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Identification of a rare variant in *TNNT3* responsible for familial dilated cardiomyopathy through whole-exome sequencing and in silico analysis

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Abstract

Background Dilated cardiomyopathy (DCM) is a prevalent etiology of heart failure, distinguished by the gradual and frequently irreversible myocardial muscle impairment. Roughly 50% of DCM occurrences stem from hereditary rare variants. In this study, our aim was to identify the genetic cause of DCM in a pedigree with several affected individuals across four generations.

Methods Whole exome sequencing was performed on the proband, with variants filtered and analyzed using in silico tools. Co-segregation analysis was conducted using Sanger sequencing. Protein structure modeling and protein–protein interaction evaluations were performed using AlphaFold3 and HADDOCK2.4, respectively.

Results We identified a missense rare variant in the *TNNT3* gene, leading to the p.Glu125Gly alteration in the Troponin T3 (TNNT3). This rare variant is strongly implicated as the causative factor for DCM in the pedigree. Several key factors underscore its significance: the rare variant co-segregates with the disease in the pedigree, is absent in 850 control samples, alters a conserved amino acid, is predicted to detrimentally affect protein function, and results in structural changes.

Conclusions Our findings suggest that *TNNT3* rare variants can induce DCM by weakening the binding energy between TNNT3 and Tropomyosin (TPM), leading to functional deficiencies in muscle contraction, as demonstrated by our structural modeling and docking studies. Troponin T is essential for the proper contraction of striated muscles and is related to cardiac development. Bioinformatics investigations have elucidated the involvement of TNNT3-related pathways, notably the Striated Muscle Contraction pathway and Cardiac Conduction. *TNNT3* resides within loci previously implicated in cardiomyopathy. Given its crucial role in muscle contractile function, rare variants in *TNNT3* hold the potential to be a significant contributing factor in the pathogenesis of DCM. A wealth of literature substantiates the correlation between troponin T and cardiac disorders. Our findings further corroborate this association.

Keywords Dilated cardiomyopathy, DCM, Whole-exome sequencing, Troponin T, *TNNT3*

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Introduction

Dilated cardiomyopathy (DCM) is a progressive and usually irreversible myocardial disorder characterized by left or biventricular dilatation and systolic dysfunction, in the absence of abnormal loading conditions and severe coronary artery disease [1–4].

DCM is a major cause of heart failure and one of the primary indications for heart transplantation globally. As a component of heart failure with reduced ejection fraction (HFrEF), DCM is identified as the most prevalent form of cardiomyopathy associated with heart failure syndromes [5, 6]. Although the exact prevalence of DCM, including genetically mediated DCM, remains unknown, recent estimates suggest that it affects about 1 in every 2500 individuals in the general population, albeit this ratio might be an underestimation. This incidence increases to 1 in every 250–400 patients with heart failure [1, 7]. Familial DCM is responsible for up to 50% of reported cases, and the prevalence of DCM is slightly higher in males, with a ratio of females to males ranging from 1:1.3 to 1:1.5 [8, 9]. While DCM can occur at any age or ethnicity, it is more commonly found in the third to fourth decades of life, and the sporadic form is more prevalent in pediatric populations [2, 10]. The majority of genetic DCM cases are inherited as an autosomal dominant trait and are characterized by incomplete and age-related penetrance, as well as variable expression. Nevertheless, there have been reports of specific forms of mitochondrial, autosomal recessive, and X-linked recessive inheritance [1, 11].

Autosomal dominant DCM cases comprise approximately 90% of familial DCM cases, while X-linked recessive and autosomal recessive cases account for less than 10% of these cases [12].

The molecular pathogenesis of DCM has been linked to rare variants in over 250 genes, most of which encode a range of sarcomeric proteins, cytoskeletal proteins, sarcolemma proteins, Z-disk proteins, desmosomal proteins, RNA binding proteins, nuclear envelope proteins, intercalated disc proteins, mitochondrial proteins, gap junction channel proteins, ion channel proteins, and transcriptional factor proteins [13]. Rare variants located in sarcomeric genes, specifically those that encode the thick and thin filament contractile proteins MYH7, MYBPC3, ACTC1, and TNNT2, have also been implicated in autosomal dominant DCM. These rare variants account for up to 5% of all cases of DCM [4]. Nevertheless, the currently acknowledged genetic etiologies are projected to provide an explanation for only 20% to 50% of cases, which highlights the need to identify more genes that contribute to DCM [13].

Studies suggest that whole exome sequencing (WES) has a high diagnostic yield in accomplishing a molecular diagnosis of genetic disorders. Accordingly, the objective of the current study was to identify causing variants of DCM in an Iranian family using WES analysis.

Materials and methods

This research was performed in accordance with the Declaration of Helsinki and with the approval of the ethics board of the Tehran University of Medical Sciences. The subjects in this study were from a family residing in Sarab city, East Azerbaijan province, Iran. The proband, a 55-year-old male patient, was referred from the Imam Khomeini Hospital Complex. Clinical evaluation included collecting detailed personal and family histories. Genomic DNA was extracted from peripheral blood samples of family members using the salting-out method. All isolated genomic DNA samples were quantified using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). Whole exome sequencing was carried out for the proband. The Agilent SureSelect Human All Exome V7 kit was used for exome capture. Sequencing was performed on an Illumina NovaSeq 6000 platform (Macrogen Facility, Seoul, Korea) with 150-bp paired-end runs and an average coverage of 100X. Raw reads in fastq format underwent quality control (QC) by FastQC software [14] and were aligned to the hg19 (GRCh37) genome reference using the Burrows–Wheeler aligner (BWA) [15] to produce a binary file (BAM). Duplicate reads were marked using Picard tools, while insertion/deletion (InDels) local realignment, base quality score recalibration and variant calling were done using Genome Analysis Toolkit (GATK) [16].

