Case Report

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Diverse Phenotypic Expression of Cardiomyopathies in a Family with TNNI3 p.Arg145Trp Mutation

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Genetic diagnosis of cardiomyopathies (CMPs) is challenging, due to the marked genetic and allelic heterogeneity and the lack of knowledge of the mutations that lead to clinical phenotypes. Here, we present the case of a large family, in which a single troponin I type 3 (TNNI3) mutation caused variable phenotypic expression, ranging from restrictive cardiomyopathy (RCMP) to hypertrophic cardiomyopathy (HCMP) to near-normal phenotype. The proband was a 57-year-old female with HCMP. Examining the family history revealed that her elder sister had expired due to severe RCMP. Using a next-generation sequencing-based gene panel to analyze the proband, we identified a known TNNI3 gene mutation, c.433C>T, which is predicted to cause an amino acid substitution (p.Arg145Trp) in the highly conserved inhibitory region of the cardiac troponin I protein. Sanger sequencing confirmed that six relatives with RCMP or near-normal phenotypes also carried this mutation. To our knowledge, this is the first genetically confirmed family with diverse phenotypic expression of CMPs in Korea. Our findings demonstrate familial implications, where a single mutation in a sarcomere protein can cause diverse phenotypic expression of cardiomyopathies. **(Korean Circ J 2017;47(2):270-277)**

KEY WORDS: Cardiomyopathies; TNNI3 mutation; Cardiomyopathy, hypertrophic; Cardiomyopathy, restrictive; Korean.

Introduction

Cardiomyopathies (CMPs) are a heterogeneous group of heart muscle diseases associated with mechanical and/or electrical dysfunction, including hypertrophic cardiomyopathy (HCMP), dilated cardiomyopathy (DCMP), restrictive cardiomyopathy (RCMP), left ventricular noncompaction (LVNC) and arrhythmogenic right ventricular dysplasia.[1] An increasing number of CMPs are now recognized to have familial forms, which result from single-gene mutations with a Mendelian inheritance pattern.[2] However, the ability to identify disease-causing mutations is quite limited, because of the marked genetic and allelic heterogeneity and the lack of complete knowledge of the mutations that lead to CMPs. To date, more than 100 genes and more than 1400 genetic variations in sarcomere proteins have been identified as causative mutations of CMPs[1)3)] Another obstacle for the genetic diagnosis of CMPs is the realization that although they are clinically distinct entities, there is a genetic overlap among CMPS. For example, although mutations in cardiac troponin are most commonly associated with HCMP and RCMP, they have also been reported in DCMP[4] and LVNC[5] Furthermore, a single mutation could cause not only a specific CMP, but also several different CMPs.[6]

Troponin (Tn) is a critical regulator of muscle contraction in cardiac muscles, and is composed of three subunits: cardiac troponin I (cTnI), cardiac troponin C, and cardiac troponin T.[4] cTnI can inhibit the actomyosin ATPase activity independently of the other Tn subunits. cTnI is encoded by the troponin I type 3 gene

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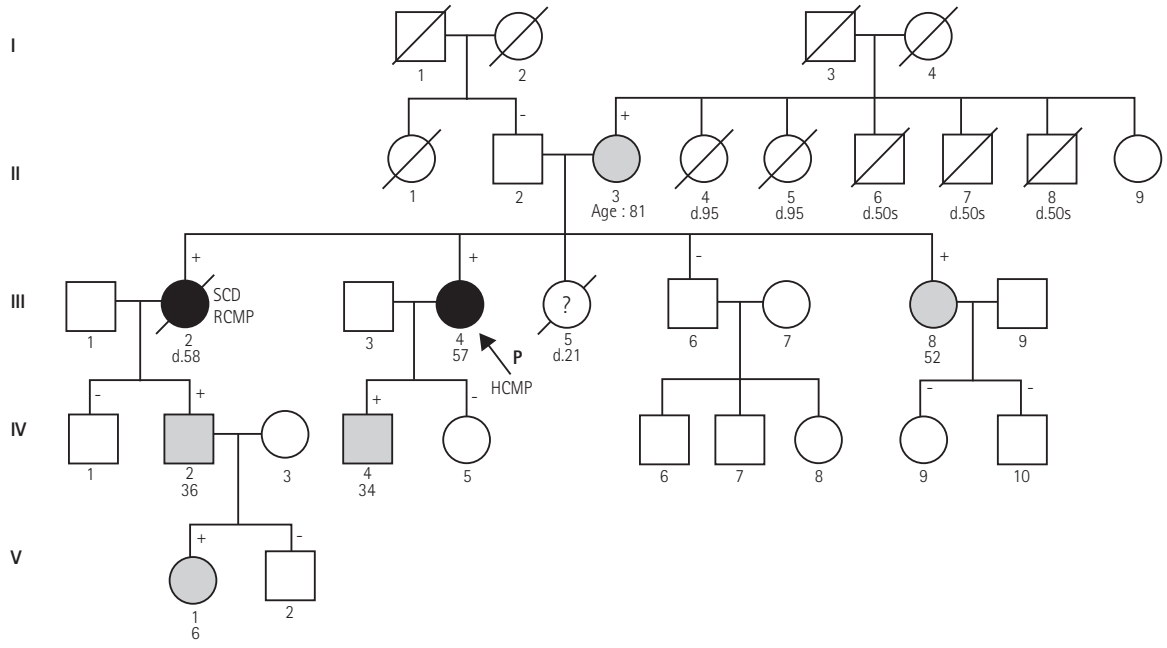

