Different contribution of sarcomere and mitochondria related gene mutations to hypertrophic cardiomyopathy

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ABSTRACT

Different contribution of sarcomere and mitochondrial related gene mutations to hypertrophic cardiomyopathy

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Backgrounds: In hypertrophic cardiomyopathy (HCM), an inherited disease, at most 60% pathogenic nuclear gene mutations are detected. Although main genetic interest has been almost limited to sarcomere gene, mitochondrial DNA (mtDNA) or related nuclear genes also contribute to cardiac energy metabolism and hypertrophy. Here, we aimed to identify the sarcomere gene and mitochondria related genetic characteristics, according to the HCM subtypes.

Materials and method: In consecutively enrolled 149 HCM patients, genetic test and transthoracic echocardiography were performed along with clinical assessment. Comprehensive HCM specific panel including 82 nuclear DNA (nDNA) (32 sarcomere genes, 6 hypertrophy inducing non-sarcomere genes, and 44 mitochondrial genes) and whole genome of mtDNA were analyzed using next generation sequencing. HCM patients were divided into two types; non-apical HCM (n=76) and apical HCM (n=73). Sequence Kernel Association Test analysis was performed to test for association of the effects of rare variants on subtype of HCM. Rare variant was defined as its minor

allele frequency less than 5.9% in current database.

Results: Among 149 patients, known pathogenic sarcomere gene mutations were detected in 35.6% (53/149) with significantly higher prevalence in non-apical HCM (34/76, 44.7%) compared to apical HCM (19/73, 26.0%) ($p=0.026$). Rare variants of 2 mitochondria-related nDNA gene, 3 mtDNA and 1 sarcomere gene were significantly associated with apical HCM but not with non-apical HCM (all $p<0.05$). Obstructive HCM was associated with rare variants of 5 sarcomere genes and 1 mitochondria-related nDNA gene (all $p<0.05$).

Conclusion: Apical HCM was related to rare variants of mitochondria-related nDNA gene or mtDNA gene, not only to classical sarcomere gene variants. Individualized approach based on biochemical background might enable individualized risk stratification and targeted therapy.

Kew words: hypertrophic cardiomyopathy, mitochondria, genetic test

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I. INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a common genetic disease that affects approximately 1 in 500 people. HCM is typically inherited with an autosomal dominant Mendelian pattern, variable expressivity, and age-related penetrance.[1] Current guidelines recommend family genetic screening if a definite pathogenic genetic mutation has been identified in a HCM patient.[2, 3] However, maternal inheritance and sporadic mutation have also been reported.[4-6] More than 1,500 individual mutations have been identified among ≥11 causative genes.[7] Currently, a pathogenic nuclear mutation is found in about 60% of HCM patients who performed genetic test, and the genetic abnormalities causing the disease in remained 40% of HCM patients are currently unknown.[8] It might be caused due to either a mistake in clinical decision or unknown additional pathogenic mutation. HCM is a disease due to abnormal hypertrophied heart, which means there might be functional disturbance in formation of normal myocardial structure. Previous genetic tests had been performed with conventional Sanger sequencing of single amplicons of sarcomere genes, which was a time consuming and expensive method with a low predictive outcome. Next Generation Sequencing (NGS) is emerging

genetic analysis technology, which is time saving, lower priced, use smaller amounts of genomic DNA, and enables to analyze a larger number of genes.[9, 10] This new methodology could increase the detection rate of genetic mutations, and identify the association between novel mutations and targeted disease.

Previous and recent studies have been based on genetic test for mainly Western population, which means that genetic data is relatively insufficient in Asian population. Moreover, it is known that genetic characteristics are different according to ethnicity, and one recent study reported about the misdiagnoses owing to racial difference in HCM.[11] Therefore, identification of genetic characteristics is necessary in Asian patients with HCM.

HCM progress with various clinical presentation such as arrhythmic sudden death, progressive diastolic heart failure and systolic heart failure, and atrial fibrillation.[8] Heart failure is caused by left ventricular (LV) outflow tract (LVOT) obstruction or diastolic dysfunction due to under-filling LV, increased myocardial O_2 demands and myocardial fibrosis in HCM patients. However, the contributors of development and severity of heart failure are not yet established. Although several studies demonstrated that some mutations were associated with adverse clinical outcome,[12-14] and one previous study reported that rare sarcomere variants were related to increase cardiovascular disease analyzed by burden testing,[15] while established evidence of genetic test for predicting the clinical outcome is lacking although conflicting data exist.

Nuclear DNA (nDNA) is located in the nucleus of eukaryotic cells, and contains 46 chromosomes. Sarcomere genes are included in nDNA. Mitochondrial DNA (mtDNA) is located in the mitochondria, and contains only one chromosome. The mitochondria are the center of metabolism, and recent data had suggested that mitochondrial dysfunction may play a key role in the pathogenesis of diabetic cardiomyopathy.[16] Mitochondrion plays a crucial role in vital functions, most importantly in oxidative phosphorylation and energy metabolism. Mitochondrion is involved in essential cellular processes including calcium signaling, apoptosis, and generation of reactive oxygen species. Mitochondrial disease includes various

clinical disorders that occur as a result of dysfunctional cellular oxidative phosphorylation due to a primary genetic defect, and it can result in the development of cardiomyopathy and arrhythmia.[17] If mitochondria disease occurs, the common cardiac presentation is myocardial hypertrophy.[18] One recent study reported the prevalence of mitochondrial DNA (mtDNA) mutation was >10% in HCM patients,[18] and another study revealed that mtDNA variants were detected in 11% of HCM patients.[19] However, there are rare studies about the prevalence of both mitochondrial related nDNA and mtDNA gene variation in HCM patients, and especially in apical HCM which is a common form in East Asia. Unknown variants associated with HCM might be able to be located in any nDNA which includes sarcomere genes, hypertrophy inducing non-sarcomere genes and mitochondrial related genes, and also in mtDNA. If there are novel genetic variants to be pathogenic for HCM, the presence of unknown genetic mutation could explain several questions; 1) the low rate of genotype positive patients although it might be obviously inherited cardiomyopathy, 2) heterogeneous reports about the association between genotype and clinical outcome, and 3) distinct morphologic characteristics such as apical HCM in Asian population. Therefore we aimed to investigate 1) the characteristics of nDNA and mtDNA gene variants with unknown pathogenicity in HCM, 2) whether apical HCM patients have a distinct genetic characteristics compare with non-apical HCM, and 3) geometric characteristics according to the genetic characteristics.

II. MATERIALS AND METHODS

1. Study population

Total 432 patients (238 of non-apical HCM, and 194 (45%) of apical HCM) were enrolled in Gangnam Severance HCM registry from 2006 to 2014. Among them, finally 149 patients (106 males, 58.8± 12.8year) were consecutively enrolled, and underwent genetic testing (Figure 1). HCM was defined as the presence of unexplained left ventricular hypertrophy (LVH) with a maximal wall thickness ≥

13mm.[20] We morphologically classified the patients with HCM into two types; apical HCM and non-apical HCM. Diagnostic criteria of apical HCM were: (1) maximal apical wall thickness ≥13mm and, (2) a ratio of maximal apical to posterior wall thickness ≥1.3.[21] Posterior wall thickness was measured at parasternal long axis view or short axis view in basal-LV level. Exclusion criteria were as follows: (1) systemic or cardiovascular disease capable of generating LV hypertrophy, (2) uncontrolled hypertension. All the patients underwent screening analysis for Fabry disease by confirming negative GLA mutation. Five year sudden cardiac death (SCD) risk was calculated using the new validated prediction model.[3]

The study protocol was approved by our institutional review board and written informed consents was obtained.

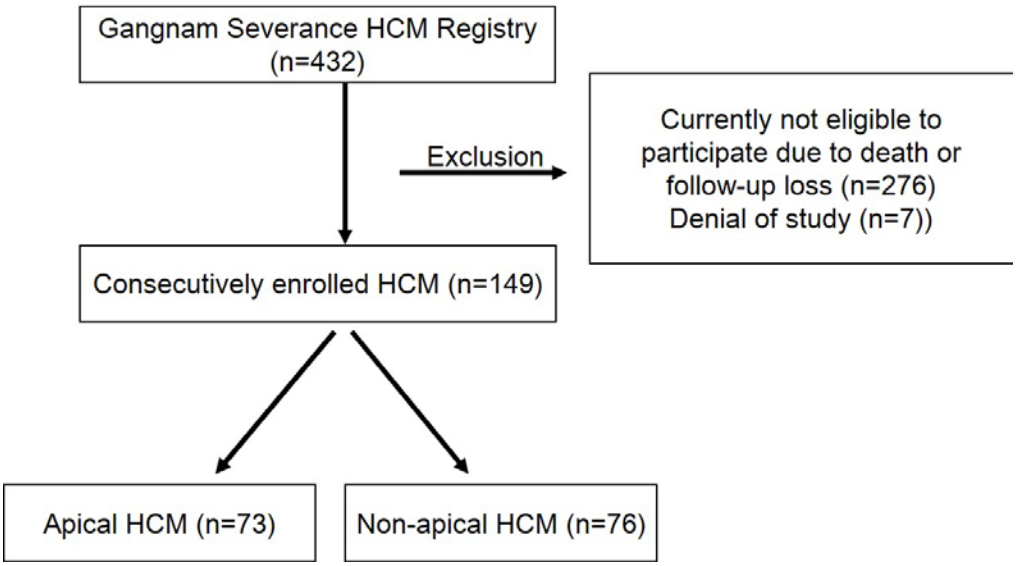

Figure 1. Flow diagram of the study population. HCM, hypertrophic cardiomyopathy.