The resultant VCF file was annotated by wANNOVAR. Nonsynonymous variants and small insertions or deletions (indels) that occurred in exonic regions, along with variants that affected splicing, were selected to identify potential causal variants. Subsequently, annotated variants were filtered by removing SNPs with minor allele frequency (MAF) > = 0.01 in the 1000 Genomes database (www.1000genomes.org), the Genome Aggregation Database (http://gnomad.broadinstitute.org/), the Exome Aggregation Consortium database (http://exac.broadinstitute.org/), and the Iranome database (http://iranome.com/), to reduce the likelihood of including benign variants. A file containing homozygous and heterozygous variations was prepared, and the file was searched to identify variations within any of the known DCM, hypertrophic cardiomyopathy (HCM), and left ventricular non-compaction (LVNC)-related genes (Table S1). We employed seven different applications to perform in silico analysis—SIFT (https://sift.bii.a-star.edu.sg), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), Mutation Taster (https://www.mutationtaster.org/), Mutation Assessor (http://mutationassessor.org/), CADD (https://

cadd.gs.washington.edu/), Alphamissense (https://alphamissense.hegelab.org/) and GERP from UCSC Genome Browser to anticipate the potential effect of the detected rare variants on protein function. Co-segregation analysis of the candidate pathogenic variants was confirmed by Sanger sequencing for the proband and available affected and unaffected family members. The experimental 3D structure of TNNT3 has not been determined, which led this study to employ Alpha-Fold3 (https://alphafoldserver.com/), a deep-learning framework for highly accurate modeling and predicting the structure of proteins and complexes [17]. The FASTA sequence of TNNT3 was retrieved from the UniProtKB database (ID: P45378) [18]. To introduce the Glu125Gly variant, the psfgen plugin in VMD1.9.3 was employed [19]. The mutant structure was minimized for 20,000 steps using the conjugate gradient method with the CHARMM36 force field for proteins [20] in the NAMD 2.13 package [21]. The 3D structure of the TPM1 dimer was obtained from the RCSB PDB database (https://www.rcsb.org/) with the PDB ID of 7UTL. This dimer includes the coordinates for two TPMs, spanning residues from 48 to 284. We named the second chain the same as the first, with residues from 332 to 568. The interaction of wild-type and mutant forms of TNNT3 with TPM were evaluated, using the HADDOCK2.4 web server [22, 23]. The active residues of TNNT3 and TPM were set from 68 to 135 [24, 25] and 220 to 284 [26–28], respectively. The passive residues representing surface neighbors of active residues, were automatically determined. Default values were used for other HADDOCK settings. All protein structures were visualized and analyzed using VMD1.9.3 [19]. LigPlot + v.2.2 was used to show the 2D plot of the interactions across the protein–protein interface [29].

Results

Clinical information

The first family member to seek medical evaluation was III.10. He experienced dyspnea on exertion and chest pain, and his left ventricular ejection fraction (LVEF) was severely reduced (LVEF = 25–30%). Coronary angiography revealed obstruction in all coronary arteries. After undergoing coronary artery bypass grafting to treat the coronary artery disease, a single chamber implantable cardioverter defibrillator (ICD) was also implanted due to the low LVEF. In the follow-up, the atrial fibrillation (AF) rhythm was electrically cardioverted with the ICD. He has been under good follow-up for at least 6 years and has shown good functional capacity while on medications. The second family member (III.12) sought medical assessment due to palpitations and a sensation of dropped beats. The ECG and 24-h ECG Holter monitoring revealed frequent premature ventricular contractions (PVC). Echocardiography showed moderate left ventricular (LV) systolic dysfunction. This patient has been on medications for 4 years and is currently medically stable. The third family member (III.4) sought medical attention due to headaches and uncontrolled hypertension. He is currently taking anti-failure medications and were admitted once for electrical cardioversion due to his condition. He has been under medical follow-up for 4 years now. The fourth family member (IV.3) experienced dyspnea on exertion and chest pain. His LVEF was severely reduced (LVEF = 25–30%) and he had significant LV systolic dysfunction. The fifth family member (III.1), experiences dyspnea on exertion and chest pain, but cardiac evaluations were not performed for her. The clinical characteristics of family members are summarized in Table 1 and the pedigree is illustrated in Fig. 1A.

Genetic finding

Representative data on exome sequencing results that evidence high-quality sequencing is presented in Table S2. From the list of DCM, LVNC, and hypertrophic cardiomyopathy-related genes, the candidate variants *SCN5A*, *TTN,* and *MYLK* were present in the proband (Table S1). A thorough literature search was conducted to identify other genes whose function may influence DCM status. This search revealed *TNNT3* as a potential candidate gene. Therefore, the variations in *SCN5A, TTN, MYLK* and *TNNT3* were considered as candidate DCM-causing rare variants to be further pursued (Table 2). Direct sequencing of these four variations in 7 members of the proband's immediate and extended family showed that a missense variant, (c.A374G:p.Glu125Gly), in the *TNNT3* gene segregated with disease status.