Fig. 1. Family affected by the c.433C>T (p.Arg145Trp) TNNI3 mutation. A black or gray symbol indicates clinically affected family members (gray: no symptoms or only mild heart problems). A diagonal line represents deceased family members, a question mark shows family members with unknown phenotypes, and the arrow indicates the index patient ("P"). A plus sign indicates the presence of a mutation, whereas minus sign indicates the absence of mutation. The age at death ("d") is shown in years. Decade of the deceased age is shown as "s". SCD: sudden cardiac death, RCMP: restrictive cardiomyopathy, HCMP: hypertrophic cardiomyopathy.

(TNNI3), which is a 5966-bp gene located on chromosome 19 consisting of eight exons and encoding a 210-amino acid protein.[7][8] Allelic variants of TNNI3 have been implicated in HCMP, RCMP, and DCMP.[4]

Recently, targeted gene panels based on a next-generation sequencing (NGS) platform has been used to analyze the exons and flanking intronic regions of genes associated with diseases of interest. Compared with whole-exome and whole-genome sequencing, targeted gene panels with a limited number of genes provide a higher depth of coverage with increasing sensitivity and specificity, and a higher capacity to interpret the findings in a clinical context.[3] Here, we report a large family, in which the p.Arg145Trp mutation of TNNI3 causes diverse phenotypes ranging from RCMP to HCMP to near-normal heart, as demonstrated with the NGS-based gene panel testing.

Case

Clinical evaluation

Clinical investigation of the proband and 13 family members was performed (Fig. 1, Table 1). A diagnosis of cardiomyopathy was made on the basis of clinical symptoms, physical as well as cardiologic examination including 12-lead electrocardiography (ECG), chest radiography, echocardiography, and whenever possible, chest contrast computed tomography (CT), cardiac magnetic resonance imaging (cardiac MRI), and laboratory studies including N-terminal of the prohormone brain natriuretic peptide (NT-proBNP). This study received the institutional review board approval (File No. 2015-07-029), and informed consent was obtained from all subjects.

NGS-based gene panel

A gene panel was designed to include 31 HCMP genes: ACTC1, ACTN2, ANKRD1, BAG3, CAV3, CRYAB, CSRP3, GLA, JPH2, LAMP2, LDB3, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOZ2, NEXN, OBSCN, PLN, PRKAG2, RYR2, TCAP, TNNC1, TNNC2, TNNI3, TNNT2, TPM1, TTR, and VCL. After obtaining informed consent, genomic DNA was extracted and captured with the Nextera Rapid Capture Custom Enrichment Kit (Illumina Inc., San Diego, CA, USA) and sequenced on a MiSeq platform. After screening all HCMP-related genes, we identified a missense mutation (c.433C>T, p.Arg145Trp) in the TNNI3 gene.