2. Genetic analysis

A. DNA preparation

Genomic DNA was extracted from EDTA-treated whole blood samples by using a QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany), on a QIAcube automatic nucleic acid extraction instrument (Qiagen), according to the manufacturer's instructions. The DNA samples were used for analysis of mtDNA and HCM gene panel.

B. Library construction and sequencing of mtDNA

The complete mtDNA was amplified by using four overlapping pairs of primers.[22] Library preparations were performed following the manufacturer's instructions (Ion XpressPlus Fragment Library Kit; Thermo Fisher Scientific) for 400 single-end reads. Library material was purified using AMPure beads (Beckman Coulter, Brea, CA, USA). For multiplexing of the samples, each DNA library was barcorded using different ligation adaptors. The fragmented and adaptor ligated libraries were selected following electrophoretic separation with the E-gel SizeSelect gel (Thermo Fisher Scientific) following the manufacturer's recommendations. Subsequent emulsion Polymerase Chain Reaction (PCR) and enrichment of the sequencing beads of the pooled libraries was performed using the OneTouch system (Thermo fisher scientific) according to the manufacturer's protocol. Finally, sequencing was done on the 318 chip using Ion PGM Hi-Q Sequencign Kit (Thermo fisher scientific) on the Ion Torrent Personal Genome Machine (PGM) (Thermo fisher scientific).

C. Data analysis of mitochondrial genome

The quality-filtered sequences were then aligned to the Revised Cambridge Reference Sequence (rCRS), and analyzed.[23] Basic data analysis was performed according to the default parameters of with the Torrent Suite Software version 5.2.1 (Life Technologies) using the plug-in VariantCaller For mtDNA that employed a TMAP Smith–Waterman alignment optimization.[24] The output of the variant caller was presented in tabular format, as a list of variations to the rCRS along with total coverage and variant frequency values. Variant detection was called to detect insertions and deletions (indels) as well as SNPs with reference to the rCRS. The visual inspection of the mapped data was performed using the Integrated Genomics

Viewer 2.3 software (IGV; Broad Institute, Cambridge, MA, USA). The mitochondrial genome databases, including MITOMAP,[25] mtDB,[26], and Phylotree[27] were referred to validate the detected variants.

D. Identification of potential pathogenic mtDNA variants

As previous study by Zaragoza et al.[28], non-haplogroup-associated novel and rare variants were further evaluated for their potential pathogenicity based on the variant's location, amino acid change and evolutionary conservation.[28]

E. HCM gene panel design

For the HCM panel targeted gene selection, a literature search from Pubmed database was performed. We designed a currently most comprehensive HCM specific panel including 82 nDNA genes (Supplementary 1). The 82 nDNA genes were selected which were reported to be related with HCM or increase LVH. Additionally, for the detection of Fabry disease, presence of GLA mutation was evaluated in outside of our lab (Genzyme Korea).

F. Library construction and sequencing of HCM gene panel

For targeted sequencing, DNA fragments were enriched by solution-based hybridization capture and sequenced with an Illumina Hiseq2500 platform (Illumina, San Diego, CA, USA) with the 2 × 150 bp paired-end read module. Genomic DNA was sheared using an Adaptive Focused Acoustics (AFA) ™ with the Covaris Focused-ultrasonicator (Covaris, Inc., Woburn, MA, USA). The quality and quantity of sheared DNA were assessed using the Agilent 2200 Tape Station system with Agilent D1000 ScreenTape (Agilent Technologies, USA) according to the manufacturer's instructions. Capture probes were customized and produced by Celemics Inc. (Korea) to cover CDS regions of 82 target genes. Purification and clean-up of samples were also performed with AMPure beads. NGS library amplification was performed using a KAPA Library Amplification Kit (Kapa Biosystems, Inc., Wilmington, MA, USA) according to the manufacturer's instructions. Library preparation, hybridization, capture procedure, and sequencing

were performed by Celemics according to the protocols recommended by the Celemics User Manual Ver 2.1 (http://www.celemics.com/home/).

G. Data analysis of HCM gene panel

Burrows-Wheeler aligner algorithm with default option was used to align reads to human reference genome sequence GRCh37.[29] SAMTools was used to convert sequence alignment map (SAM) file to BAM format.[30] Sorting and removing duplications were performed using Picard tool (http://broadinstitute.github.io/picard/). GATK was used to perform indel realigning and base quality score re-calibration.[31] Variants were annotated with ANNOVAR[32] and Variant Effect Predictor (http://asia.ensembl.org/info/docs/tools/vep/index.html). Variants were further filtered with altered allele frequency > 30%, > 50x coverage, and population frequency less than 0.01 in the 1000 Genome Project, ESP6500, and ExAC databases. Prediction of mutation's pathogenicity was performed using Alamut® Visual software (Interactive Biosoftware, Rouen, France). For missense mutations, the pathogenicity of the pathogenic variants was predicted with SIFT (http://sift.jcvi.org/) and Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/). The classification of variants was based on American College of Medical Genetics and Genomics (ACMG) standards and guidelines.[33]

3. Conventional echocardiographic analysis

We retrospectively analyzed echocardiographic images diagnosed as HCM. Echocardiography study was performed with a commercially available machine. Comprehensive echo-Doppler evaluation was performed according to current American Society of Echocardiography (ASE) guidelines.[34] A routine standard echocardiography study was performed for measurement of systolic and diastolic parameters: left atrial (LA) diameters, volume, LV end-systolic and diastolic dimensions, mitral inflow velocities and mitral annulus velocities. LA volume was measured at end-systole, and LA volume index (LAVI) was calculated as LA volume/body surface area. Peak early (E) and late (A) diastolic mitral inflow velocities were measured in apical 4-chamber view. Tissue Doppler interrogation

was done in septal mitral annulus in apical 4-chamber view, then peak systolic mitral annulus velocity (s') and early diastolic mitral annulus peak velocity (e') were measured, and the ratio of E/e' was calculated. The presence of diastolic dysfunction was defined if more than half of the parameters meet the four recommended variables; 1) mitral annular septal e' < 7 cm/sec or lateral e' <10 cm/sec, 2) average E/e' ratio > 14, 3) LAVI > 34 mL/m^2, and 4) peak TR velocity > 2.8 m/sec.[35] Teichlholz method was used to calculate LV ejection fraction and in cases with LV ejection fraction less than 50%, biplane Simpson's method was used. LV wall thickness was measured in all cross-sectional planes. The maximal thickness of LV was defined as the largest dimension evident at any site within LV chamber. Posterior wall thickness was measured at parasternal long axis view or short axis view. The continuous wave Doppler was used to measure peak velocity across the LVOT, and the pressure gradient (PG) was calculated by using the Bernoulli equation, 4 x (peak velocity across the LVOT)^2.[36] It was measured at resting, and during valsalva maneuver. LVOT obstruction was defined as a systolic PG of 30mmHg or higher across the LVOT. The PG was also measured at mid-level of LV cavity, and mid-LV obstruction was defined in the same way. To improve LV border definition, contrast echocardiography was performed in patients with poor-defined LV border.

Apical HCM comprised pure type and mixed type. Pure types presented with hypertrophy that was confined to below the papillary muscle level, and mixed types presented with asymmetrical hypertrophy of the interventricular septum, where hypertrophy was greatest in the apical segments but did not extend to basal segments. This classification is consistent with a previous study.[37, 38] We also classified the patients with HCM according to the presence of LVOT or mid-LV obstruction, either resting or dynamic condition; hypertrophic obstructive cardiomyopathy (HOCM) and hypertrophic non-obstructive cardiomyopathy (HNCM). Anterior mitral leaflet lengths (AML) were measured in both parasternal long axis (PLX) view (Figure2A) and apical 3 chamber views (Figure 2B). In 3 chamber view and PLX view, AML were measured in diastole with the leaflets

maximally extended parallel to the anterior septum and defined as the distance from the most distal extent of anterior leaflet to its insertion into the posterior aortic wall. For their comparison indexed by body surface area were used.

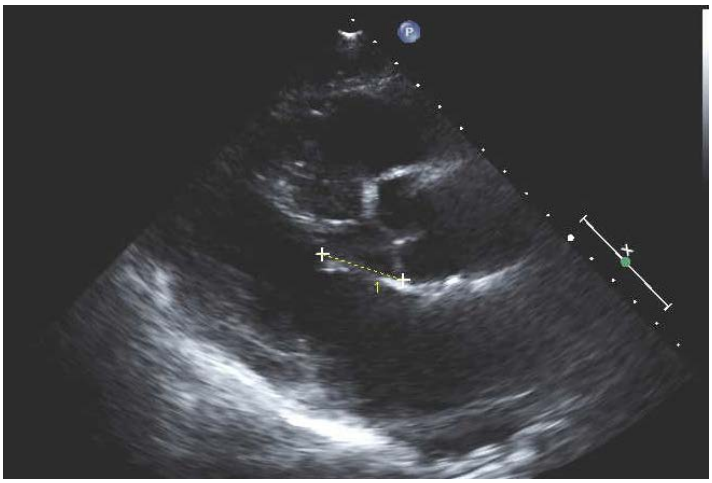

Figure 2A

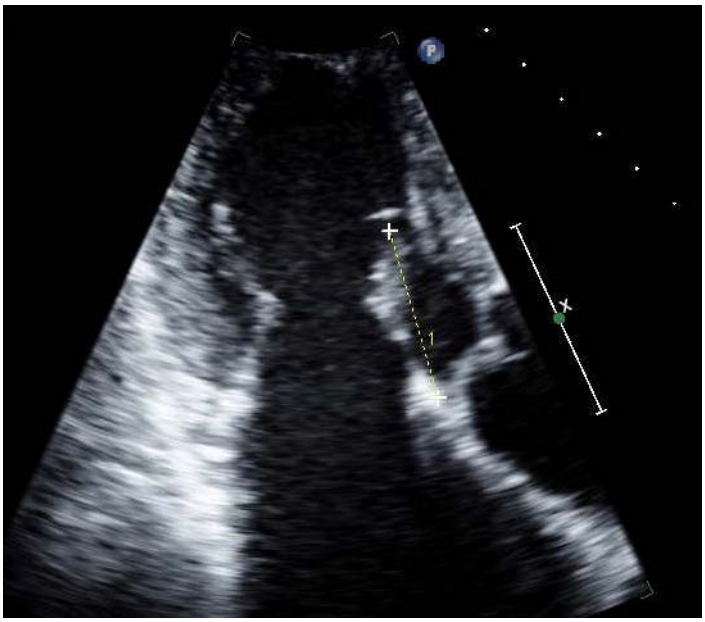

Figure 2B

Figure 2. Measurement of anterior mitral leaflet lengths (AML) in parasternal long axis view (2A) and apical 3 chamber views (2B). The leaflet lengths were measured in diastole with the leaflets maximally extended parallel to the anterior septum.