To assess the frequency of this variant in the general population, it was compared against the genomAD and ExAC databases, where it was found to have a frequency of 0.000012 and 0.000009, respectively. It is worth noting that this variant was not detected in the 1000 Genomes Project or the Iranome databases. The *in-silico* analyses performed on the variant using the prediction tools SIFT, Mutation Taster, CADD and Alphamissense indicated a pathogenic potential, with scores of SIFT (D), Mutation Taster (D), CADD (29.9) and Alphamissense (likely pathogenic). These scores suggest that the variant may have a deleterious effect. Moreover, the GERP tool identified the region containing the variant as evolutionarily conserved (Table 3), further supporting the notion that the variant may be detrimental. Considering these findings, it is anticipated that the variant is deleterious. In addition, it is worth mentioning that the variant met the criteria outlined by the American College of Medical Genetics and Genomics (ACMG) and was categorized as likely pathogenic (Table S3).

Table 1 Clinical data of patients of the studied family

Origin of family	**Sarab, Iran**					
Patient ID	**III.1**	**III.4**	**III.6**	**III.10**	**III.12**	**IV.3**
Sex	Female	Male	Male	Male	Male	Male
Age at examination (years)	N/A	40	Deceased	57	52	32
Age at onset (years)	N/A	38	56	54	50	31
Disease duration (years)	N/A	2	4	3	2	1
Rhythm in ECG	N/A	Sinus rhythm, persistent atrial fibrillation (Electrically cardioverted)	N/A	Sinus rhythm, persistent atrial fibrillation (Electrically cardioverted)	Sinus rhythm, frequent PVC	Sinus rhythm
LV size in imaging	N/A	Mild LVE	N/A	Mild LVE	Normal	Moderate LVE
LVEF% in imaging	N/A	30%	N/A	40%	36%	24%
Scar in Cardiac MRI	N/A	N/A	N/A	N/A	N/A	Patchy mid wall in inferoseptum
Coronary artery disease	N/A	–	N/A	+ (3VD)	–	–
Gene	*TNNT3*	*TNNT3*	–	*TNNT3*	*TNNT3*	*TNNT3*
Chromosome Change (hg19)	c.A374G	c.A374G	–	c.A374G	c.A374G	c.A374G
Protein change	p.E125G	p.E125G	–	p.E125G	p.E125G	p.E125G
Mutation type	Missense	Missense	–	Missense	Missense	Missense
Zygosity	Heterozygous	Heterozygous	–	Heterozygous	Heterozygous	Heterozygous
Confirmation method	S.S	S.S	–	S.S	WES and S.S	S.S

WES: Whole Exome Sequencing/ S.S: Sanger Sequencing/ LV: Left ventricle/ LVE: Left ventricular enlargement/ LVEF: Left ventricular ejection fraction (55% < Normal range < 65%)/ N/A: Not available /3VD: 3 vessel diseases

To validate the candidate variant in the pedigree, Sanger sequencing was conducted on a total of five affected family members and three unaffected family members over 40 years of age. The results of the sequencing revealed that the index patient (III.12), his brothers (III.4 and III.10), his cousin (III.1), and his nephew (IV.3) all displayed the rare variant in a heterozygous state (AG), While the other unaffected siblings of the proband—III.2, III.3, and III.7, aged 47, 45, and 58, respectively—showed a wild-type genotype for the variant (AA). This information is illustrated in Fig. 1B.

In silico protein modeling of TNNT3

The best model of TNNT3 was obtained from the AlphaFold server. The complex formed by TNNT3 with TNNI, TNNC, tropomyosin, and actin plays a crucial role in regulating muscle contraction and relaxation through calcium-mediated interactions and coordination among its subunits. As the Glu125Gly variant occurs within the TNNT3 binding site for tropomyosin, this interaction is evaluated in both the wild-type and mutant forms of TNNT3. The most reliable conformation of the complex between wild-type and mutant TNNT3 with TPM yielded HADDOCK scores of -140.6 ± 22.8 and -116.1 ± 8.5, respectively. The HADDOCK score is a weighted sum of intermolecular electrostatic (Elec), van der Waals (vdW), desolvation (Dsolv), and buried surface area (BSA). Both complexes were aligned based on the TNNT3 backbone, as presented in Fig. 2A. It is evident that in the mutant form, TPM is located farther away from TNNT3, with an RMSD value of 3.98 Å between TPMs. Specifically, residue His275 is within a distance of less than 3 Å from residue 125 in the wild-type complex, and 6.5 Å in the mutant complex (Fig. 2B, C). Glutamic acid is a polar amino acid with a large side chain, whereas glycine is the smallest amino acid with no side chain. As shown in Fig. 3, a hydrogen bond between Glu125 and His275 is present in the wild-type, but absent in the mutant. More details about the differences between the non-covalent interactions on the surface of these two proteins can be seen in Fig. 3. Consequently, this rare variant induces structural alterations, resulting in weaker binding energy between TNNT3 and TPM, thereby leading to functional insufficiency.