Case presentation

The proband (III:4) was a 57-year-old woman. At the age of 43 years, she was diagnosed for the first time with asymptomatic cardiomegaly

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Table1. Clinical data of affected individuals in a family with the c.433C>T mutation

		Age/ sex	Symptoms	LVEDD (mm)	LVESD (mm)	MWT (mm)	IVSd (mm)	LVPWd (mm)	LA (mm)	E (m/s)	A (m/s)	DT (msec)	e′ (m/s)	a′ (m/s)	E/e′	LAVI (mL/m^2)	RVSP (mmHg)	NT-proBNP (pg/mL)	ECG
III:2	SCD RCMP	58/F	DOE (NYHA II)	56	39	10	8	8	60 RA 94	0.59	NA	126	0.06	NA	10.5	122	51	1441	AF RVH
III:4	HCMP Alive	57/F	Palpitation discomfort, mild dyspnea	48	32	20	14	9	46	0.74	0.33	113	0.05	0.08	14.0	38	49	1100	NSR→AF Incomplete RBBB
II:3	Alive	81/F	None	49	29	9	8	8	50	0.59	0.68	250	0.06	0.12	10.7	42	25	388	NSR Non-specific ST-T change
III:8	Alive	52/F	None	43	26	9	7	7	39	0.73	1.01	196	0.01	0.12	7.5	29	NA	47	NSR Non-specific ST-T change
IV:2	Alive	36/M	Palpitation	49	28	10	9	9	34	0.88	0.69	230	0.12	0.10	7.2	20	NA	5	NSR Non-specific ST-T change
IV:4	Alive	34/M	None	47	25	9	8	8	35	0.87	0.51	210	0.12	0.11	7.4	33	21	28	NSR Non-specific ST-T change
V:1	Alive	6/F	None																

LVEDD: left ventricular end-diastolic dimension, LVESD: left ventricular end-systolic dimension, MWT: maximal left ventricle wall thickness, IVSd: interventricular septal thickness in diastolic phase, LVPWd: left ventricular posterior wall thickness in diastolic phase, LA: left atrium, E: early diastolic filling, A: atrial filling, DT: deceleration time, e′ : early diastolic mitral annulus motion (velocity), a′: late diastolic mitral annulus motion (velocity), LAVI: left atrium volume index, RVSP: right ventricular systolic pressure (by tricuspid regurgitation maximum velocity), NT-proBNP: N-terminal prohormone of brain natriuretic peptide, ECG: electrocardiogram, SCD: sudden cardiac death, RCMP: restrictive cardiomyopathy, F: female, M: male, DOE: dyspnea on exertion, RA: right atrium, NA: left atrium, AF: atrial fibrillation, RVH: right ventricular hypertrophy, HCMP: hypertrophic cardiomyopathy, NSR: normal sinus rhythm, AF: atrial fibrillation, RBBB: right bundle branch