2A. AML measurement in parasternal long axis view

2B. AML measurement in apical 3 chamber view

4. Cardiovascular Magnetic Resonance (CMR) analysis

Among 149 patients, CMR was performed in 54 patients. CMR was performed with a 1.5-T scanner (Magnetom Avanto®; Siemens Medical Solutions, Erlangen, Germany) with a phased array body coil. The LV 2-, 3-, 4-chamber and short axis views were obtained using cine images with steady-state free precession (SSFP) sequence. The acquisition parameters were: repetition time (TR) = 40.2 ms, echo time (TE) = 1.13 ms, flip angle = 80°, 25 phases, slice thickness = 6 mm, slice gap = 2 mm, acquisition matrix = 192 x 109, and field of view=308 x 379 mm. Late gadolinium enhancement (LGE) imaging with a magnitude- and phase-sensitive inversion recovery prepared fast gradient echo sequence was obtained in 10 minutes after administration of 0.2 mmol/kg of a gadolinium-based contrast agent (gadoterate dimeglumine; Dotarem, Guerbet, France). LGE imaging was obtained in the same axis and slice thickness used in the cine imaging. A bolus of contrast media was intravenously administered at 2 mL/sec, followed by 20 mL of normal saline at 4 mL/sec through a 20-gauge cannula in the antecubital vein using a power injector (Nemoto; Nemoto Kyorindo, Tokyo, Japan). The appropriate inversion time before LGE-imaging was determined using a fast gradient echo sequence with inversion times varying from 150-650 msec to null the signal from the normal myocardium. The LGE imaging parameters were: TR=495 ms, TE=3.36 ms, flip angle=25°, acquisition matrix=256 x 156; and field of view=300 x 370 mm. Native-T1 mapping with a modified Look and Locker technique were performed during the mid-diastolic phase and the post-T1 mapping was performed 15 minutes after the contrast media injection, using the same slice axis and parameters as pre-T1 mapping.[39] Quantitative T2 mapping imaging was performed before contrast media injection with a T2-prepared SSFP pulse sequence along the same short-axis planes as used for cine imaging. A motion correction algorithm provided by the vendor was used to reduce motion artifacts. The acquisition parameters for T2 mapping were as follows: T2 preparation times = 0 ms, 24 ms, and 55 ms; TR = 3 × R-R ms; TE = 0 ms, 24 ms, and 55 ms; acquisition matrix = 126 × 192;

acquisition time = 7 × R-R; single-shot acquisition; flip angle = 70°; and bandwidth = 916 Hz/pixel. T2-pixel maps were generated after motion correction using commercially available software on the scanner's workstation (Syngo; Siemens Medical Solutions, Erlangen, Germany).[40]

The endocardial and epicardial borders were contoured using a semi-automated method (Argus®, Siemens, Germany or Qmass® MR 7.5, Medis, Leiden, Netherland), and then LV end-diastolic volume (LVEDV) and LV end-systolic volume (LVESV) were measured. To determine the end-diastolic LV mass, the difference between the epicardial and endocardial areas for all slices was multiplied by the slice thickness and section gap and then multiplied by the specific gravity of the myocardium (1.05 g/mL). Papillary muscle mass was included in the LV cavity and excluded from the LV mass measurements. Stroke volume was calculated as LVEDV minus LVESV, and LV ejection fraction was calculated as (100 x stroke volume)/LVEDV. LV mass index was calculated by LV mass/body surface area. Whole LV was divided into 16 regional segments according to AHA guideline and maximal thickness within each segment was measured.[41]

From the LGE images, LV was divided into 16 segments[41]. Presence of LGE involvement in each segment and total number of LGE involving segment were measured. In addition, pattern of LGE and the percentage of LGE in LV mass were measured using dedicated quantitative analysis software (Qmass®MR 7.5, Medis, Leiden, Netherland).[39] In each short-axis slice image, boundaries of contrast-enhanced areas were automatically traced. On LGE-MR images, myocardium with abnormal enhancement was defined as the area of hyper-enhancement more than five standard deviations from the remote myocardium. Remote myocardium was defined as non-enhanced myocardium opposite to the hyperenhanced myocardium on the LGE-MR images.[42] The maximum signal was determined by computer-assisted window thresholding of the enhanced area. Obvious artifacts such as those caused by motion were excluded by highlighting them using a tool from the software package. Total LGE amount was calculated by summation of all slice volumes of enhancement.[43]

Region of interest was placed in each segment. The myocardial extracellular volume fraction (ECV) was calculated as follows: ECV = (ΔR1 of myocardium / ΔR1 of LV blood pool) × (1 - hematocrit), R1 = 1 / T1, ΔR1 = Post-contrast R1 - Pre-contrast R1.[44] The T2, T1 and ECV values were measured according to AHA myocardial segmentation with the exception of the apex. ECV measurement was possible in 55 patients due to lack of hematocrit value in others.

5. Assessment of systemic mitochondrial dysfunction

For the evaluation of systemic involvement of mitochondrial dysfunction, detailed paper questionnaires were completed by the subjects. The questionnaire comprised evaluation for neurologic, gastrointestinal, endocrine and ophthalmologic involvements. For the neurologic involvement, 10 questions comprised headache, previous history of stroke, history of epilepsy or seizure, motor weakness of extremities, sensory changes, presence of diplopia, gait disturbance, dysphasia and hearing difficulties, were replied. For the gastrointestinal involvement, 3 questions comprised constipation, diarrhea or dysphagia were obtained. For the endocrinal abnormalities, 3 questions comprised history of diabetes, thyroid disease and infertility, were responded. For the ophthalmologic abnormalities, 3 questions comprised visual disturbance, blurred vision and ptosis were answered. Finally 19 questions were answered from all the subjects. The numbers of “yes” were summed, and then the score was used for the analysis.

6. Statistics

We conducted gene based analyses to test for association of the effects of rare variants on type of HCM and clinical parameters such as LAVI. Using all identified SNPs in 82 genes HCM panel and whole mtDNA sequencing, we performed a gene-based analysis of rare variants with the Sequence Kernel Association Test (SKAT).[45-47] For this association analysis, the rare variant were defined as genetic variants with MAF <5.9% ($MAF < 1/\sqrt{(2N)} = 0.0583$).[48] A single variant test analysis is the standard approach testing for association between genetic variants. We analyzed low-frequency variants. Potential confounding factors (age, sex, HTN,

DM and eGFR) were used as covariates. We set the significance level at 0.05 for gene based analysis. Clinical data was analyzed using different method. Continuous variables that are normally distributed are reported as mean ± SD or 95% confidence interval (CI). Student t-test was used to compare the means of continuous variables that were approximately normally distributed between the two groups. Normality was determined using the Kolmogorov-Smirnov goodness-of-fit test. Categorical variables are reported as count (percentage) and are compared using Fisher's exact test. All clinical statistical analyses were performed using the SPSS version 19.0 statistical package (IBM, Markham, Canada). A two sided p-value of < 0.05 was considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the database. All authors have read and agreed to the manuscript as written.

III. RESULTS

1. Baseline characteristics

The baseline characteristics of the patients are described in Table 1. Mean age of enrolled patients was 58.8±12.8 years. Of the patients, 106 (71.1%) were male. Eighty-three (55.7%) of patients had well-controlled hypertension. No patients had GLA mutation for Fabry disease. Seventy-three patients, 49% of total HCM (73/149) were classified as apical HCM group. Male was more prevalent in apical HCM group than non-apical HCM group (79.5% vs. 63.2%, p=0.031). Five-year SCD risk was higher in non-apical HCM group compared with apical HCM group (2.75±1.54 vs. 1.78±0.81%, p=0.002). SCD of 2nd degree relatives and non-sustained ventricular tachycardia in 24 hour holter monitoring was more frequent in non-apical HCM group than apical HCM group, but it was not statistically significant There were no significant difference in age, history of hypertension, diabetes mellitus and coronary artery disease between non-apical HCM and apical HCM group (all p>0.05).

