septoplasty.

The 3-month-old daughter, the proband, was born at 39 weeks gestation and came to attention due to sudden death at 3 months of age. Prenatal ultrasounds were unremarkable and there were no dysmorphic features. She was found limp and cyanotic in her car seat. She had been healthy, active, gaining weight since birth and had breast fed 30 min prior to sudden death. Autopsy showed endocardial fibroelastosis, as well as renal lipidosis and hepatic steatosis with no other findings. Investigations for infectious agents were negative. Metabolic disorders of cellular energy production were initially suspected, but not confirmed. Prenatal echocardiography showed moderate cardiomegaly with moderate to severe biventricular systolic dysfunction. There were deep pockets of trabeculations in the apex of the right and left ventricles. There was moderate tricuspid regurgitation. Outflow tract and aortic anatomy were normal. Both pregnancies affected with foetal demise had similar echocardiographic findings. Paternity testing was not done.

The focus was shifted to suspecting a familial dilated cardiomyopathy after the mother's two subsequent pregnancies resulted in stillbirth related to prenatal onset of dilated cardiomyopathy. She was seen at Vancouver Island Medical Genetics Program and exome analysis was initially requested on DNA banked from the proband. Subsequently, familial variant testing was performed including samples from both stillborn children. The woman had a pregnancy that resulted in a live birth before the birth of the proband. The pedigree of the family is outlined in figure 1a.

Whole-exome sequencing

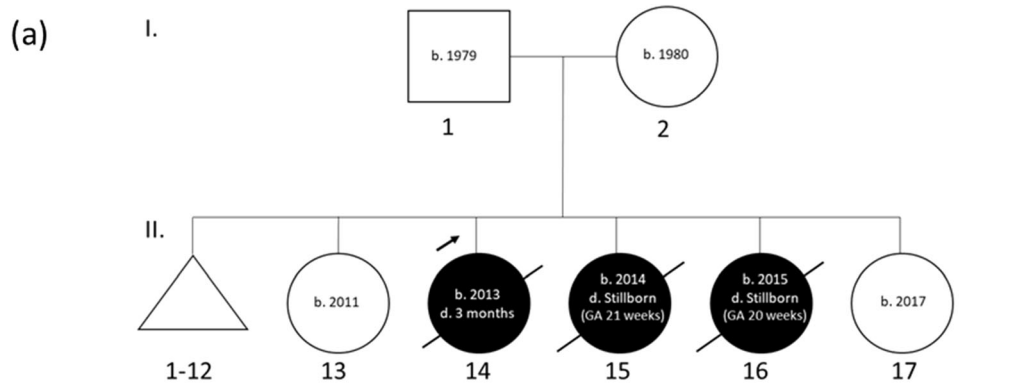
Whole-exome sequencing (WES) for the proband was done as a duo with a sample of DNA from the first stillbirth included with the analysis. WES identified the *TNNC1* variant. Familial variant testing by Sanger sequencing was performed for the second stillbirth (positive), and both parents (negative). Additional, familial testing for the TNNC1 variant was performed for the two unaffected (living) siblings and both were negative.

WES was performed by enriching for coding exons with target specific baits using a solution-based hybridization. Sequencing was performed using lllumina Hi-Seq sequencing instrumentation. The minimum average exome wide depth of coverage was 85×. The sequencing data was analysed using the custom software package Carpe Novo for filtration and annotation of variants identified.

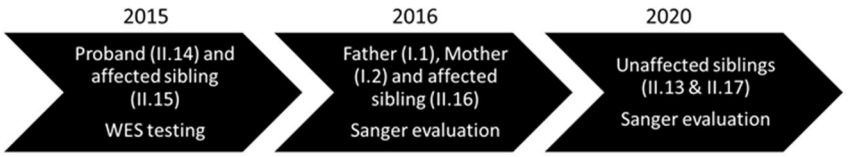
Sanger sequencing

Inheritance and segregation were evaluated by Sanger sequencing using the primer sequences TNNC1_E3-M13F 5′-CTCGTTGTAAAACGACGGCCAGTCTTATCAGAAG AGGACCCCAGG and TNNC1_E3-M13R 5′-CTGCTCA GGAAACAGCTATGACCTTACAGAGGCCAGGGTAGG TAC following established processes in the laboratory. Electropherograms were evaluated using Mutation Surveyor software.

The study was approved by the institutional review board of the Medical College of Wisconsin. Informed consent was obtained from the parents.



(b)



Variant	Proband II.14	II.15	I.1	I.2	II.16	II.13	II.17
TNNC1 c.100G>C	Heterozygous	Heterozygous	Wild type	Wild type	Heterozygous	Wild type	Wild type

(c)