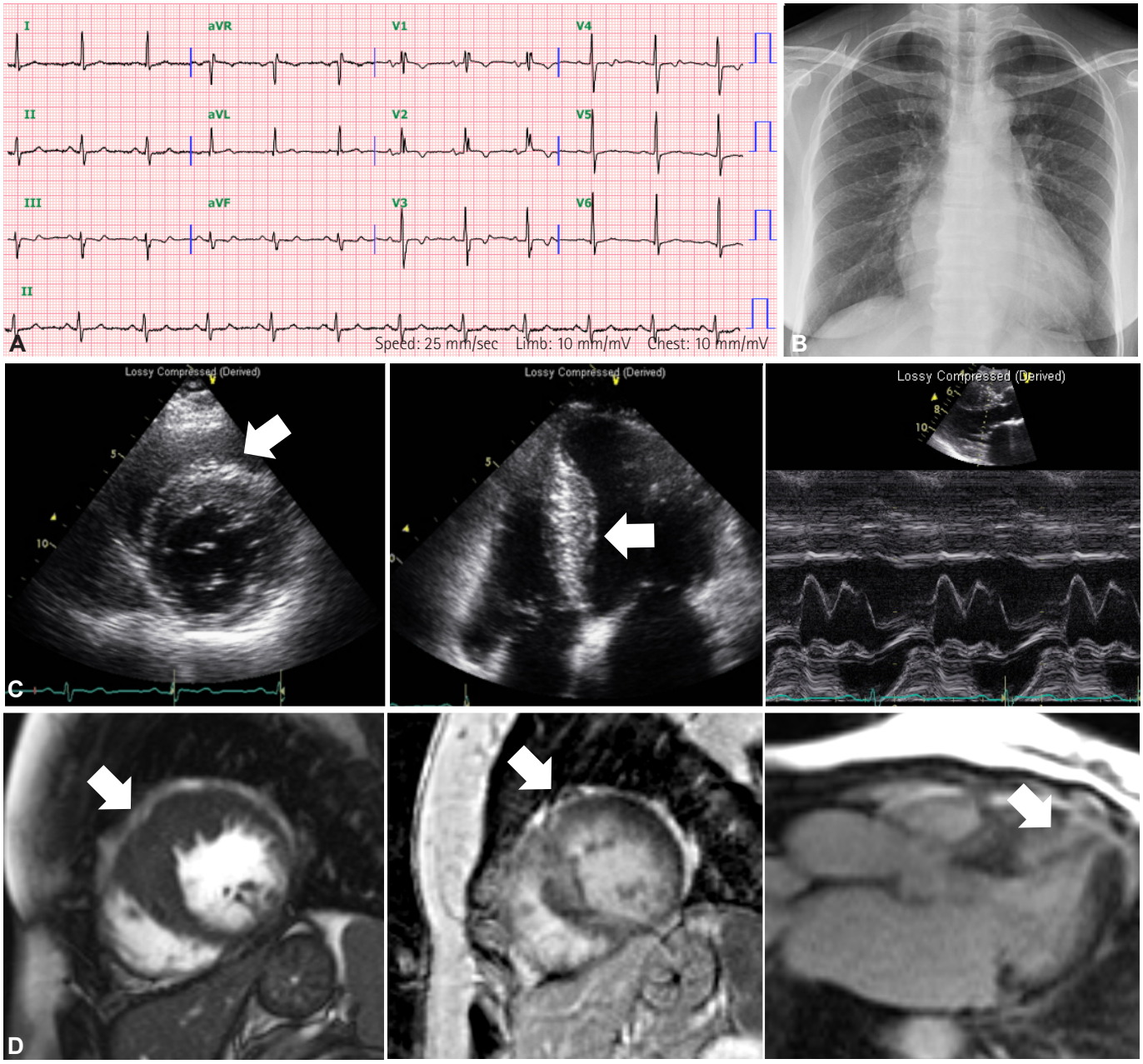

Fig. 2. Imaging findings of proband (III:4) as a 57-year-old woman. (A) Incomplete RBBB, non-specific ST-T change and regular sinus rhythm were seen on initial ECG. (B) Slight cardiomegaly (cardiothoracic ratio was 0.62) without pulmonary edema was detected on chest radiographs. (C) In the echocardiographic examination, the upper two panels showed increased thickness of the interventricular septum to a maximum of 20 mm (white arrows), consistent with HCMP. However, there was no systolic anterior motion of the mitral valve (lower panel). (D) Cine short axis view using a balanced steady-state free precession sequence revealed thickening of the apical septal and anterior wall of the left ventricle (white arrow in the first panel). Delayed enhancement images using the Turbo-FLASH sequence showed hyper-enhancement in the same area (white arrow in the second and third panel). RBBB: bundle branch block, ST-T: ST-T segment, ECG: electrocardiogram, HCMP: hypertrophic cardiomyopathy.

(cardiothoracic ratio was 0.62) on chest radiograph (Fig. 2B), and later diagnosed with HCMP at another hospital. At the age of 51 years, she visited our hospital. Initially, ECG showed a normal sinus rhythm with incomplete right bundle branch block, left atrial enlargement and anterolateral T wave abnormalities (Fig. 2A). Echocardiographic examination revealed asymmetrical septal hypertrophy without left ventricle (LV) outflow tract obstruction, accompanied by left atrial enlargement, restrictive filling pattern, normal LV systolic function and normal cavity size (Fig. 2C). Cardiac MRI also showed delayed hyper-enhancement, which reflects fibrotic change in the