Discussion

In this study, we utilized whole exome sequencing as a methodology to explore the genetic etiology of DCM in an Iranian family with multiple affected members. Our investigation led us to discover a heterozygous rare variant (exon12:c.A374G:p.Glu125Gly) within the *TNNT3* gene. To the best of our understanding, this specific rare variant has not been previously documented as a causative factor for DCM in any publicly accessible databases

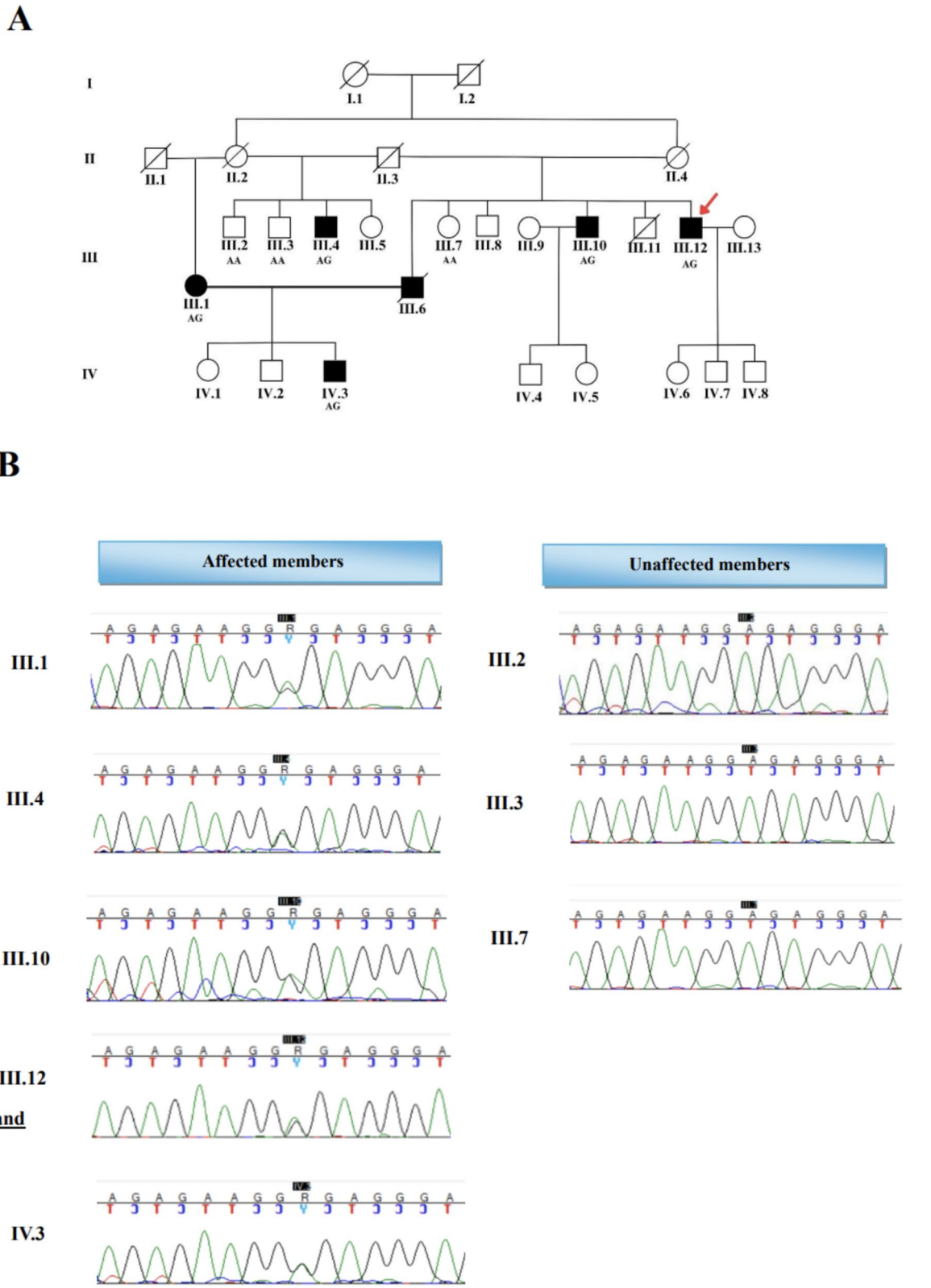

Fig. 1 **A** Pedigree of the studied family. Filled circles and squares: DCM affected; proband is identified with arrow. Genotypes of respective putative causative gene in individuals are shown. AG Heterozygous mutant genotype, AA normal genotype. **B** Sequence chromatograms of *TNNT3* mutated and wild-type genotypes

Table 2 Candidate variants information

Chr	Position	Gene	AA change	Zygosity	dbSNP	Co-Segregation
11	1,955,213	*TNNT3*	exon12:c.A374G: p.Glu125Gly	Het	rs747984101	+
3	38,591,972	*SCN5A*	exon27:c.C5837T:p.Ser1946Phe	Het	rs199473332	–
3	123,359,274	*MYLK*	exon27:c.T4169C:p.Met1390Thr	Het	–	–
2	179,457,531	*TTN*	exon128:c.C32120T:p.Pro10707Leu	Het	rs72646840	–

The table represents the candidate variants' information and the results of their co-segregation with the disease in the studied pedigree. The (+) symbol shows that the rare variant co-segregated with the disease, and the (–) indicates that it did not. Het: heterozygous

Table 3 TNNT3 amino acid conservation across different species

Human	E	R	A	E	Q	Q	R	I	R	A	E	K	E	R	E	R	Q	N
Chimp	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Gorilla	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Orangutan	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Gibbon	**D**	.	.	.	**H**	**H**	.	.	**P**	.	.	.	**D**	.	.	.	.	.
Zebrafish	.	.	.	.	.	.	.	.	.	**T**	.	**Q**	.	**K**	.	.	**H**	**A**
Crab-eating-macaque	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Baboon	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Green_monkey	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Marmoset	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Squirrel_monkey	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Bushbaby	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Chinese_tree_shrew	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Squirrel	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Lesser_Egyptian_jerboa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Prairie_vole	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Chinese_hamster	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Golden_hamster	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Mouse	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Rat	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Naked_mole-rat	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Guinea_pig	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Chinchilla	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Brush-tailed_rat	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Multiple alignment shows the amino acid conservation in different species. The candidate rare variant of *TNNT3* is illustrated in the red box. The dot indicates that the amino acid is the same as that of human [69]

or scientific literatures. Co-segregation analysis was conducted to validate the candidate variant in this family, the analysis revealed the presence of the *TNNT3* rare variant exclusively in the affected family members, while it was absent in both the unaffected family members and the 800 control individuals. Based on these findings, it is strongly indicated that the identified *TNNT3* rare variant is highly likely to be the underlying cause of DCM in this Iranian family.

