

# Deletion in *TNNI3* gene is associated with restrictive cardiomyopathy <sup>☆☆☆</sup>

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## Abstract

In dilated and hypertrophic cardiomyopathies, over ten disease-causing genes have been identified in each entity. In contrast, mutations in only desmin and cardiac troponin T and I (*TNNI3*) have been shown to cause restrictive cardiomyopathy (RCM). We applied a candidate gene approach and identified a novel one nucleotide deletion, resulting in frame shift and predicted formation of a premature stop codon, deletion of part of exon 7 and all exon 8, and truncation of significant C-terminal portion of *TNNI3*. Western blot analysis showed approximately 50% reduction of total troponin I content in myocardial tissue. The clinical hallmark was a restrictive type of cardiac hemodynamics, and congestive heart failure, leading to the death of the patient at the age of 28.

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Although our knowledge on the genetic causes and pathogenesis of hypertrophic and dilated cardiomyopathies is expanding, little is known about molecular defects, pathogenetic pathways and genotype–phenotype correlations in RCM. To date, mutations in three genes, desmin, troponin T and cardiac troponin I (*TNNI3*), have been described as possible causes of primary RCM [1–3]. Recently, RCM was also described to be part of the phenotype in Emery–Dreifuss muscular dystrophy caused by lamin A/C gene mutations [4]. Here we describe an idiopathic case of RCM. We applied a candidate gene approach in order to search for the possible genetic cause of the disease and a novel single nucleotide deletion in *TNNI3*, resulting in a premature stop codon, was identified.

Approval for the study was obtained from the local ethics committees in St. Petersburg and Stockholm. The index case, a 28 year-old woman, died due to progression of congestive heart failure. She first presented at the age of 23 with spontaneous episodes of chest pain. At that time ECG showed progressive increase of P wave duration and amplitude, right shift of the QRS axis (90°), and right ventricular hypertrophy. Echocardiographic examinations revealed typical signs of restrictive filling

pattern with E/A ratio decreasing from 3.8 to 2.3, deceleration time progressing from 86 to 135 ms and isovolumic relaxation time was 55 ms. The patient was diagnosed with restrictive cardiomyopathy in accordance with the following echocardiographic criteria: 1) restrictive left ventricular filling pattern in the absence of obvious known cause for it, and 2) exclusion of constrictive pericarditis. Subsequent echocardiographic examinations during the following 6 years revealed a progressive (concentric) remodeling of the left ventricle with initially normal

Table 1  
Echocardiographic data of proband, obtained during 6 years follow-up

Parameter	1996	1997	1999	2002 Jan	2002 Feb
LVESD (mm)	24	28	27	21	19
LVEDD (mm)	38	38	35	34	32
IVSd (mm)	11	10	10	9	8
LVPWd (mm)	11	10	10	11	10
LAD (mm)	54	55	66	84	100
RAD (mm)	21/42	23/54	38/74	56.2/77	60/78
PAPs (mmHg)	28	28	38	58	51
IVS/LVPW	1	1	1	1	0.8
LV mass (g)	145	132	114	107	81
LVMI (g/m <sup>2</sup> )	99	91	80	74	54
EF/FS	66/35	66/35	78/36	78/37	78/38

EF — ejection fraction, FS — fractional shortening, IVSd — interventricular septum measured in end diastole, LAD — left atrium dimension, LV mass — left ventricular mass, LVEDD — left ventricular end systolic dimension, LVESD — left ventricular end systolic dimension, LVMI — left ventricular mass index, LVPWd — left ventricular posterior wall thickness in end diastole, PAPs — pulmonary artery pressure, RAD — right atrium dimension, calculated in two perpendicular positions: interatrial septum–lateral wall and tricuspidal valve–superior wall.

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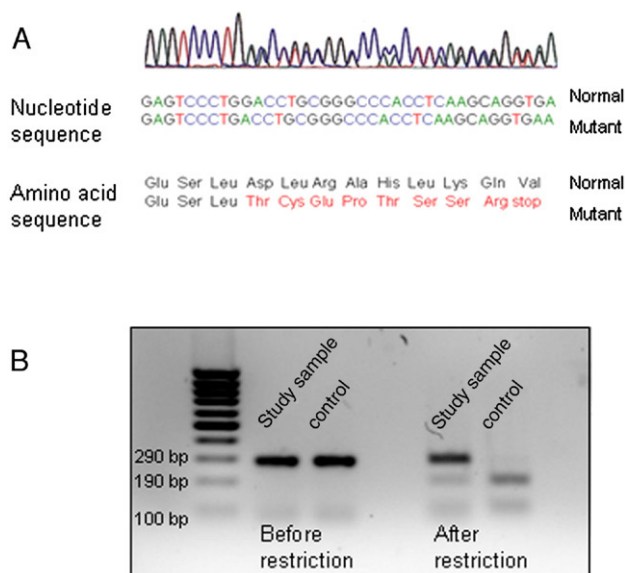


Fig. 1. Genetic studies of *TNNI3* in patient with restrictive cardiomyopathy. A. Sequence of *TNNI3* gene. In the patient DNA a one nucleotide (G) deletion was detected at position 4762 with an anticipated frame shift in exon 7, causing formation of stop codon ( $\Delta 168\text{fs} \times 176$ ). B. Restriction analysis of study and control samples. Deletion of nucleotide G at position 4762 breaks the recognition site for *AvaII* restriction enzyme. This results in the absence of restriction in the mutant allele, leading to partial restriction in the study sample, compared to full restriction of both alleles in the control sample.

and finally decreased left ventricular mass index (Table 1). The systolic function remained preserved during the entire follow-up period.