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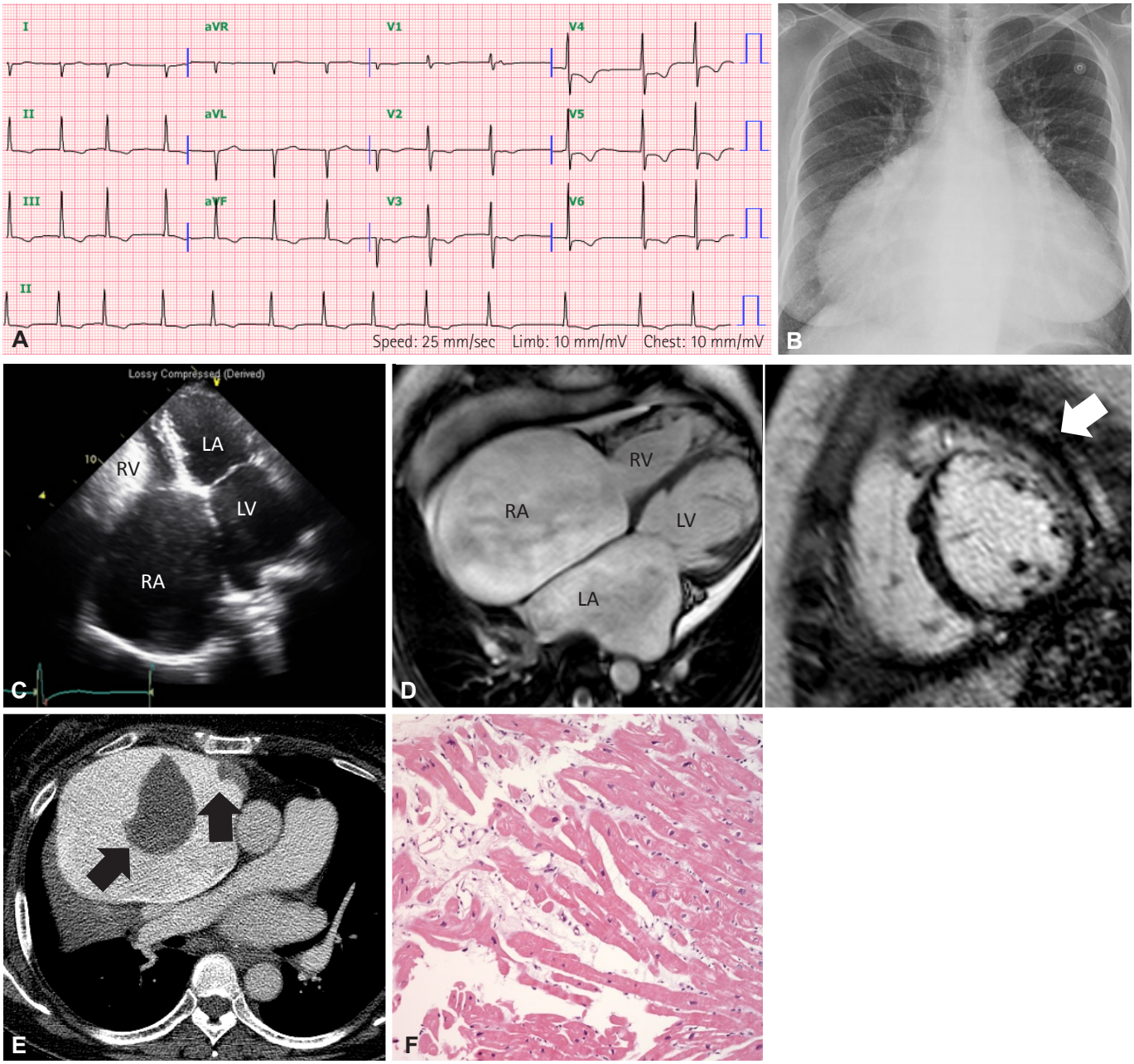

Fig. 3. Imaging findings of patient's older sister (III:2). (A) ECG showed atrial fibrillation and right ventricular hypertrophy. (B) Marked cardiomegaly was seen (cardiothoracic ratio was 0.83) in chest radiograph. (C) In echocardiographic examination, huge enlargement of both atria with preserved left ventricular systolic function was observed, consistent with RCMP. (D) Four-chamber view using balanced steady-state free precession sequence revealed marked dilatation of LA and RA without ventricular dilatation (first panel). Delayed enhancement images using Turbo-FLASH sequence showed hyper-enhancement at anterior and antero-lateral wall of mid-ventricular level (white arrow). (E) Contrast-enhanced chest computed tomography demonstrated two low-attenuation and irregular-shaped masses (black arrows) in the huge right atrium, which were suspicious of thrombi. (F) Endomyocardial biopsy of the right ventricle. Myocytes showed mild focal disarray and increased nucleus size. The interstitium was widened and edematous (hematoxylin and eosin, 200x). ECG: electrocardiography, RCMP: restrictive cardiomyopathy, LA: left atrium, RA: right atrium, LV: left ventricle, RV: right ventricle.

myocardium in the hypertrophied segments (Fig. 2D). An arrhythmic event (atrial tachycardia), followed by an event of paroxysmal atrial fibrillation, was found on intermittent Holter monitoring. The patient's clinical features were compatible with those of HCMP.