DCM is linked to a broad range of genetic variations involving numerous genes, as revealed by our latest inquiry made on 16th April 2023, from the DisGeNET database (https://disgenet.com), which reported correlation of over 500 genes with pathogenesis of DCM. The extensive genetic heterogeneity certainly makes the genetic analysis of this condition quite challenging [1, 30, 31]. It seems that the majority of causing rare variants in DCM are impacting the structure or function of

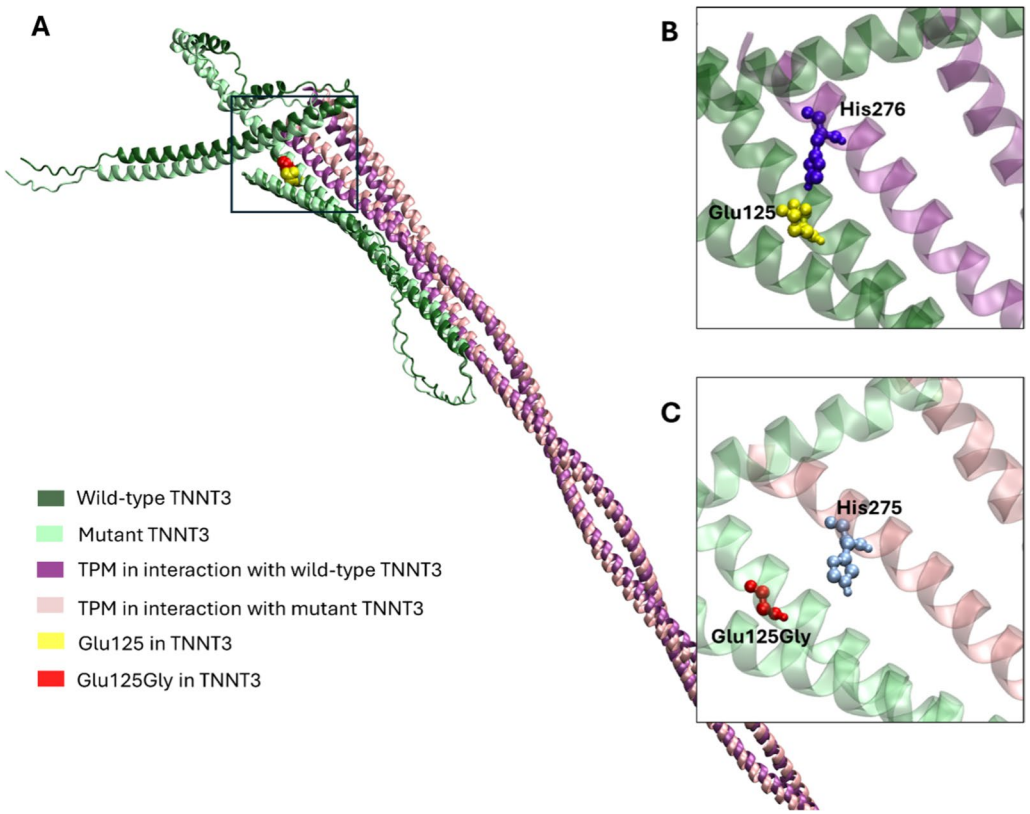

Fig. 2 Structural Changes in TNNT3 Due to the Glu125Gly variant. **A** Complex structure of wild-type and mutant forms of TNNT3 with TPM. Zoomed-in view of the interaction around residue 125 in (**B**) the wild-type and (**C**) the mutant

important components, such as the sarcomere, cytoskeleton, and ion channels [2, 32].

Approximately 10% of DCM cases can be attributed to sarcomeric rare variants, which give rise to impairments in the generation and transmission of force within the cardiac muscle. Moreover, ongoing investigations are progressively illuminating the relationship between sarcomeric rare variants and the development of a dilated phenotype [11, 33, 34].

Cardiomyopathies, including DCM, have been associated with various rare variants in troponin genes which are a group of sarcomeric genes [25, 35]. The Troponin complex is a crucial regulatory protein involved in the calcium-mediated regulation of muscle contraction and relaxation in both skeletal and cardiac muscle tissues. Specifically in cardiac muscle, it plays a critical role in the transition from diastole to systole [25, 35–37]. Troponin T (TnT), the largest subunit in the troponin complex, plays a crucial structural role. It anchors the troponin complex to the thin filament by binding to tropomyosin and ensures that the core domain of troponin is properly aligned with the actin filament [38].

Studies have identified point rare variants occurring in the middle and C-terminal regions of cardiac troponin T (cTnT) as causative factors for cardiomyopathies. These regions are particularly susceptible to structural alterations, and their dysfunction has been implicated in the development of cardiomyopathies. These structural changes within the protein could impact its stability and its interactions with other proteins, potentially leading to reduced functionality. Furthermore, abnormal splicing of the N-terminal region of cardiac TnT has also been identified as a potential mechanism leading to cardiomyopathy [25].