Of the 149 patients, 142 patients completed questionnaire for mitochondrial related

symptom. Mean score of questionnaire was 1.7±2.0 (1.0±1.4 for neurologic symptom; 0.2±0.4 for gastrointestinal symptom; 0.3±0.6 for endocrinologic symptom; 0.2±0.5 for ophthalmologic symptom). When comparing the score between apical HCM and non-apical HCM, the score was not significantly different.

Table 1. Baseline characteristics according to hypertrophy pattern

Characteristics	Total (n=149)	Apical HCM (n=73)	Non-apical HCM (n=76)	P value
Age, years	58.8±12.8	59.2±12.5	58.4±13.2	0.729
Female, n (%)	43 (28.9%)	15 (20.5%)	28 (36.8%)	0.031
SBP, mmHg	121±19	122±20	119±18	0.285
DBP, mmHg	73±13	74±12	72±14	0.480
eGFR, ml/min/1.73m^2	82.51±14.30	80.55±14.02	84.45±14.40	0.108
Hypertension, n (%)	83 (55.7%)	42 (57.5%)	41 (53.9%)	0.742
Diabetes mellitus, n (%)	28 (18.8%)	18 (24.7%)	10 (13.2%)	0.094
Stroke, n (%)	7 (4.9%)	3 (4.3%)	4 (5.6%)	>0.999
CAD, n (%)	13 (10.9%)	5 (8.8%)	8 (12.9%)	0.563
Scores for mitochondrial related symptom	1.7±2.0	1.6±1.8	1.9±2.2	0.478
FHx of SCD-1st	9 (6.0%)	3 (4.0%)	6 (8.2%)	0.261
FHx of SCD-2nd	9 (6.0%)	2 (2.6%)	7 (9.6%)	0.090
Unexplained syncope, n (%)	6 (4.0%)	2 (2.6%)	4 (5.3%)	0.353
NSVT, n (%)*	12 (17.9%)	2 (7.7%)	10 (24.4%)	0.076
5-year SCD risk, %*	2.35±1.37	1.78±0.81	2.75±1.54	0.002
Atrial fibrillation, n (%)	15 (10.1%)	5 (6.8%)	10 (13.2%)	0.157

HCM, hypertrophic cardiomyopathy; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated Glomerular Filtration Rate; CAD, coronary artery disease with luminal narrowing>50%; PCI, percutaneous intervention; FHx, family history; SCD, sudden cardiac death; 1st , 1st degree family; 2nd, 2nd degree family; NSVT, non-sustained ventricular tachycardia;

*Analysis of 67 patients for whom 24 hour Holter test was performed; 41 and 26 patients in non-apical HCM and apical HCM, respectively.

2. Echocardiographic and CMR analysis

The echocardiographic and CMR analysis of the patients are described in Table 2 and Table 3. Non-apical HCM group showed significantly higher maximal wall thickness compared with apical HCM group (20.2±3.8 vs. 17.5±2.5mm, p<0.001). Apical HCM group had larger LV EDD compared with non-apical HCM group (47.03±4.05 vs. 44.84±4.87mm, p=0.003), while LV ESD and EF were not significantly different between two groups (p>0.05). Non-apical HCM group had higher right ventricular systolic pressure (28.6±9.3 vs. 24.6±6.4mmHg, p=0.004), larger LAV (75.7±40.0 vs. 58.7±22.6ml, p=0.002), LAVI (44.1±23.3 vs. 32.8±13.1 ml/m^2, p<0.001), lower e' velocity (4.6±1.7 vs. 5.4±1.2 cm/sec, p=0.002) and higher E/e' (16±6 vs. 13±4, p<0.001) compared with apical HCM group. Resting peak trans-LVOT pressure gradient was higher (15.45±18.90 vs. 9.22±10.83mmHg, p=0.015), and LVOT or mid-LV obstruction was more frequent (34.2 vs. 16.4%, p=0.015) in non-apical HCM group compared with apical HCM group. MR degree was relatively higher in non-apical HCM group than apical HCM group (p>0.05).

CMR analysis revealed that LV mass and LV mass index was higher in non-apical HCM group compared with apical HCM group (92.1±29.1 vs. 70.7±15.7g/m^2, p=0.002). Non-apical HCM group showed higher prevalence of LGE compared with apical HCM group (63% vs. 37%, p=0.013). Percentage of LGE (9.7±9.6% vs. 2.5±4.6%, p=0.001) and LGE involving segment number (4.30±3.96 vs. 1.22±1.91, p=0.001) were significantly higher in non-apical HCM group compared with apical HCM group. Non-apical HCM group has significantly higher average ECV (32.6±4.8 vs. 28.1±2.6%, p=0.001) and T2 value (56.7±3.7 vs. 54.4±2.6ms, p=0.015), and higher tendency of native T1 (1046.8±45.9 vs. 1022.3±46.3ms, p=0.061) than apical HCM group. ECV was correlated with 5-year SCD risk (r=0.450, p=0.031), while neither the presence of LGE nor % LGE mass was not correlated with 5-year SCD risk. (Figure 3)

Table 2. Echocardiographic analysis according to hypertrophy pattern

Characteristics	Total (n=149)	Apical HCM (n=73)	Non-apical HCM (n=76)	P value
LVEDD, mm	45.9±4.6	47.0±4.1	44.8±4.9	0.003
LVESD, mm	29.3±4.2	29.9±3.4	28.8±4.8	0.113
LAV, mL	67.4±33.8	58.7±22.6	75.7±40.0	0.002
LAVI, mL/m^2	38.5±19.8	32.8±13.1	44.1±23.3	<0.001
MR degree*	0.48±0.31	0.44±0.22	0.53±0.37	0.083
MR ≥ mild grade, n (%)	11 (7.4%)	3 (4.1%)	8 (10.5%)	0.210
MR ≥ moderate grade, n (%)	3 (2.0%)	0 (0%)	3 (3.9%)	0.245
LVEF, %	67.7±7.3	68.5±6.2	66.9±8.2	0.181
S', cm/s	7.1±1.9	7.0±1.6	6.6±1.8	0.119
E/e'	13.7±5.3	12.7±4.2	16.3±5.7	<0.001
RVSP, mmHg	23.0±11.6	24.6±6.4	28.6±9.3	0.004
Maximal thickness, mm	18.9±3.5	17.5±2.5	20.2±3.8	<0.001
PPG at resting, mmHg	12.40±15.74	9.22±10.83	15.45±18.90	0.015
PPG during Valsalva, mmHg	23.43±30.52	17.75±18.75	27.11±35.81	0.105
LVOT or mid-LV obstruction, n (%)	38 (25.5%)	12 (16.4%)	26 (34.2%)	0.015

LV, left ventricle; EDD, end-diastolic dimension; ESD, end-systolic dimension; LAV, left atrial volume; LAVI, left atrial volume index; MR, mitral regurgitation; EF, ejection fraction; s', systolic mitral annular velocity; E/e', the ratio of mitral peak velocity of early filling (E) to early diastolic mitral annular velocity (e'); RVSP, right ventricular systolic pressure; PPG, trans-LV outflow tract peak pressure gradient; LVOTO, left ventricular outflow tract obstruction

*MR degree was scored as described following; 0, no MR; 0.5, trivial MR; 1, mild MR; 2, moderate MR

Table 3. CMR analysis according to hypertrophy pattern

Parameters	Total (n=54)	Apical HCM (n=27)	Non-apical HCM (n=27)	P value
LVEDV, ml	136.3±24.5	133.9±24.7	138.7±24.6	0.473
LVESV, ml	48.4±15.51	49.4±16.1	47.4±15.1	0.649
LVEF, %	65.1±8.0	64.0±24.7	66.1±9.2	0.335
LV mass, g	146.0±46.3	131.9±34.9	160.2±52.2	0.023
LV mass index, g/m^2	81.4±25.6	70.7±15.7	92.1±29.1	0.001
Presence of LGE, n (%)	30 (55.6%)	10 (37.0%)	20 (74.1%)	0.013
Percent LV LGE amount, %	6.1±8.3	2.5±4.6	9.7±9.6	0.001
Number of LGE segments	2.76±3.45	1.22±1.91	4.30±3.96	0.001
Average Native T1, ms	1034.1±47.3	1022.3±46.3	1046.8±45.9	0.061
Average ECV, %	30.2±4.3	28.1±2.6	32.6±4.8	<0.001
Average T2, ms	55.5±3.4	54.4±2.6	56.7±3.7	0.015

LV, left ventricle; EDV, end-diastolic volume; ESV, end-systolic volume; EF, ejection fraction; LGE, late gadolinium enhancement; T1, T1 relaxation time; ECV, extracellular volume; T2, T2 relaxation time

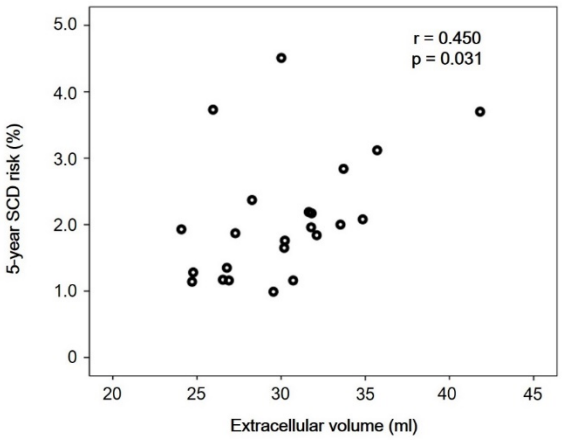

Figure 3. Correlation between ECV and 5-year SCD risk

ECV, extracellular volume; SCD, sudden cardiac death

3. Genetic characteristics

A. Sarcomere mutations according to phenotype

In 149 patients, 13 sarcomere gene mutations were detected in 35.6% (53/149) as described at Table 5 and Figure 4. Detailed profiles of detected sarcomere genes are described in Table 4. Sarcomere mutations were detected with significantly higher prevalence in non-apical HCM (34/76, 44.7%) compared to apical HCM (19/73, 26.0%; p=0.026). Sarcomere mutations were most frequently detected in *MYH7* and *MYBPC3* with a prevalence of 10.1% (15/149) and 8.7% (13/149), respectively. Among the cases with sarcomere gene mutations, the prevalence of two major genes, *MYH7* and *MYBPC3*, was 51% (27/53). In non-apical HCM, *MYH7* (12/34, 35%) and *MYBPC3* (8/34, 24%) were the major two mutation genes, while the most cases of mutations were detected in *TNNI3* (37%, 7/19), followed by *MYBPC3* (26%, 5/19) and *MYH7* (16%, 3/19) in apical HCM. One patient met criteria of end-stage HCM with reduced EF of 34%, and mutation was detected at *MYPN* sarcomere gene. One apical HCM patient had double mutations both in *MYH7* and *MYBPC3* (Table 5).