Family history taking revealed that the patient's older sister (III:2) also had cardiomyopathy. The sister had symptoms of heart failure

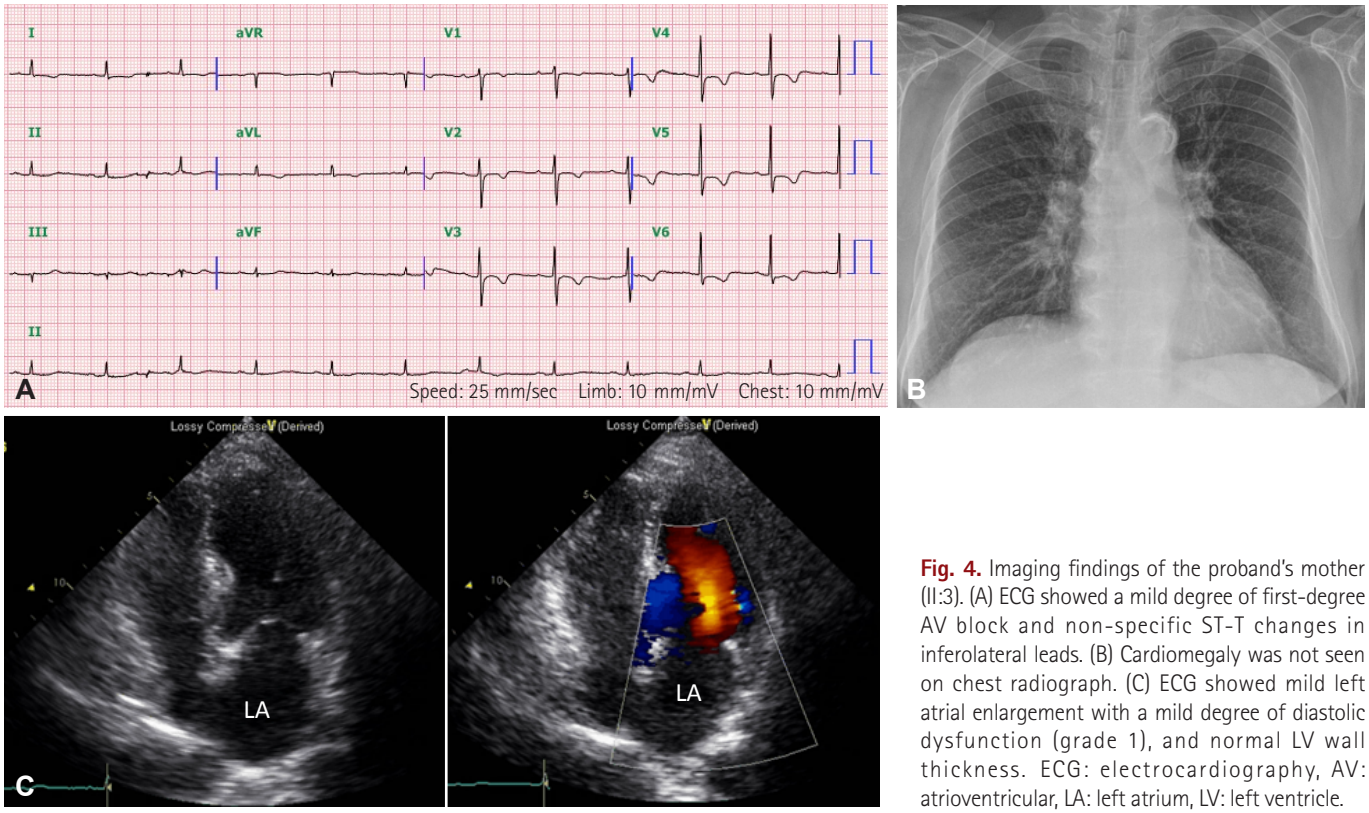

Fig. 4. Imaging findings of the proband's mother (II:3). (A) ECG showed a mild degree of first-degree AV block and non-specific ST-T changes in inferolateral leads. (B) Cardiomegaly was not seen on chest radiograph. (C) ECG showed mild left atrial enlargement with a mild degree of diastolic dysfunction (grade 1), and normal LV wall thickness. ECG: electrocardiography, AV: atrioventricular, LA: left atrium, LV: left ventricle.

from the age of 51. At the age of 54 years, she visited our hospital. ECG showed atrial fibrillation and ST-T abnormalities in inferolateral leads without low voltage in the limb leads (Fig. 3A). Severe cardiomegaly was noted in the chest radiograph (cardiothoracic ratio was 0.83) (Fig. 3B). Echocardiographic examination showed severe bi-atrial enlargement and a restrictive filling pattern accompanied by normal LV systolic function and wall thickness (Fig. 3C). In addition, cardiac MRI revealed mid and basal wall myocardial fibrosis from the basal to mid anterior, anterolateral and anteroseptal segments of the LV (Fig. 3D). Endomyocardial biopsy from the right ventricle showed nonspecific microscopic findings, such as mild focal disarray of myocytes with karyomegaly and interstitial widening (Fig. 3F). She succumbed to sudden death at the age of 58 while awaiting cardiac transplantation. Her last chest contrast CT, performed a few days before her death, revealed interval-increased extent of markedly dilated both atria, with two large thrombi in the right atrium (Fig. 3E). Her clinical features were typical of RCMP.

