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# Exploring the c.406 C > T variant in *TNNI3* gene: pathogenic insights into restrictive cardiomyopathy

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## Abstract

**Background** Restrictive cardiomyopathy (RCM) is a rare cardiac disorder characterized by diastolic dysfunction and myocardial stiffness, frequently associated with genetic variants. We aimed to explore the genetic basis of RCM in a diagnosed patient through comprehensive genetic analysis.

**Methods** Whole exome sequencing (WES) was conducted on the proband, followed by Sanger sequencing for variant confirmation and familial segregation analysis. In silico tools and structural protein modeling were employed to assess the functional impact of the identified variant.

**Results** The c.406 C > T variant, classified as likely pathogenic, results in a truncated TNNI3 protein. Bioinformatics analysis highlighted significant structural disruptions, likely impairing sarcomere function. The patient presented with growth retardation, progressive dyspnea, and echocardiographic findings consistent with RCM. Both parents were heterozygous carriers, supporting an autosomal recessive inheritance pattern. The homozygosity of the novel variant identified in this study is a critical factor in the genotype–phenotype correlation observed in this case.

**Conclusion** This study identified the novel c.406 C > T variant in TNNI3 as a potential pathogenic driver of RCM, emphasizing the critical role of genetic evaluations in early diagnosis and management of inherited cardiomyopathies. Further studies are warranted to explore therapeutic interventions targeting TNNI3-related pathologies.

**Keywords** Restrictive cardiomyopathy, Mutation, Variant, Whole exome sequencing, TNNI3

## Introduction

Restrictive cardiomyopathy (RCM) is a rare yet serious form of cardiomyopathy, characterized by impaired diastolic filling due to increased stiffness of the ventricular walls [1]. Unlike other cardiomyopathies, RCM is distinguished by its restrictive nature, wherein the heart muscle becomes rigid and less compliant, leading to impaired ventricular filling and reduced cardiac output. This condition often results in symptoms of heart failure, including dyspnea, fatigue, and edema [2]. The pathophysiology of RCM is complex, and can be influenced by various factors, including genetic variants, infiltrative diseases,

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and fibrosis [3]. Genetic variants play a crucial role in the development and progression of RCM by shedding light on the underlying molecular mechanisms of the disease [4]. Variants in genes associated with cardiomyopathy can lead to altered protein function disrupted cellular processes, and ultimately contribute to the pathological features observed in RCM [5]. The *TNNI3* gene (OMIM:115210), part of the titin family, encodes a protein that plays a critical role in maintaining the structural integrity and function of cardiac muscle cells [6]. Titin, a large multifunctional protein, is essential for muscle contraction and elasticity, and its proper functioning is vital for normal cardiac physiology [7]. Variants in *TNNI3* has been implicated in altering the normal function of titin, potentially disrupting its role in muscle contraction and elasticity [8]. The prevalence and spectrum of gene variants associated with RCM were systematically described. This section aims to synthesize the latest research findings regarding next-generation sequencing (NGS) in individual diagnosed with inherited cardiomyopathies and differing levels of growth retardation. Additionally, we will examine the obstacles faced in the clinical implementation of NGS and consider its potential role in advancing our comprehension of the genetic underpinnings of cardiovascular disease (CVD).

## Materials and methods

### Clinical examination

The patient was the first child born to a healthy family of four. The parents were not- consanguineous, and his younger brother showed no abnormalities or signs of the same illness. The patient showed some degree of growth retardation at the age of four. She presented with a height of 92 cm and weight of 12 kg at age four. These anthropometric findings were consistent with moderate growth retardation. Therefore she was referred to a nutritionist; but she didn't show any improvement in her growth situation until the age of six, when she started to show symptoms consistent with progressive dyspnea on exertion, which had worsened gradually over the past 12 months. Upon visiting a local hospital, the patient underwent a thorough examination and was diagnosed with heart failure, subsequently receiving supportive treatment. Further diagnostic evaluations at better-equipped facilities were recommended for a comprehensive understanding of her condition. She was managed conservatively with a treatment plan aimed at alleviating symptom, with a referral to a specialized tertiary care center for additional advanced testing and access to resources necessary for establishing a definitive diagnosis. At the hospital, an echocardiographic examination revealed signs compatible with RCM, leading to a referral to Rajaie Cardiovascular Institute, Tehran, Iran, for further evaluation, especially a genetic consultion.