Sarcomere gene mutation-positive group was younger (55±13 vs. 61±12 years, p=0.004), less prevalence of hypertension (37.7 vs. 65.6%, p=0.001), more prevalence of female (40 vs. 23%, p=0.038). Sarcomere mutation-positive group had higher tendency of sudden cardiac death history in 1st degree family (11 vs. 3%, p=0.054). Sarcomere gene mutation-positive group had a higher LAV (77.2±45.0 vs. 61.9±24.0ml, p=0.025), LAVI (44.96±25.67 vs. 34.98±14.59 ml/m^2, p=0.011) and RVSP (29.41±8.21 vs. 25.16±7.87mmHg, p=0.004) compared with sarcomere gene mutation-negative group as described at Table 6. Sarcomere gene mutation-positive group showed a higher tendency of diastolic dysfunction, although it was statistically insignificant (39.6 vs. 26.0%, p=0.098). The presence of diabetes mellitus, which could cause hypertrophy, was not different between two groups. There was also no significant different in the score for mitochondrial related symptom based on questionnaires between two groups. There were no significant

differences in maximal wall thickness, LV EDD, ESD, EF and E/e' between the two groups (all $p>0.05$). CMR analysis showed no difference of LV mass, LV mass index, presence of LGE, native T1, ECV and T2 between two groups as described at Table 6. When sarcomere gene mutation-positive group was further divided into thick filament mutation group and thin or non-thick filament mutation group, thick filament group had higher prevalence of women and unexplained syncope, while thin filament mutation group had higher LAV and LAVI as shown in table 7.

Table 4. Detailed description of detected sarcomere gene mutations

Gene	NM_number	Amino Acid Change	DNA Change	Zygosity	dbSNP	Classification
ACTC1	NM_005159.4	p.Ile311Val	c.A931G	Heterozygous	Novel	Likely pathogenic
BAG3	NM_004281.3	p.Arg218Trp	c.652C>T	Heterozygous	rs397514506	Likely pathogenic
CRYAB	NM_001289807.1	p.Arg157His	c.470G>A	Heterozygous	rs141638421	Pathogenic
MYPN	NM_001256267.1	p.Ala461Val	c.1382C>T	Heterozygous	Novel	Likely pathogenic
MYPN	NM_001256267.1	p.Pro1100Leu	c.3299C>T	Heterozygous	Novel	Likely pathogenic
MYBPC3	NM_000256.3	p.Glu1269*	c.3805G>T	Heterozygous	Novel	Pathogenic
MYBPC3	NM_000256.3	p.Glu838*	c.2512G>T	Heterozygous	Novel	Pathogenic
MYBPC3	NM_000256.3	p.Glu60*	c.178G>T	Heterozygous	Novel	Pathogenic
MYBPC3	NM_000256.3	p.Phe29Serfs*10	c.86delT	Heterozygous	Novel	Pathogenic
MYBPC3	NM_000256.3	p.Ala1255Profs*76	c.3763delG	Heterozygous	rs786204362	Likely pathogenic
MYBPC3	NM_000256.3	p.Arg1033Gln	c.3098G>A	Heterozygous	rs397516003	Likely pathogenic
MYBPC3	NM_000256.3	p.Arg945Glyfs	c.2833_2834del	Heterozygous	rs397515987	Pathogenic
MYBPC3	NM_000256.3	p.Arg820Gln	c.2459G>A	Heterozygous	rs2856655	Likely pathogenic
MYBPC3	NM_000256.3	p.Arg502Gln	c.1505G>A	Heterozygous	rs397515907	Pathogenic
MYBPC3	NM_000256.3	p.Arg495Gln	c.1484G>A	Heterozygous	rs200411226	Likely pathogenic
MYH6	NM_002471.3	p.Gln1065His	c.3195G>C	Heterozygous	rs267606904	Likely pathogenic
MYH6	NM_002471.3	p.Arg795Gln	c.2384G>A	Heterozygous	rs267606907	Pathogenic

MYH6	NM_002471.3	p.Glu808Gly	c.2423A>G	Heterozygous	Novel	Likely pathogenic
MYH7	NM_000257.3	p.Thr1377Met	c.4130C>T	Heterozygous	rs397516201	Pathogenic
MYH7	NM_000257.3	p.Arg870Cys	c.2608C>T	Heterozygous	rs138049878	Pathogenic
MYH7	NM_000257.3	p.Arg442Cys	c.1324C>T	Heterozygous	rs148808089	Likely pathogenic
MYH7	NM_000257.3	p.Arg869Pro	c.2606G>C	Heterozygous	Novel	Likely pathogenic
MYH7	NM_000257.3	p.Lys450Asn	c.1350G>T	Heterozygous	Novel	Likely pathogenic
MYH7	NM_000257.3	p.Lys1022Glu	c.3064A>G	Heterozygous	Novel	Likely pathogenic
MYH7	NM_000257.3	p.Lys994Arg	c.2981A>G	Heterozygous	Novel	Likely pathogenic
MYH7	NM_000257.3	p.Leu476Phe	c.1426C>T	Heterozygous	Novel	Likely pathogenic
MYH7	NM_000257.3	p.Ala200Val	c.599C>T	Heterozygous	Novel	Likely pathogenic
MYOM1	NM_003803.3	p.Ser451Phefs*27	c.1351dupT	Heterozygous	Novel	Likely pathogenic
MYL3	NM_000258.2	p.Ala57Gly	c.170C>G	Heterozygous	rs139794067	Pathogenic
TCAP	NM_003673.3	p. Pro142Arg	c.425C>G	Heterozygous	Novel	Likely pathogenic
TNNC1	NM_003280.2	p.Ala8Val	c.23C>T	Heterozygous	rs267607125	Likely pathogenic
TNNI3	NM_000363.4	p.Arg162Pro	c.485G>C	Heterozygous	rs397516354	Likely pathogenic
TNNI3	NM_000363.4	p.Arg145Gln	c.434G>A	Heterozygous	rs397516349	Pathogenic
TNNI3	NM_000363.4	p.Arg145Gly	c.433C>G	Heterozygous	rs104894724	Pathogenic
TTN	NM_003319.4	p.Ser4417Asn	c.13250G>A	Heterozygous	rs147879266	Pathogenic

NM number, National Center for Biotechnology Information (NCBI) reference sequence

Table 5. Distribution of sarcomere gene mutations in HCM patients

Gene	Full-name (coding protein)	Total (n=149)	Non-apical HCM (n=76)	Apical HCM (n=73)	P value
*MYH7**	(β)-myosin heavy chain-7	15	12	3*	
*MYBPC3**	myosin binding protein C	13	8	5*	
TNNI3	cardiac troponin I	12	5	7	
MYH6	(α)-myosin heavy chain	4	2	2	
MYPN	myopalladin	2	1	1	
MYL3	myosin light chain 3	1	0	1	
MYOM1	Myomesin-1 (connect to titin)	1	1	0	
TCAP	Telethonin	1	1	0	
TNNC1	Troponin C	1	1	0	
TTN	Titin	1	0	1	
ACTC1	cardiac muscle alpha actin	1	1	0	
BAG3	BCL2 associated athanogene 3	1	1	0	
CRYAB	Alpha-crystallin B chain	1	1	0	
Total		54	34	19	0.016