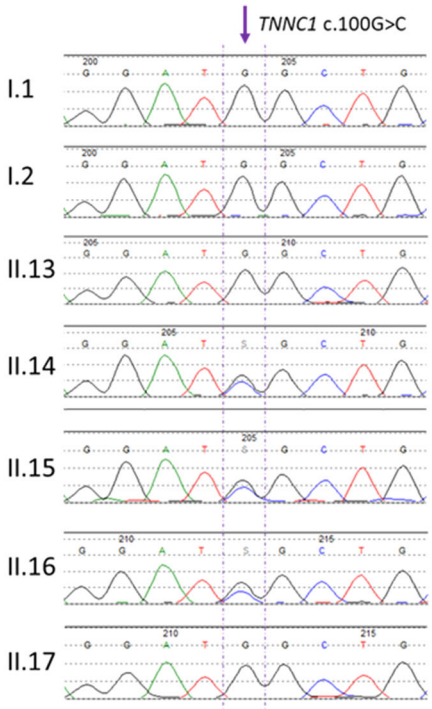


Figure 1. Pedigree and genetic analysis. (a) Pedigree of the family. There was no known family history of dilated cardiomyopathy. The proband has unaffected parents with 12 miscarriages, two stillborn and two unaffected siblings. (b) Genetic analysis: WES for the proband (II.14) was done as a duo with a sample of DNA from the first stillbirth included with the analysis (II.15), identifying the *TNNC1* variant; c.100G > C (p.Gly34Arg). Inheritance, and segregation analysis was done by Sanger sequencing. (c) Electropherograms of Sanger sequence. The heterozygous *TNNC1 de novo* variant in the proband, c.100G > C (NM_003280), as indicated by the vertical dotted lines on the electropherogram was evaluated in parents and all siblings (affected and unaffected) using Sanger sequencing. The variant is shown to be present only in the proband and the two affected siblings (II.14, II.15 and II.16).

Table 1. Variant identified in the proband and the first stillborn child (II.2 & II.3 in figure 1a).

Gene	Chr.	cDNA change	Protein change	Variant type	Classification	Zygosity	Parent of origin
TNNC1	3	c.100G > C	p.Gly34Arg	Missense	Likely pathogenic*	Heterozygous	*De novo*

*Updated to likely pathogenic after inheritance and segregation analysis.

Results

Cytogenetic and molecular analysis

Karyotypes were normal for both stillbirths and the microarray report indicated degraded DNA. WES was performed as a duo on the banked genomic DNA sample from the 3-month-old female, proband (figure 1a, II.14), along with a sample of DNA from the first stillbirth (figure 1a, II.15). While no CNVs were identified using WES, the heterozygous variant c.100G > C (p.Gly34Arg) in the *TNNC1* gene (NM_003280) was identified in both the proband and the first stillborn child, table 1, which was classified as a variant of unknown significance (VUS) following the American College of Medical Genetics and Genomics (ACMG) guidelines (Richards *et al.* 2015).

Segregation analysis

Inheritance and segregation of the *TNNC1* variant was evaluated in the second stillbirth (figure 1a, II.16) and both parents (figure 1a, I.1 and I.2) along with the two unaffected (living) siblings (figure 1a, II.13 and II.17), by Sanger sequencing. As shown in Sanger chromatographs, figure 1c, the *TNNC1* variant, c.100G > C (p.Gly34Arg) was identified only in the affected individuals in the family i.e., the proband and the two stillborn children (figure 1a, II.14, II.15 and II.16). The *TNNC1* variant, c.100G > C (p.Gly34Arg) variant was not identified either in the parents demonstrating it to be *de novo*, or the two live unaffected siblings of the proband. The presence of same *de novo* variant in all the three of the affected individuals suggests germline mosaicism.

Discussion

We report a pedigree with a novel variant in the *TNNC1* gene which fits very well with the phenotype of dilated cardiomyopathy observed for the three affected siblings. The apparently *de novo* nature of the variant present for all three affected siblings supports germline mosaicism.

The *TNNC1* variant, c.100G > C (p.Gly34Arg) changes amino acid glycine at codon 34 to arginine, a change from a nonpolar to polar amino acid. The amino acid glycine is highly conserved among species, and the observed change occurs in the EF-hand domain of the cTnC protein, associated with calcium binding. Studies suggest that mutant cTnC that occurs due to single amino acid changes causes disease via a dominant negative mechanism (Reinoso *et al.* 2021), in contrast to genes encoding myosin-binding protein C and titin, which typically produce truncating variants and elicit disease via haploinsufficiency (Yotti *et al.* 2019). It is hypothesized that upon incorporation into the contractile apparatus, cTnC mutants trigger contractile dysfunction due to altered interactions with cTnT, cTnI or Ca2+ binding affinity, or a combination of scenarios (Reinoso *et al.* 2021).

The p.Gly34Arg change has not been previously reported in patients with cardiomyopathies but another amino acid change at the same codon, p.Gly34Ser has been previously reported in a newborn with cardiomegaly identified through X-ray and presenting with clinical signs of cardiac failure (Hassoun *et al.* 2021). Histology was consistent with endocardial fibroelastosis and distinctive microangiopathy, analogous to the autopsy results of our proband. The variant has not been listed in the Genome Aggregation Database (https://gnomad.broadinstitute.org). Based on the ACMG guidelines (Richards *et al.* 2015), the collective evidence presented here along with the segregation data led to the reclassification of the *TNNC1* variant p.Gly34Arg to likely pathogenic.

Currently, a limited number of genes are known to cause dilated cardiomyopathy in foetuses, infants and children. This family expands the phenotype associated with *TNNC1* to include severe cardiomyopathy in the prenatal or early infantile period. This has important implications in the genetic counselling of families with a *TNNC1* pathogenic or likely pathogenic variant. The identification of a probable germline mosaic variant presented here and recent inclusion of *TNNC1* to the ACMG's recommended list for reporting secondary findings (Miller *et al.* 2022), suggests that consideration of possible germline mosaicism and family counselling in evaluating variants in *TNNC1*.

All data included in this study are available upon request from the corresponding author.

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