Studies have indicated that the primary mechanism responsible for the pathogenesis of DCM associated with cTnT rare variant is the desensitization of Ca^{2+}, and/or alter myosin ATPase activity in cardiac muscle. This leads to impaired force generation during cardiac muscle contraction [25, 35]. Studies have revealed that rare variants in the cTnT gene account for approximately 15% of familial hypertrophic cardiomyopathy (FHCM) cases [39], and about 3% of familial DCM cases [40]. Moreover, these rare variants have also been associated with other cardiac conditions, such as restrictive cardiomyopathy (RCM) and LVNC, as highlighted in various studies [39].

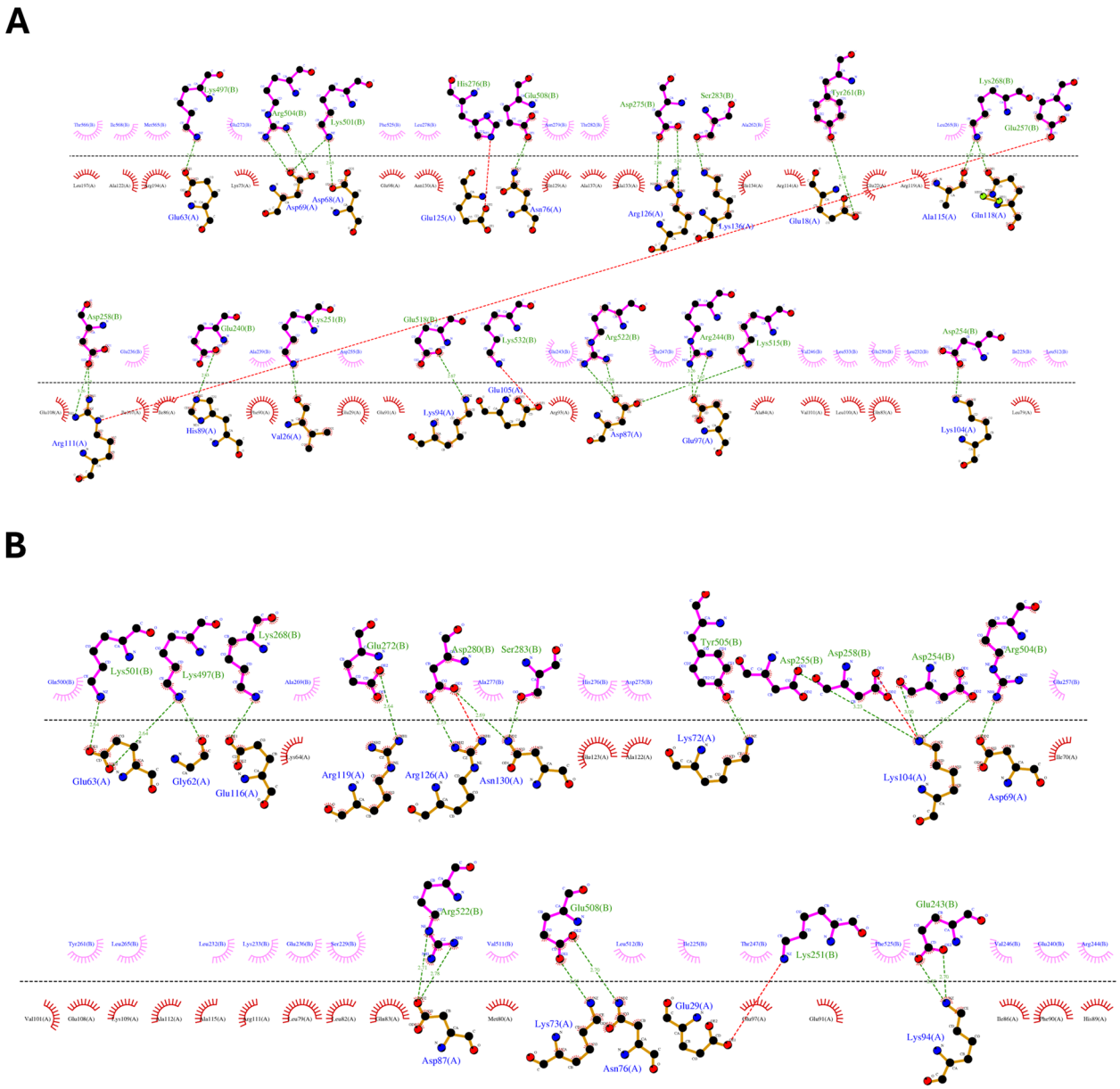

Fig. 3 Interactions across the protein–protein interface. Residue-residue non-covalent interaction between the (**A**) wild-type and (**B**) mutant forms of TNNT3 (chain A) and TPM (chain B) are shown. The TPM dimer includes two TPMs with residues spanning from 48 to 284. We named the second chain the same as the first, designating it as chain B, with residues from 332 to 568. The horizontal dashed line represents the interface. Residues which are in hydrophobic contacts and hydrogen bonds are depicted in red spoked arcs and green dotted lines, respectively. Nitrogen, oxygen, carbon, and other atoms are colored in blue, red, black, and green, respectively. The hydrogen bond between Glu125 and His275 is highlighted

TNNT3, also known as Troponin T isoform 3, is an integral component of the Troponin complex. Human fast skeletal troponin T (*TNNT3*) is a protein-coding gene located on chromosome 11p15.5. This gene consists of 19 exons and 18 introns, among these exons, 11 (exons 1–3, 9–15, and 18) undergo constitutive splicing [25, 41]. The rare variant we discovered is in exon 12, which is part of the conserved middle region of *TNNT3* (Fig. 4).