*One patient had double mutations on *MYH7* and *MYBPC3*

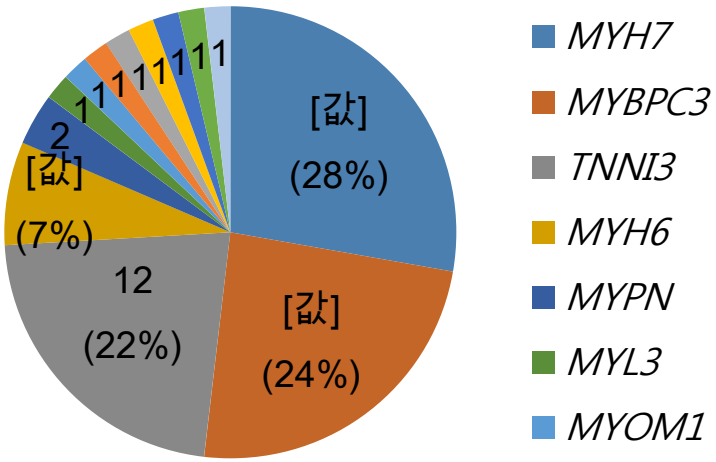

Figure 4A

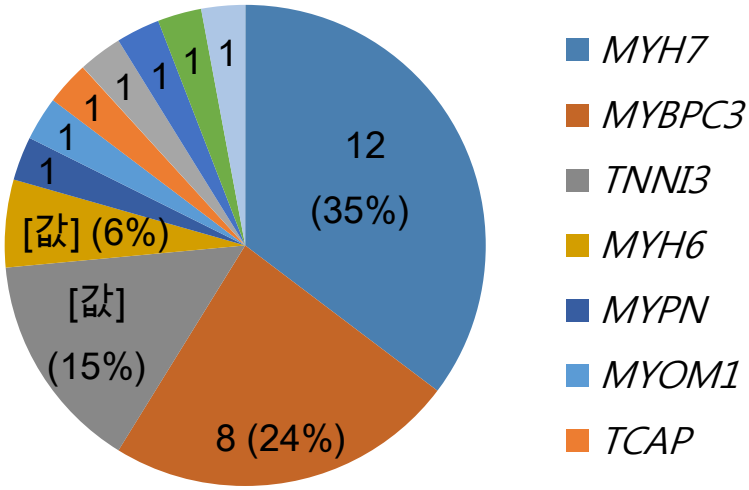

Figure 4B

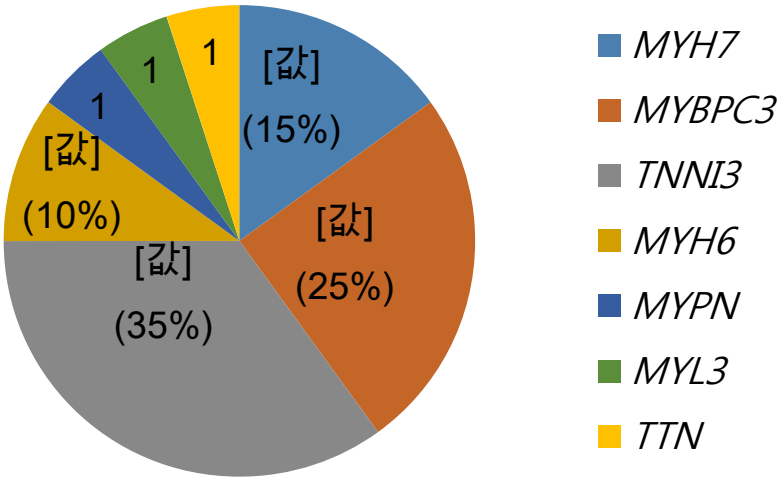

Figure 4C

Figure 4.Prevalence of sarcomere gene mutations according to HCM phenotypes.

4A. Sarcomere gene mutations in total HCM patients

4B. Sarcomere gene mutations in non-apical HCM patient

4C. Sarcomere gene mutations in apical HCM patients

Table 6. Clinical characteristics according to sarcomere gene mutations

Characteristics	With sarcomere gene mutation (n=53)	Without sarcomere gene mutation (n=96)	P value
Age, years	54.5±13.5	61.1±11.9	0.002
Women, n (%)	21 (39.6%)	22 (22.9%)	0.038
Hypertension, n (%)	20 (37.7%)	63 (65.6%)	0.001
Diabetes, n (%)	8 (15.1%)	20 (20.8%)	0.512
Body surface area, m^2	1.73±0.19	1.80±0.20	0.044
eGFR, ml/min/1.73m^2	83.33±13.05	81.86±14.90	0.427
Scores for mitochondrial related symptoms	1.5±1.8	1.9±2.1	0.349
Atrial fibrillation, n (%)	8 (15.1%)	7 (7.3%)	0.158
FHx of SCD-1st *	6 (11.3%)	3 (3.2%)	0.070
FHx of SCD-2nd *	3 (5.7%)	6 (6.3%)	>0.999
Unexplained syncope, n (%)	4 (7.5%)	2 (2.1%)	0.188
NSVT, n (%)$^{€}$	3 (11.1%)	9 (22.5%)	0.335
5-year SCD risk, %$^{€}$	2.50±1.68	2.25±1.13	0.471
Echocardiographic analysis			
Apical HCM, n (%)	19 (35.8%)	54 (56.3%)	0.026
LVOT or mid-LV obstruction, n (%)	12 (22.6%)	26 (27.1%)	0.565
PPG at resting, mmHg	11.21±17.81	13.05±14.53	0.496
PPG during Valsalva, mmHg	20.58±33.56	24.86±29.01	0.478
LV EDD, mm	45.45±4.81	46.17±4.49	0.367
LV ESD, mm	28.55±4.68	29.73±3.84	0.099
LAV, ml	77.2±45.0	61.9±24.0	0.025

LAVI, ml/m^2	44.96±25.67	34.98±14.59	0.011
MR degree*	0.53±0.36	0.46±0.28	0.188
MR ≥ mild grade, n (%)	5 (9.4%)	6 (6.3%)	0.522
MR ≥ moderate grade, n (%)	2 (3.8%)	1 (1.0%)	0.553
EF, %	67.9±8.2	67.5±6.8	0.725
S', cm/s	6.7±1.6	6.9±1.8	0.527
E/e'	15.2±5.5	14.1±5.2	0.237
Advanced diastolic dysfunction, n (%)	21 (39.6%)	25 (26.0%)	0.098
RVSP, mmHg	29.41±8.21	25.16±7.87	0.004
Maximal LV wall thickness, mm	18.93±3.55	18.87±3.49	0.921
CMR analysis			
LVEDV, ml	136.5±21.7	136.2±26.0	0.959
LVESV, ml	49.0±18.0	48.1±14.4	0.852
LVEF-CMR, %	64.9±11.0	65.1±6.4	0.935
LV mass, g	132.0±38.7	152.5±48.5	0.133
LV mass index, g/m^2	76.9±25.7	83.4±25.6	0.385
Presence of LGE, n (%)	11 (65)	19 (51)	0.268
%LGE mass	8.9±11.4	4.9±6.2	0.188
Number LGE segment	3.3±3.5	2.5±3.4	0.445
Native T1, ms	1028.0±60.4	1036.8±40.8	0.538
ECV, %	30.5±4.6	30.1±4.3	0.752
T2, ms	56.3±3.8	55.1±3.2	0.259

eGFR, estimated Glomerular Filtration Rate; FHx, family history; SCD, sudden cardiac death; NSVT, non-sustained ventricular tachycardia; HCM, hypertrophic cardiomyopathy; LVOT, left ventricular outflow tract; LV, left ventricle; EF, ejection fraction; EDD, end-diastolic dimension; ESD, end-systolic dimension; LAVI, left atrial volume index; RVSP, right ventricular systolic pressure; E/e', the

ratio of mitral peak velocity of early filling (E) to early diastolic mitral annular velocity (e'); diastolic dysfunction, defined if more than half of the parameters meet the four recommended variables; 1) mitral annular septal e' < 7 cm/sec or lateral e' <10 cm/sec, 2) average E/e' ratio > 14, 3) left atrial volume index > 34 mL/m^2, and 4) peak TR velocity > 2.8 m/sec.[35]

*Analysis was done in 148 patients because one patient refused family history taking

€ Analysis of 67 patients for whom 24 hour Holter test was performed; 41 and 26 patients in non-apical HCM and apical HCM, respectively.

Table 7. Comparisons between thick filament and non-thick filament mutation group

	No sarcomere gene mutation group (n=96)	Thick filament mutation group (n=32)	Thin or non-thick filament mutation group (n=21)	P value
Age, years	61.1±11.9	55.8±13.6†	52.7±13.4*	0.007
Women, n (%)	22 (23)	18 (56)	3 (14)*	<0.001
Hypertension, n (%)	63 (66)	12 (38)†	8 (38)†	0.005
Diabetes, n (%)	20 (21)	3 (9)	5 (24)	0.291
Body surface area, m^2	1.80±0.20	1.69±0.20†	1.79±0.15	0.026
AF, n (%)	7 (7)	4 (13)	4 (19)	0.235
FHx of SCD-1st, n (%)	3 (3)	4 (13)	2 (10)	0.125
FHx of SCD-2nd, n (%)	6 (6)	1 (3)	2 (10)	0.627
Syncope, n (%)	2 (2)	4 (13)	0 (0)	0.021
NSVT, n(%)(n=63)	9 (23)	0 (0)†	3 (25)	0.119
5-year SCD risk, % (n=63)	2.25±1.13	2.32±1.64	2.74±1.78	0.576
Echocardiography	No sarcomere gene mutation group (n=96)	Thick filament mutation group (n=32)	Thin or non-thick filament mutation group (n=21)	P
ApHCM, n (%)	54 (56)	10 (31)†	9 (43)	0.041
PPG-rest, mmHg	13.1±14.5	12.7±22.3	8.9±6.7	0.554
DLVOTO, n (%)	26 (27)	8 (25)	4 (19)	0.744
LVEDD, mm	46.2±4.5	44.6±4.8	46.7±4.7	0.180
LVESD, mm	29.7±3.8	27.5±4.4†	30.1±4.8*	0.019