Family history revealed that a younger sister (III:5) of the proband had suddenly expired at the age of 21. Family genetic analysis further revealed five other affected members (II:3, III:8, IV:2, IV:4, V:1), and cardiac evaluation was performed for all family members, except one (V:1). Notably, the proband's mother (II:3) (81 years old) did show subtle cardiac abnormalities. Her ECG showed a mild degree of first degree AV block and non-specific ST-T changes in the inferolateral leads (Fig. 4A). Echocardiography showed a mild degree of left atrial enlargement with mild diastolic dysfunction (Fig. 4C). The other members (III:8, IV:2, IV:4) showed non-specific ST-T changes in ECG. Echocardiography revealed no significant abnormal findings except mild diastolic dysfunction in III:8.

Sanger sequencing of the TNNI3 gene mutation

The TNNI3 mutation, p.Arg145Trp, was validated by Sanger sequencing in the proband (III:4) and her available family members (Fig. 5). To investigate the genetic diagnosis of the affected individual who had already died (III:2), genomic DNA was isolated from frozen heart tissue from an earlier biopsy. Exon 6 of TNNI3 was amplified using primer sets designed by the authors: TNNI3-e6-F, 5′-ggggattcagttccaggatt-3′,TNNI3-e6-R,5′-gcatttctgaggacccctta-3′, TNNI3-e6-tissue-F, 5′-aggatggaggagttgggtgt-3′, TNNI3-e6-tissue-R, 5′-aggtccagggactccttagc-3′. The PCR products were sequenced on the ABI Prism 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). DNA sequences were analyzed

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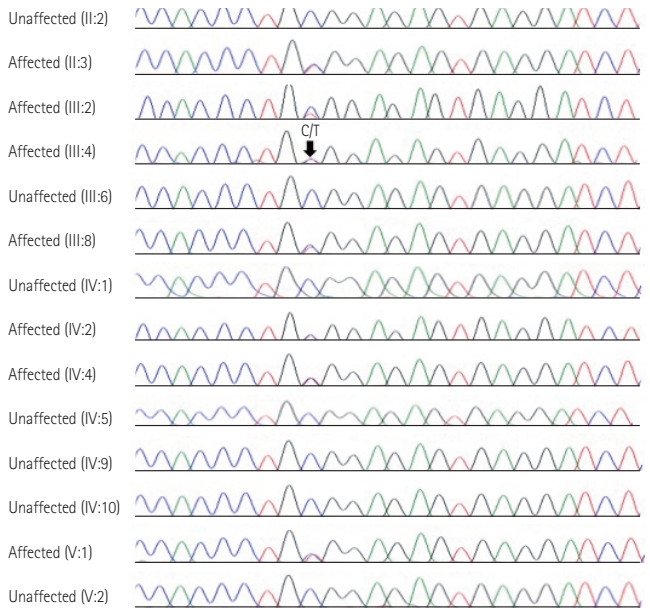

Fig. 5. Sequence analysis of the TNNI3 gene. Chromatograms show the heterozygous nonsynonymous mutation (c.433C>T, p.Arg145Trp) of the TNNI3 gene in the proband (III:4), individuals II:3, III:2, III:8, IV:2, IV:4, and V:1, and the normal sequence in individuals II:2, III:6, IV:1, IV:5, IV:9, IV:10, and V:2 (arrow).

by comparison with reference sequence of TNNI3, NM_000363.4.