### Exon enrichment and next-generation sequencing

Genomic DNA was extracted from peripheral whole blood using the salting-out method [9]. This fragmented DNA then utilized to determine the genetic basis of the disorder. Whole exome sequencing (WES) was conducted by Illumina HiSeq 6000 platform at a mean read depth of 150× (Macrogen, Inc., Netherland) and raw data was analyzed by Cardiogenetic Research Center, Rajaie Cardiovascular Institute, Tehran, Iran. To validate the candidate variants identified through WES, Sanger sequencing was conducted.

### Variant validation

The identified candidate causative variants associated with RCM were ultimately confirmed in the family through Sanger Sequencing using specific primers (forward primer: ggtctccctgtttttggttcc, reverse primer: ctcaggcctagggttgttgg for *TNNI3* gene on a SimpliAmp Thermal Cycler (Thermo Fisher Scientific). PCR products were sequenced on an ABI Sequencer 3500XL PE (Applied Biosystems) and analyzed by Bioedit tool [10].

### Bioinformatics approach to predict damaging effect of idenified variant

Bioinformatics approach was used to predicting the functional impact of identified variant is crucial for understanding their potential role in disease. To this end, we employed a bioinformatics approach utilizing five established in silico prediction tools: MutationTaster [11], PolyPhen-2, SIFT (Sorting Intolerant From Tolerant), FATHMM (Functional Analysis through Hidden Markov Models), and CADD [12]. In addition to these predictive analyses, the pathogenicity of variants was evaluated according to the American College of Medical Genetics and Genomics (ACMG) guidelines. By integrating the predictions from these bioinformatics tools with the ACMG guidelines, we aim to enhance our understanding of how specific genetic alterations contribute to inherited cardiomyopathies [13].

### Protein network analysis

Network Analysis Structural modeling of the *TNNI3* protein was performed using the SWISS-MODEL server ( http://swissmodel.expasy.org/) to evaluate the potent ial effects of identified variants. The canonical FASTA sequence of the *TNNI3* protein was retrieved from the UniProt database (UniProt ID: P19429) (http://www.uniprot.org/). This sequence served as the foundation for building a three-dimensional model of the protein, allowing for a better understanding of its structural conformation, functional domains and conservation of the amino acid in this particular residue. The SWISS-MODEL server employs homology modeling techniques, which leverage known protein structures to predict the 3D

conformation of target proteins. By inputting the *TNNI3* sequence, we generated a structural model that reflects the expected spatial arrangement of its amino acids. This model is essential for visualizing how specific variants may alter the protein's structure and, consequently, its function. In summary, combining structural modeling with network analysis allows for a comprehensive evaluation of how specific genetic variants in *TNNI3* may influence protein function and contribute to inherited cardiomyopathies.

## Results

### Patient characteristics and family history

The patient, a 4-year-old girl, presented with progressive growth retardation. At the time of presentation, her weight was about 12 kg, and her height was about 80 cm, both significantly below the normal range for her age. In response to these concerns, her parents sought the advice of a local nutritionist. Despite undergoing dietary therapy, including various food supplements, the patient exhibited no signs of improvement. Her condition worsened, accompanied by symptoms of dyspnea and fatigue, until the age of 6. Subsequently, she was referred to a local cardiologist for further evaluation. Based on the echocardiographic findings and other examinations, the cardiologist suspected RCM. Due to these findings, the patient was referred to the Rajaie Cardiovascular Institute in Tehran, Iran. A comprehensive initial workup, including blood tests and urinalysis, revealed no additional abnormalities. Consequently, a specialized echocardiogram was performed, which corroborated the initial diagnosis of RCM. Echocardiography revealed findings consistent with restrictive cardiomyopathy, characterized by preserved systolic function and diastolic dysfunction. The study also indicated significant enlargement of both the left and right atria, along with signs of elevated filling pressures (Fig. 1). Transthoracic echocardiography revealed preserved left ventricular systolic function with an ejection fraction (EF) of 40%. Diastolic function was significantly impaired, as evidenced by an elevated E/A ratio of 2.1. The left atrium was dilated, with a left atrial volume index (LAVI) of 44 $mL/m^2$, and the right atrium was also enlarged, measuring 22 $cm^2$ in area. There was evidence of mild left ventricular enlargement, and wall thicknesses were within normal limits. Mild tricuspid regurgitation was present, along with moderate mitral regurgitation. These findings suggest a pathophysiological mechanism of impaired ventricular filling, likely due to myocardial stiffness. As a result, the patient was considered for genetic consultation to investigate potential underlying etiology.