LAV, mL	61.9±24.0	73.0±37.2	83.6±55.2*	0.015
LAVI, mL/m^2	35.0±14.6	44.2±25.0†	46.1±27.3*	0.011
Advanced diastolic dysfunction, n (%)	25 (26.0%)	13 (40.6%)	8 (38.1%)	0.250
MR grade	0.46±0.28	0.50±0.36	0.57±0.36	0.301
LV ejection fraction, %	67.5±6.8	68.2±7.5	67.5±9.4	0.889
S', cm/s	6.9±1.8	6.7±1.6	6.7±1.7	0.817
E/e'	14.1±5.2	15.7±5.8	14.5±5.2	0.359
RVSP, mmHg	25.1±7.9	30.0±9.1†	28.6±7.0	0.012
Maximal thickness, mm	18.9±3.5	19.3±3.3	18.3±3.9	0.577
CMR-index (n=54)				
LVEDV, ml	136.2±26.0	138.2±28.5	134.6±12.0	0.955
LVESV, ml	48.1±14.4	48.1±19.0	50.0±18.1	0.951
LVEF-CMR,%	65.1±6.4	65.9±9.5	63.9±13.1	0.877
LV mass, g	152.5±48.5	111.5±26.4†	155.2±38.4*	0.045
LV mass index, g/m^2	83.4±25.6	63.3±18.6†	92.1±24.6*	0.043
Presence of LGE, n (%)	19 (51)	5 (56)	6 (75)	0.475
%LGE mass	4.9±6.2	4.9±5.9	13.4±14.6*†	0.024
Number LGE segment	2.5±3.4	2.0±2.2	4.8±4.3	0.195
Average native T1, ms	1036.8±40.8	1030.2±71.2	1025.8±52.4	0.815
Average ECV, %	30.1±4.3	29.5±2.9	32.0±6.3	0.604
Average T2, ms	55.1±3.2	55.8±2.4	56.9±5.0	0.432

Thick filament gene includes *MYH7, MYBP-3, MYH6* and *MYL3*; *p<0.05 vs. thick filament mutation; †p<0.05 vs. no sarcomere mutation group

B. Rare variants analysis according to phenotype

Rare variants (MAF<5.9%) analysis revealed the association between genetic mutation and the phenotype of HCM described at Table 8. Rare variants in 2 mitochondrial genes (*PCCB*, p=0.011; COQ4, p=0.012, respectively), 3 mtDNA genes (*MT-tRNA*, p=0.013; *MT-ND4*, p=0.039; *MT-RNR1*, p=0.043; respectively) and 1 sarcomere genes were significantly associated with apical HCM. (Table 8A) Rare variants in 5 sarcomere genes (*MYBPC3*, p=0.006; *CAV3*, p=0.019; *ACTC1*, p=0.041; *TCAP*, p=0.045; *CRYAB*, p=0.046, respectively) and 1 mitochondrial related nDNA (*NDUFA2*, p=0.046) were associated with obstructive HCM. (Table 8B) The prevalence of each rare variant is described at Table 8.

Table 8. Rare variant associations according to phenotypes

8A. Associations of significant rare variants to apical HCM

Gene	Classification	P value
PCCB	Mitochondrial	0.011
COQ4	Mitochondrial	0.012
MT-tRNA (s(AGY)	mtDNA	0.013
MT-ND4 (12S)	mtDNA	0.039
MT-RNR1	mtDNA	0.043
VCL	Sarcomere	0.040

8B. Associations of significant rare variants to obstructive HCM compared with non-obstructive HCM

Gene	Classification	P value
MYBPC3	Sarcomere	0.006
CAV3	Sarcomere	0.019
ACTC1	Sarcomere	0.041
TCAP	Sarcomere	0.045
NDUFA2	Mitochondrial	0.046
CRYAB	Sarcomere	0.046

C. Rare variants according to diastolic function

We analyzed the relationship between genetic characteristics and diastolic function. According to current guideline[35] as described in method, the patients were divided into two groups with diastolic dysfunction group (n=46) or without diastolic dysfunction (n=103). Rare variants in 1 sarcomere gene (*MYL3*, p=0.043), 1 non-sarcomere gene (*FHL1*, p=0.030), 3 mitochondrial genes (*SLC25A20*, p=0.044; *COX15*, p=0.037; *SCO2*, p=0.029; respectively) and 2 mtDNA (*16S*, p=0.043; *G*, p=0.040; respectively) were significantly associated with the presence of diastolic dysfunction. (Table 9A) Rare variants in 1 sarcomere gene (*MYOZ2*, p=0.039), 1 mitochondrial gene (*COA5*, p=0.010) and 3 mtDNA (*16S/RNR3*, p=0.028; *ND2*, p=0.008, *C/Y*, p=0.028, respectively) were associated with larger left atrial dimension (LAD≥40mm) (Table 9B), and 1 sarcomere gene (*MYPN*, p=0.038) and 1 mtDNA (*P/ATT*, p=0.016) were associated with larger LAVI (≥34ml/m^2). (Table 9C) There were dense network of rare variants between various phenotype and clinical characteristics. Clusters of nDNA and mtDNA variants were identified at Figure 5.

Table 9. Genes with rare variants according to clinical situations

9A. Associations of significant rare variants to diastolic dysfunction

Gene	Classification	P for rare variant
MYL3	Sarcomere	0.043
SLC25A20	mitochondrial	0.044
COX15	mitochondrial	0.037
SCO2	mitochondrial	0.029
FHL1	Non sarcomere	0.030
16S	mtDNA	0.0432
G	mtDNA	0.040

DD, Diastolic dysfunction; advanced DD was defined if more than half of the parameters meet the four recommended variables; 1) mitral annular septal e' < 7 cm/sec or lateral e' <10 cm/sec, 2) average E/e' ratio > 14, 3) left atrial volume index > 34 mL/m^2, and 4) peak TR velocity > 2.8 m/sec[35]

9B. Associations of significant rare variants to LAD (left atrial dimension) ≥40mm

Gene	Classification	P for rare variant
COA5	mitochondrial	0.010
MYOZ2	Sarcomere	0.039
16S/RNR3	mtDNA	0.028
ND2	mtDNA	0.008
C/Y	mtDNA	0.028

LAD, left atrial dimension

9C. Prevalence and associations of significant rare variants to LAVI ≥34ml/m^2

Gene	Classification	P for rare variant
MYPN	sarcomere	0.038
P/ATT	mtDNA	0.016

LAVI, left atrial volume index

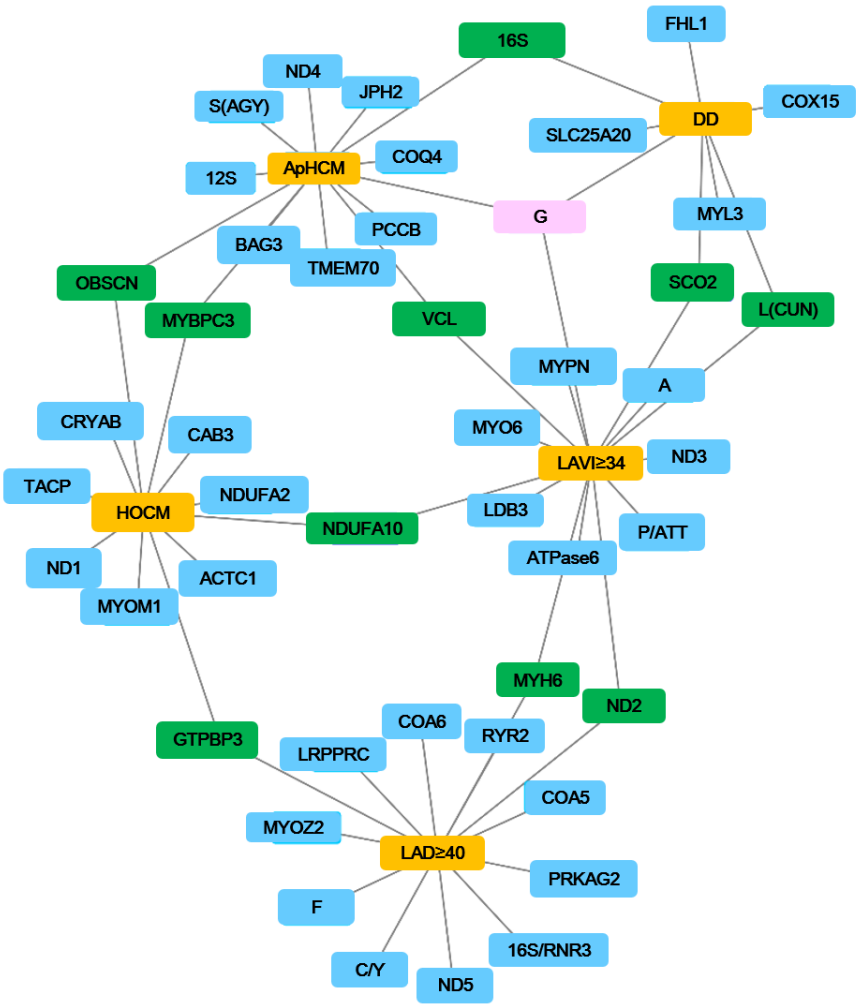

Figure 5. Network of 4 pathways enriched for apical HCM, obstructive HCM, enlarged LA (LAD≥40mm) and advanced diastolic dysfunction. The color codes are; blue, genes involved in 1 pathway; navy, genes involved in ≥2 pathways; violet, genes involved in ≥3 pathways.