Proteomics studies

TNNT3, a tissue-specific isoform of troponin T, is predominantly expressed in adult human fast skeletal muscle. However, research has revealed a developmentally regulated switch between fetal/neonatal and adult

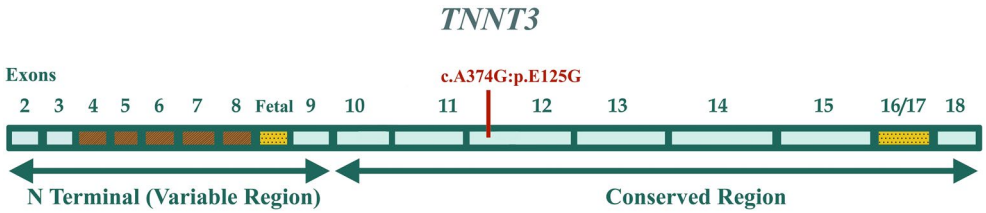

Fig. 4 Schematic view of the *TNNT3* exons. The identified rare variant is located in exon 12 which is in the conserved region. The brown boxes indicate alternatively spliced exons. The developmentally regulated exons are in yellow

troponin T type 3, a phenomenon that has been well-documented across numerous studies and databases [42]. Interestingly, despite its primary association with adult fast skeletal muscle, TNNT3 exhibits unexpected expression patterns during fetal cardiac development. Immunohistochemistry studies have confirmed that fast skeletal muscle troponin TNNT3 is specifically expressed in EC6 cells of the fetal heart, challenging the conventional understanding of TNNT3's tissue specificity [43]. Further supporting this discovery, several databases indicate TNNT3 protein expression in the heart: 1. the PHAROS database reports a fetal cardiac protein expression value of 1.921251 for TNNT3 [44]. 2. Jensenlab assigns a heart protein expression value of 2.954 to TNNT3 [45, 46]. 3. ProteomicsDB indicates a cardiac expression intensity of 3.56 for the TNNT3 protein [47, 48]. These findings collectively suggest that TNNT3's role may extend beyond its well-established function in adult fast skeletal muscle, particularly during fetal cardiac tissue development.

Transcriptomics studies

In addition to proteomics studies, the investigation of mRNA expression using RNAseq, Microarray, and SAGE techniques in normal human tissues reveals significant cardiac expression of the *TNNT3* gene [49]. A recent study analyzed expression data from GTEx V8 and found that the *TNNT3* gene is significantly up-regulated in both cardiac and skeletal muscle tissues [50]. In addition, *TNNT3* was identified as being highly expressed in left ventricular (LV) tissue through prioritization methods that included expression quantitative trait loci (eQTL) and transcriptome-wide association studies (TWAS) [51]. In a study of the developing human heart, comparing ventricular myocardium at 9 and 16 week gestational age (WGA), spanning late first to early second trimester, downregulated differentially expressed gene network analysis identified *TNNT3* as a central hub gene. Gene Ontology analysis suggested significant alterations in actin-mediated cell contraction processes ($q < 0.0001$), highlighting key genetic changes in cardiac tissue during this critical developmental period [52].

Moreover, transcriptional analysis of fetal cardiac populations at the single-cell level provides additional evidence supporting the notion that troponins, including *TNNT3*, are not exclusive to muscle cells but are also expressed in non-muscle cell types such as endothelial cells [43]. Single-cell expression data (https://www.proteinatlas.org/ENSG00000130595-TNNT3) has shown high expression of *TNNT3* in cardiac endothelial cells. Multiple studies have demonstrated that endothelial dysfunction is associated with the development of dilated cardiomyopathy (DCM) [53]. In addition, endothelial–mesenchymal transition has been identified as a potential mechanism in the pathogenesis of myocardial fibrosis and remodeling in DCM progression, suggesting a promising therapeutic target for DCM treatment [54].

An analysis of an in-house RNA-seq data set comprising normal left ventricle samples provides substantial evidence supporting the involvement of *TNNT3* in cardiomyopathy. Furthermore, this rare variant was not found in an in-house database comprising 20,000 Iranian individuals.

Animal model studies

Wang et al. (2016) identified *TNNT3* as one of the sarcomeric genes downregulated in atrial septal defect (ASD) tissues in 6–12-month-old infants compared to normal controls from fetuses at 30–33 week gestational age. Their animal study demonstrated that *Tnnt3* showed dynamic expression across mouse embryonic stages, with a notable peak at E13.5. Gene network analysis indicated that *Tnnt3* was among the genes with the most interactions with other differentially expressed genes. Gene Ontology analysis suggested *Tnnt3*'s involvement in biological processes related to cardiac muscle contraction or morphogenesis [55].

The Tnnt3 protein has been identified as an important factor in cardiac development and maturation. Gomes et al. (2016) demonstrated through immunoblotting that Tnnt3 is expressed in neonatal mouse hearts, along with the Tnnt1 (slow skeletal) and Tnnt2 (cardiac) isoforms. Importantly, the study revealed that Tnnt3 levels decrease throughout postnatal development in mice, suggesting its potential as a marker for cardiac maturation. The researchers noted that Tnnt3 is found at the highest levels in immature hearts,

indicating the transition between fetal/neonatal and adult cardiac phenotypes. This finding underscores the role of Tnnt3 as a potential biomarker for cardiac developmental stages. In addition, the study highlighted that expression patterns of Tnnt3 can vary between species, with adult zebrafish hearts predominantly expressing tnnt3b, the ortholog of mammalian *TNNT3* [42].