### Genetic findings

We conducted WES on the affected individual and identified a novel homozygous variant in the *TNNI3* gene, specifically c.406 C > T:p.R136*. Sanger sequencing confirmed this finding, revealing that individual's parents were heterozygous carriers (Fig. 2-AB). Following WES, no additional rare variants classified as potentially pathogenic based on the ACMG/AMP guidelines were detected in any genes currently associated with RCM. This inheritance pattern supports an autosomal recessive mode of transmission for the associated phenotype. In silico analysis of the c.406 C > T variant was performed using several bioinformatics tools. The results indicated that this variant is predicted to be disease-causing according to MutationTaster. Additionally, the CADD score for this variant was calculated to be 14.02, suggesting a significant impact on gene function. Furthermore, according to the ACMG guidelines, this variant was classified as likely pathogenic. These findings collect.ively underscore the potential role of the *TNNI3* c.406 C > T variant in the observed clinical phenotype and contribute to a broader understanding of the genetic basis of the disease. To further investigate the functional implications of the identified variant, we conducted protein analysis using UniProt and multiple sequence alignments across different species. The results showed that the arginine residue at position 136 is highly conserved among various species, indicating its importance in maintaining protein structure and function. Structural modeling was employed to analyze the impact of the c.406 C > T variant on the *TNNI3* protein. The results demonstrated that this variant leads to a truncated protein, which is likely to disrupt the normal folding and stability of the protein. Such truncation can severely impair the protein's functional capabilities, as essential domains may be lost, ultimately affecting cardiac muscle contraction (Fig. 2-CD).

## Discussion

The identification of the c.406 C > T variant in the *TNNI3* gene in our patient highlights its potential pathogenic role in RCM. This study contributes to the expanding body of research linking *TNNI3* variants to severe cardiac conditions, particularly those affecting diastolic function. Our findings are consistent with previous studies that underscore the role of *TNNI3* gene in maintaining sarcomeric integrity and cardiac muscle elasticity, which are critical for normal ventricular compliance [14]. The pathogenicity predicted through in silico analysis, suggested that the c.406 C > T variant may disrupt the structural and functional properties of the *TNNI3* protein, thereby contributing to the restrictive phenotype observed. The clinical presentation of growth retardation and progressive dyspnea in our patient highlights the diverse phenotypic manifestations associated with