The family history revealed that the father of the proband died from sudden cardiac arrest at the age of 29. It is interesting to note that two female family members, including the

proband, had both been pregnant with subsequent intrauterine fetal death in the third trimester, and, further, that sudden death occurred in two more family members before 1 year of age. No DNA was available for mutation analysis from any of these family members.

A deletion of one single nucleotide in exon 7 of *TNNI3* (nt4762delG according to the Gene Bank sequence X90780) was detected by DHPLC and confirmed by direct cycle sequencing in the index patient (Fig. 1A). The deletion breaks the recognition site for *AvaII* restriction enzyme, which was used for additional confirmation of the mutation (Fig. 1B). The deletion causes a frame shift in codon 168, and is predicted to lead to the formation of a premature stop codon at position 176 (D168 fs X176) (Gene Bank accession number DQ303406). This mutation was not found in two healthy members of the family, or in 100 analyzed healthy controls (200 chromosomes).

Western blot analysis performed with three different antibodies, detecting different troponin I epitopes, showed an approximately 50% decrease in troponin I content in the study sample, compared to the control (Fig. 2A,B,C). Although the frame shift mutation introducing a premature stop codon at position 176 suggests formation of a truncated protein, lacking 57 amino acids at the C-terminal end, no extra band of lower molecular weight, corresponding to the truncated mutant protein, was detected with any of the antibodies used. The reason why we were not able to detect a mutant truncated form of troponin I is not clear, but is likely due to rapid degradation of the mutant protein.

Cardiomyopathies with mutations in troponin I can combine morphological and echocardiographic signs of restrictive and hypertrophic phenotype with one of them prevailing [2]. One of the possible explanations for this variation in phenotype may lie in the mechanism of actin–troponin–tropomyosin complex interaction. We hypothesize

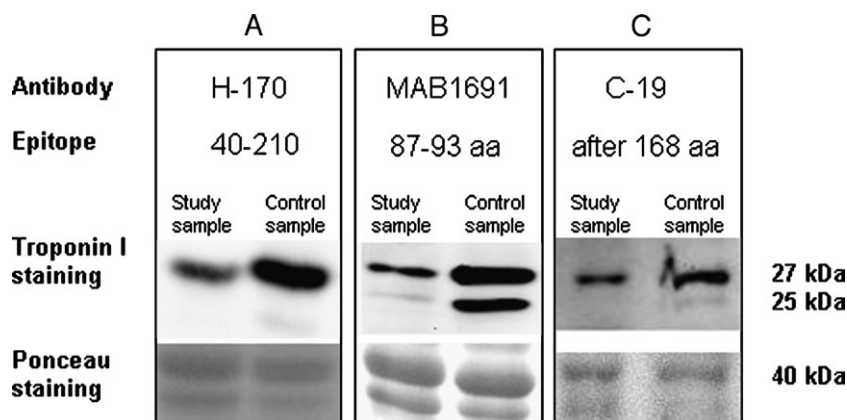


Fig. 2. Western blot analysis of troponin I. Illustration of troponin I protein expression in patient and control samples, detected with three different antibodies. The lower molecular weight band (25 kDa) indicates the ischemic ‘stunned’ form of troponin I. Ponceau membrane staining demonstrates equal sample loading, also confirmed by quantification using computer software. Lane A: H-170 antibody, raised against amino acids 40–210, is expected to detect only full-length troponin I. Amount of full-length troponin I in the study sample corresponds to 49% of the control sample. Lane B: MAB1691 antibody, raised against amino acids 87–93, is expected to detect both normal and mutant proteins. The amount full-length troponin I (27 kDa) in the study sample corresponds to 47% of the control sample. No truncated protein is detected. Lane C: C-19 antibody, raised against troponin I epitope located after amino acid 168, is expected to detect normal full-length and stunned forms of troponin I. Amount of full-length troponin I in the study sample corresponds to 51% of the control sample.

that changes in actin-binding affinity, affinity to troponin C, and ability to inhibit thin filaments during diastole, caused by certain *TNNI3* mutations, lead to an altered interaction within the actin–troponin–tropomyosin complex, and thus cause either severe diastolic dysfunction and restrictive cardiomyopathy, or myocardial hypertrophy.

In our case, the deletion induced a frame shift from residue 168 and formation of stop codon in position 176 expected to result in the truncation of major C-terminal part of cardiac troponin I. This might lead to a nearly total absence of the second actin- and troponin C-binding region of the protein. Western blot analysis showed an approximate 50% decrease in total troponin I content, but failed to detect mutant truncated form of the protein. This argues for a haploinsufficiency mechanism of the mutation, rather than a dominant-negative effect. The lack of inhibitory effect of the troponin–tropomyosin complex on the thin filaments could, therefore cause impaired active relaxation of myocardium and a restrictive filling pattern.

In conclusion, we describe a case of RCM with a novel *TNNI3* mutation. We hypothesize that the mutation has a

haploinsufficiency mechanism and hence accounts for abnormal relaxation of the myocardium and restrictive hemodynamics.

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