Results showed that the proband (III:4) was heterozygous for the missense mutation (c.433C>T; p.Arg145Trp) in the TNNI3 gene. The family study showed that her mother (II:3) carried the mutation, indicating maternal transmission. Her two sisters (III:2 and III:8), her second nephew (IV:2) and his daughter (V:2), and the proband's son (IV:4) also had the same TNNI3 mutation. According to a literature review and mutation database, the TNNI3 c.433C>T (p.Arg145Trp) mutation has been previously described in patients with RCMP,[6] and its pathogenicity has been validated in vivo and in vitro by independent functional assays.[9-11]

Discussion

In this report, we present a family carrying a TNNI3 mutation producing variable phenotypic expression. Although the TNNI3 c.433C>T (p.Arg145Trp) mutation has been reported in patients with isolated RCMP,[4][6] no variable expressivity in a single family has been noted in such cases to date. It was previously thought that HCMP, DCMP, and RCMP were discrete and separate CMPs, but the finding of a single mutation in a sarcomere protein that is associated with phenotypic variability in CMPs, indicates that CMP comprises a spectrum of hereditary cardiac contractile protein diseases.[4][6]

The cTnI is the inhibitory subunit of Tn, the thin filament regulatory complex that confers calcium-sensitivity to striated muscle actomyosin ATPase activity. Wild-type human cTnI inhibited ATPase activity in a Ca^{2+} concentration-dependent manner.[9] It has been reported that allelic variants of TNNI3 mutation provoke HCMP (74%), RCMP (23%), and rarely DCMP (3%).[4] The p.Arg145Trp mutation identified in our study occurs within the highly conserved inhibitory region, which is critical to the biological activity of TnI.[12][13] Using actomyosin ATPase assays, Gomes et al.[9] demonstrated that p.Arg145Trp has a reduced ability to inhibit ATPase activity in the absence of Ca^{2+} and, furthermore, that the mutant was unable to fully relax contraction in porcine-skinned fibers in the absence of Ca^{2+}. p.Arg145Trp mutation also led to an increase in the Ca^{2+} sensitivity of force development compared with wild type cTnI.[9]

The clinical features of this family are interesting for several reasons. First, it is well-known that the p.Arg145Trp mutation in the TNNI3 gene could induce either HCMP or RCMP in a single family. The mixed appearance of HCMP and RCMP had previously only been reported in a case with the p.Asp190His mutation (not the p.Arg145Trp mutation) in the TNNI3 gene.[6] The second important observation is that the majority of the individuals carrying the p.Arg145Trp mutation showed a near-normal phenotype of the heart at the time of presentation. In particular, the proband's mother (II:3) was healthy without any signs of CMP, except for subtle changes in her ECG and echocardiogram, even at the advanced age of 81. Therefore, the prognosis of RCMP with p.Arg145Trp mutation could be more favorable than that of other TNNI3 mutations. RCMP is a rare and distinct form of CMP that is characterized by diastolic dysfunction but intact systolic function, until the later stages of the disease.[1] Among the different CMPs, RCMP has the worst prognosis.[14][15] According to published results,[6] RCMP patients with the p.Arg145Trp mutation showed a later onset of the disease in comparison with those having other TNNI3 mutations, including p.Arg192His, p.Lys178Glu, p.Ala171Thr, and p.Leu144Gln (median age, 69 years vs. 25 years). In our cases, one affected individual (III:2) died at 58 years, whereas other individuals who harbored the TNNI3 p.Arg154Trp mutation were all asymptomatic at the time of presentation, suggesting phenotypic diversity exists in prognosis as well as subtypes of CMPs.

It is still unclear why the same mutation causes different CMP phenotypes. It has been suggested that different mutations produce different sensitivities to Ca^{2+}; high sensitivity leads to HCMP or RCMP, and low sensitivity to DCMP.[4] However, the lack of a molecular understanding of the TNNI3 mutations and their functional consequences has limited our ability to address how phenotypic diversity arises from the same gene mutation. It is

possible that even in families with Mendelian inheritance of CMP, more complex genetic or environmental interactions are involved in determining the clinical phenotype.[4] In fact, it has been reported that 9.5% of Chinese HCMP patients harbor multiple mutations of sarcomere proteins, and the number of mutations was positively correlated with the maximum wall thickness.[16] Therefore, we could not exclude the possibility of the presence of another gene mutation in patients with severe phenotypes. Also, it is highly speculative that the non-appearance of the clinical expression of CMP in the mother may be the consequence of the opposed influence explicated by another hypothetical gene sequence having a protective effect.

In conclusion, we report a large family with the TNNI3 p.Arg145Trp mutation and a diverse range of phenotypes from RCMP to HCMP to near-normal heart. To our knowledge, this is the first genetically confirmed CMP case with diverse clinical expression in the Korean population. An interesting question for future research will involve determining how mutations in the same gene can cause a range of distinct phenotypes.

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