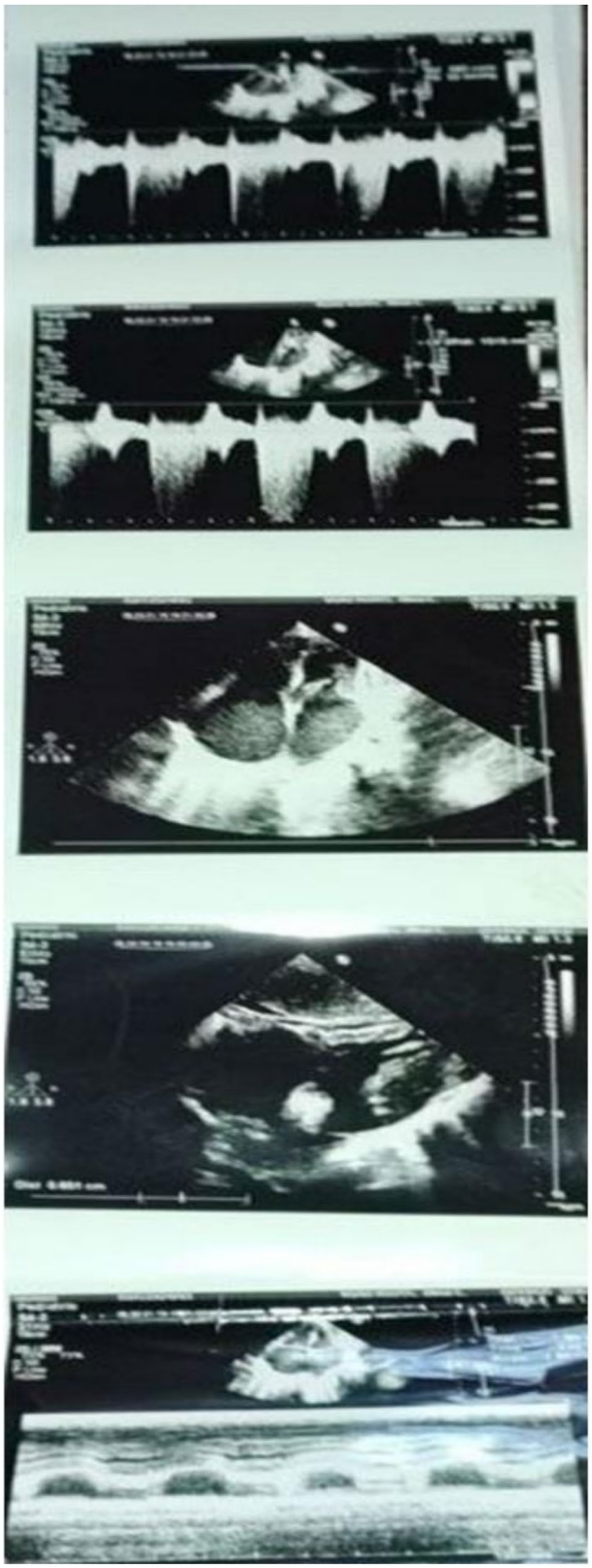

**Fig. 1** The patients echocardiography. Transthoracic echocardiography showed preserved left ventricular systolic function with an ejection fraction (EF) of 40%. Diastolic function was markedly impaired, indicated by an elevated E/A ratio of 2.1. Left atrial dilation was observed, with a left atrial volume index (LAVI) of 44 mL/m$^2$, and right atrial enlargement, with an area of 22 cm$^2$. Mild left ventricular enlargement was noted, with wall thicknesses remaining within normal ranges. Mild tricuspid regurgitation and moderate mitral regurgitation were also present