Previous studies have shown that during normal cardiac development in mice, the transcription factor Prox1 plays a role in repressing the expression of genes related to fast-twitch contractile proteins, such as Tnnt3, Tnni2, and Myl1. The loss of Prox1 is sufficient to induce a shift from slow to fast-twitch fiber type and contractility in mouse skeletal muscle. Furthermore, cardiac-specific Prox1 knockout mice, which survive to adulthood, exhibit a significant increase in the expression of fast-twitch skeletal genes within cardiac tissue, leading to severe DCM [56]. Based on these findings, overexpression of *TNNT3* may be associated with the development of DCM.

Studies have also described the conditional ablation of Hira in the cardiogenic mesoderm of mice, resulting in surface edema, ventricular and atrial septal defects, and embryonic lethality. Dysregulation of a subset of cardiac genes, notably the upregulation of troponins *Tnni2* and *Tnnt3*, which are crucial for cardiac contractility, was identified. HIRA binds to GAGA-rich DNA loci in the embryonic heart, particularly to an enhancer of *Tnni2* and *Tnnt3* (TTe), which is also bound by the transcription factor NKX2.5, an established cardiomyopathy gene [57, 58]. This expanded understanding of *TNNT3* expression patterns opens new avenues for research into its potential functions in heart development and disease.

Computational modeling

According to the gene ontology (GO) annotations, the *TNNT3* gene product is involved in actin binding and tropomyosin binding [49]. Based on reactome functional interactions (FIs) prediction, TNNT3 has a protein–protein interaction with TPM1, with a functional interaction score (FI score) of 0.78 out of 0.98 [59]. Computational modeling and docking results in this study provided deeper insights into the structural and functional implications of the Glu125Gly rare variant in *TNNT3*. The significantly less negative HADDOCK score for the mutant complex compared to the wild-type underscores the destabilizing effect of this rare variant on the interaction between TNNT3 and tropomyosin 1, essential for muscle contraction regulation. The increased RMSD in the mutant form suggests considerable conformational changes, potentially leading to impaired function.

Associated diseases and pathways

Diseases associated with *TNNT3* include Distal Arthrogryposis, Type 2B2, and Type 1A [60]. Although rare, there have been reports of a link between Arthrogryposis and the development of DCM [61, 62]. Several studies have documented the coexistence of cardiomyopathy in individuals with skeletal dysfunction [63–65]. This gene is associated with various pathways, including the striated muscle contraction pathway and cardiac conduction [49]. A homozygous splice site variant in *TNNT3* was identified in a patient with Nemaline myopathy and Distal Arthrogryposis [66]. Machine learning analysis has identified *TNNT3* as a key gene linked to sudden death (SD). Its expression in both cardiac and skeletal muscle suggests a critical role in SD pathophysiology. Variants in *TNNT3* may cause myocardial changes leading to SD, making it a promising biomarker for SD risk and emphasizing its significance in cardiac function [67].

In a study conducted by Sasse-Klassen et al. (2004), an extended family affected by autosomal dominant LVNC was investigated. The study implicated locus 11p15 as the likely cause of the disease within the family, though no specific candidate gene was identified [68]. According to OMIM (https://www.omim.org/), this region has been designated as LVNC2, associated with LVNC (MIM#609470). Interestingly, our research findings indicate that the *TNNT3* gene, situated precisely within this locus, shows promising potential as a candidate for DCM.

Conclusion

Collectively, the findings discussed highlight the importance of TNNT3 in cardiac development. Our investigation has detected a rare *TNNT3* variant in a patient with DCM, suggesting that variants in this gene may lead to myocardial changes that result in DCM. This study not only identifies a specific genetic variant but also expands our understanding of the genetic basis of DCM, adding to the growing pool of potential variants linked to this disorder.

Abbreviations

DCM	Dilated cardiomyopathy
TNNT3	Troponin T3
TPM	Tropomyosin
WES	Whole exome sequencing
HCM	Hypertrophic cardiomyopathy
LVNC	Left ventricular non-compaction
RCM	Restrictive cardiomyopathy
GATK	Genome analysis toolkit
LVEF	Left ventricular ejection fraction
ICD	Implantable cardioverter defibrillator
ACMG	American College of Medical Genetics and Genomics
cTnT	Cardiac troponin T

Supplementary Information

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Additional file 1.

Additional file 2.

Additional file 3.

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Author contributions
M.J. and V.B. performed all wet lab experiments and data analysis, protein modeling, literature review, and writing of the manuscript; K.I. performed the conception and design of the research, writing of the manuscript; N. S. performed protein modeling, writing of the manuscript; R. M. performed patient identification, patient follow-ups, clinical and paraclinical examinations and studies.

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Availability of data and materials
The datasets generated and/or analyzed during the current study are available in the Sequence Read Archives (SRA) repository with the BioProject accession number PRJNA1128708.

Declarations

Ethics approval and consent to participate
The study received approval from the Iran National Science Foundation (Grant number: 98007928) and the Research Ethics Committee of Tehran University of Medical Sciences. This experiment was conducted in accordance with the Declaration of Helsinki. All participants provided their written informed consent to participate in the study.

Consent for publication
Written informed consent for the publication of clinical details was obtained from all study participants.

Competing interests
The authors declare no competing interests.

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