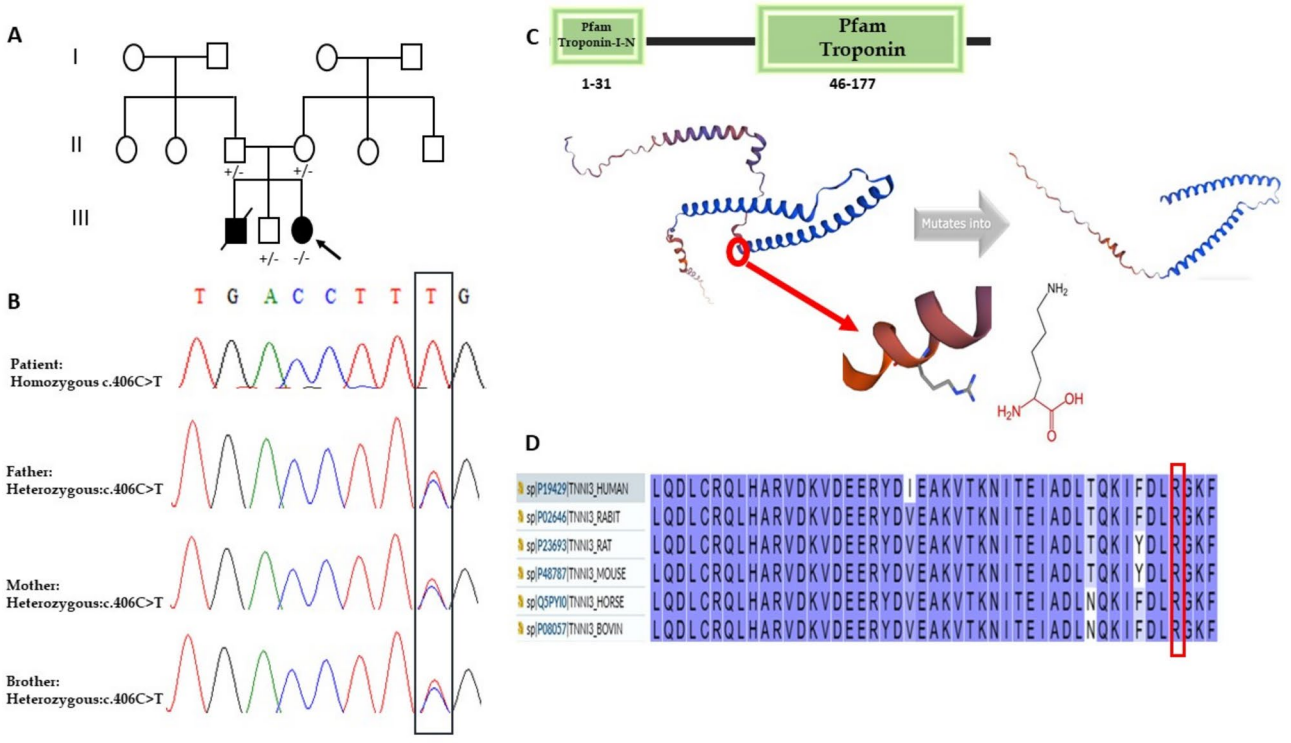


**Fig. 2** (**A**) Pedigrees for families affected by cardiomyopathy. (**B**) Molecular analysis of the *TNNI3* gene revealed homozygous and heterozygous missense variants of c.406 C > T in family. (**C**) Structural representation of the TNNI3 protein, illustrating the variant of the amino acid arginine to stop codon, this alternation highlights the impact of genetic change on protein synthesis and function. The large arrow highlights the location of the variant, which corresponds to an alpha helix region. (**D**) Diagram showing the conversation of specific amino acid residue across different species, the high light position indicates critical amino acid that is preserved due to their essential role in protein function and structural integrity

*TNNI3*-related RCM. This aligns with previous research indicating that *TNNI3* variants may not only impact cardiac function but also have broader systemic implications [15]. Given the rarity and the genetic heterogeneity of RCM, the identification of specific gene variants is crucial for refining diagnostic process and guiding treatment options. Our findings reinforce the importance of comprehensive genetic evaluations in RCM patients, as it may reveal underlying genetic factors that influence both prognosis and management approaches. Several studies on *TNNI3* variants have reported associations with severe diastolic dysfunction, even in pediatric patients, which parallels our case early-onset presentation [16]. For instance, studies by Ditaranto et al.., documented *TNNI3* variants may result in early and progressive forms of RCM that frequently present in childhood or adolescence with signs of heart failure, growth retardation, and systemic symptoms [17]. Our patient's clinical presentation—early-onset restrictive cardiomyopathy with progressive dyspnea, significant biatrial enlargement, preserved systolic function, and moderate growth retardation—shares key features with cases reported by van den Wijngaard et al., in which *TNNI3* mutations caused severe pediatric RCM [18]. This suggests a potentially more severe loss-of-function mechanism and highlights a broader systemic involvement, including growth delay, which was not emphasized in previous reports. Our findings show notable clinical overlap with the case reported by Shah et al., where a dominant-negative missense mutation in *TNNI3* (p.Lys178Glu) caused severe, early-onset restrictive cardiomyopathy, marked by dyspnea, biatrial enlargement, and preserved systolic function [19]. Similar to our patient, the disease manifested in early childhood and progressed rapidly. However, unlike the heterozygous missense mutation reported by Shah et al., our case involves a homozygous nonsense variant (p.Arg136), likely resulting in a complete loss of protein function. Additionally, our patient exhibited growth retardation, which was not observed in Shah's case. These differences suggest that loss-of-function mutations may result in broader systemic effects and a distinct pathophysiological mechanism compared to dominant-negative missense variants. Our findings further support these observations, as the patient displayed significant growth delays and progressively worsening dyspnea, reflecting the hallmark features of *TNNI3*-linked RCM. This emphasizes the importance of early recognition and intervention to mitigate adverse outcomes. Additionally, while most previously documented *TNNI3*-related studies focus on hypertrophic or dilated phenotypes, our

findings contribute to the growing subset of restrictive presentations. This aligns with a shift observed in more recent studies that recognize *TNNI3* variants as contributors to RCM, expanding the phenotypic spectrum associated with this gene beyond hypertrophic and dilated cardiomyopathies [20]. The management of *TNNI3*-associated RCM remains challenging due to the lack of targeted therapies and the disease's rapid progression in pediatric cases [21]. Current strategies are primarily supportive and focus on alleviating symptoms of heart failure using diuretics to manage fluid overload, and in some cases, beta-blockers or ACE inhibitors to reduce afterload and improve diastolic filling [22]. However, these interventions have limited efficacy in altering disease progression. The evolving understanding of *TNNI3* variants in different cardiomyopathy phenotypes highlights the need for a nuanced approach to genetic counseling and management strategies for affected families In this study, we utilized WES to investigate an Iranian family with a history of RCM and identified a novel missense deleterious variant, R136*, in the *TNNI3* gene that causes RCM in the family. This homozygous c.406 C > T variant, reported here for the first time, was confirmed in the proband through WES and validated in the family using Sanger sequencing, revealing its paternal origin. Our findings highlight the value of genetic testing not only for diagnosis but also for understanding inheritance patterns within families affected by RCM.

This study has several limitations. First, it is based on a single case, which limits the generalizability of the findings and prevents robust genotype–phenotype correlations. Functional validation of the identified *TNNI3* variant was not performed, so the pathogenic mechanism remains speculative and inferred primarily from in silico predictions and structural modeling. Additionally, the lack of myocardial biopsy or protein expression data restricts direct insight into the impact of the nonsense mutation on cardiac tissue architecture and function. Finally, long-term follow-up data are unavailable, limiting assessment of the natural disease course and treatment response in this specific genetic context.

## Conclusion

In conclusion, our study reinforces the association between *TNNI3* variants and RCM while highlighting the clinical importance of recognizing the diverse phenotypic expressions linked to these genetic variants. The homozygosity of the identified novel variant plays a pivotal role in the phenotypic manifestation observed in this case. Emphasizing this point strengthens the understanding of the genotype–phenotype relationship in the context of the disease. The identification of novel variants, such as c.406 C > T, expands our understanding of *TNNI3*-related pathologies and emphasizes the need for ongoing research into the genetic underpinnings of cardiomyopathies. Future studies should aim on elucidating the mechanisms through which these variants disrupt cardiac function and exploring potential therapeutic avenues for affected individuals.

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**Author contributions**
TM and HH drafted the work. SK designed the project and performed WES analysis. MM surveyed the patient clinically. All authors reviewed the manuscript.

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**Data availability**
No datasets were generated or analysed during the current study.

## Declarations

**Ethics approval and consent to participate**
Our study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of Rajaie Cardiovascular Institute, Tehran, Iran (IR.RHC.REC.1403.111). Informed consent to participate was obtained from all of the participants in the study. Informed consent to participate was obtained from the parents or legal guardians of any participant under the age of 16.

**Consent for publication**
Written informed consent for publication of clinical details and/or clinical images was obtained from the parents of the patient.

**Competing interests**
The authors declare no competing interests.

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