4. Mitral valve geometry and genetic characteristics

AML of HCM was significantly longer than controls (2.83±0.36 vs. 2.36±0.31 cm in 3CH, p<0.001). AML-3CH length was significantly correlated to body surface area (BSA, r=0.351, p<0.001), maximal wall thickness (r=0.208, p=0.016), left atrial volume (r=0.250, p=0.002) in echocardiography, left ventricular (LV) end-diastolic volume (r=0.436, p=0.001) and LV mass in CMR (r=0.373, p=0.005) in CMR. . Indexed AML length by BSA of non-apical HCM was significantly longer than apical HCM (1.65±0.23 vs. 1.57±0.20 cm/m^2 in 3CH, p=0.046) along with higher prevalence of sarcomere gene mutations (45% vs. 25%, p=0.016). (Table 10) Sarcomere mutation (+) group has longer indexed AML length in total (1.66±0.23 vs. 1.59±0.21 cm/m^2 in 3CH, p=0.048) and in non-apical HCM subgroup (1.81±0.24 vs. 1.70±0.22 cm/m^2 in average, p=0.038) but not in apical HCM subgroup (1.68±0.19 vs. 1.68±0.21 cm/m^2 in average, p=0963). (Table 11) Thin or non-thick filament gene mutation group had a higher average AML length than sarcomere mutation (-) group and thick filament gene mutation group (32.0±3.8 vs. 30.1±3.4 vs. 29.1±3.4mm in thin or non-thick filament gene mutation group, sarcomere mutation (-) group and thick filament gene mutation group, respectively, p=0.017).

AML length was correlated with LV mass, EDV, ESV and LAD (all p<0.05), but indexed AML length (AMLAML/BSA) was not related with LV mass, EDV, ESV and LAD (all p>0.05). LV mass index, LAV and LAVI were correlated with indexed AML (all p<0.05). Peak trans-LVOT PG was correlated with indexed AML length (p<0.05). LAV was correlated with AML and indexed AML (all p<0.05). Increased MR degree was correlated with longer AML and indexed AML (all p>0.05). Sarcomere gene mutation was correlated with indexed AML length measured at apical 3 chamber view. (Table 13A) In multivariate linear regression, all the variables exhibiting a significant relationship with indexed AML length at univariate analysis were included in each models with (Model 1,2 and 3) or without (Model 4, 5 and 6) sarcomere gene mutation. In Model 1, 2 and 3, LVMI, indexed EDD and MR grade were significantly correlated with indexed AML length (all

p<0.05). Sarcomere gene mutation was not correlated with indexed AML length. The models without sarcomere gene mutation (Model 4, 5 and 6) showed that LVMI, indexed EDD, peak trans-LVOT PG and MR grade had significant correlation with indexed AML length (all p<0.05). (Table 13B)

Table 10. Anterior leaflet length of MV according to hypertrophy pattern

Characteristics	Control (n=30)	Total (n=149)	P value	Apical HCM (n=73)	Non-apical HCM (n=76)	P value
AML –PLX, mm	26.4±2.9	32.0±4.4	<0.001	32.3±4.5	31.8±4.3	0.506
AML-3CH, mm	23.6±3.1	28.3±3.6	<0.001	28.3±3.3	28.4±3.8	0.844
AML-average, mm	25.0±2.4	30.2±3.5	<0.001	30.3±3.4	30.1±3.6	0.757
iAML-PLX, mm/m^2	14.0±2.0	18.2±2.7	<0.001	18.0±2.6	18.5±2.9	0.256
iAML-3CH, mm/m^2	12.5±1.8	16.1±2.2	<0.001	15.7±2.0	16.5±2.3	0.045
iAML-average, mm/m^2	13.3±1.6	17.2±2.2	<0.001	16.8±2.0	17.5±2.4	0.081

AML, anterior mitral leaflet length; PLX, parasternal long axis; 3CH, apical three chamber; iAML, indexed anterior mitral leaflet length (AML/body surface area)

Table 11. Comparison of anterior mitral leaflet length according to sarcomere gene mutation

11A. Comparisons in total HCM

Characteristics	With sarcomere gene mutation (n=53)	Without sarcomere gene mutation (n=96)	P value
AML –PLX, mm	32.0±4.4	32.1±4.4	0.954
AML-3CH, mm	28.4±3.9	28.2±3.4	0.737
AML-average, mm	30.3±3.8	30.1±3.4	0.052
iAML-PLX, mm/m^2	18.7±2.8	18.0±2.7	0.163
iAML-3CH, mm/m^2	16.6±2.3	15.9±2.1	0.048
iAML-average, mm/m^2	17.6±2.3	16.9±2.1	0.052

AML, anterior mitral leaflet length; PLX, parasternal long axis; 3CH, apical three chamber; iAML, indexed anterior mitral leaflet length (AML/body surface area)

11B. Comparisons in non-apical HCM

Characteristics	With sarcomere gene mutation (n=34)	Without sarcomere gene mutation (n=42)	P value
AML –PLX, mm	32.2±4.3	31.4±4.3	0.411
AML-3CH, mm	28.6±4.1	28.2±3.5	0.612
AML-average, mm	30.4±3.9	29.8±3.3	0.445
iAML-PLX, mm/m^2	19.2±3.0	17.9±2.7	0.048
iAML-3CH, mm/m^2	17.0±2.2	16.0±2.4	0.086
iAML-average, mm/m^2	18.1±2.4	17.0±2.2	0.038

Table 12. Comparisons of anterior mitral leaflet lengths between thick filament and non-thick filament mutation group

	No sarcomere gene mutation group (n=96)	Thick filament mutation group (n=32)	Thin or non-thick filament mutation group (n=21)	P value
AML –PLX, mm	32.1±4.4	30.8±3.9	33.9±4.4*	0.043
AML-3CH, mm	28.2±3.4	27.6±3.5	29.9±4.2*	0.076
AML-average, mm	30.1±3.4	29.1±3.4	32.0±3.8*†	0.017
iAML-PLX, mm/m^2	18.0±2.7	18.4±2.6	19.1±3.2	0.109
iAML-3CH, mm/m^2	15.9±2.1	16.5±2.3	16.8±2.3	0.260
iAML-average, mm/m^2	16.9±2.1	17.4±2.2	17.9±2.5†	0.125

AML, anterior mitral leaflet length; PLX, parasternal long axis; 3CH, apical three chamber; iAML, indexed anterior mitral leaflet length (AML/body surface area); Thick filament gene includes *MYH7, MYBP-3, MYH6* and *MYL3*; *p<0.05 vs. thick filament mutation; †p<0.05 vs. no sarcomere mutation group

Table 13. Univariate and multivariate analysis for of correlation for anterior mitral leaflet lengths

13A. Univariate analysis of correlation for anterior mitral leaflet lengths

	AML-PLX	AML-3ch	AML-average	Indexed AML-PLX	Indexed AML-3ch	Indexed AML-average
	r (p value)					
Age	-0.077 (0.178)	-0.140 (0.092)	-0.118 (0.153)	0.208 (0.012)*	0.189 (0.022)*	0.223 (0.007)**
BSA	0.296 (0.000)**	0.351 (0.000)**	0.363 (0.000)**	-0.490 (0.000)**	-0.515 (0.000)**	-0.559 (0.000)**
eGFR	0.100 (0.246)	0.134 (0.118)	0.147 (0.086)	0.035 (0.688)	0.063 (0.468)	0.071 (0.410)
LV mass-CMR	0.357 (0.009)**	0.373 (0.005)**	0.392 (0.003)**	0.178 (0.201)	0.188 (0.174)	0.190 (0.169)
LVMI-CMR	0.287 (0.037)*	0.292 (0.032)*	0.313 (0.021)*	0.337 (0.014)*	0.361 (0.007)**	0.370 (0.006)**
LVEDD	0.194 (0.018)*	0.119 (0.154)	0.193 (0.019)*	-0.125 (0.132)	-0.205 (0.013)*	-0.167 (0.042)*
LVESD	0.175 (0.034)*	0.153 (0.065)	0.197 (0.016)*	-0.057 (0.491)	-0.084 (0.313)	-0.066 (0.426)
LVEDD index	-0.125 (0.132)	-0.230 (0.005)**	-0.185 (0.025)**	0.362 (0.000)**	0.318 (0.000)**	0.390 (0.000)**
LVEDV-CMR	0.407 (0.003)**	0.436 (0.001)**	0.473 (0.000)**	0.008 (0.957)	0.003 (0.985)	0.021 (0.880)
LVESV-CMR	0.315 (0.022)*	0.422 (0.001)**	0.409 (0.002)**	-0.043 (0.759(	0.018 (0.897)	-0.004 (0.980)
LVEF-CMR	-0.129 (0.120)	-0.156 (0.060)	-0.164 (0.046)*	-0.127 (0.125)	-0.157 (0.059)	-0.162 (0.049)
LGE	-0.107 (0.445)	-0.005 (0.969)	-0.050 (0.717)	0.063 (0,655)	0.164 (0.235)	0.133 (0.337)
LAVI	0.090 (0.276)	0.152 (0.067)	0.136 (0.098)	0.256 (0.002)**	0.337 (0.000)**	0.327 (0.000)**
LAV	0.172 (0.037)*	0.250 (0.002)**	0.237 (0.004)**	0.150 (0.070)	0.230 (0.005)**	0.208 (0.011)*
LAD	0.259 (0.002)**	0.304 (0.000)**	0.325 (0.000)**	0.078 (0.349)	0.121 (0.148)	0.113 (0.173)

Max-T	0.154 (0.272)	0.097 (0.484)	0.135 (0.330)	0.272 (0.049)*	0.218 (0.113)	0.262 (0.056)
LVOT- PPG	0.009 (0.917)	0.052 (0.534)	0.017 (0.834)	0.164 (0.047)*	0.225 (0.006)**	0.198 (0.016)*
MR grade	0.091 (0.274)	0.177 (0.032)*	0.146 (0.076)	0.273 (0.001)**	0.392 (0.000)**	0.361 (0.000)**
Mild MR	0.161 (0.051)	0.238 (0.004)*	0.220 (0.007)**	0.232(0.005)**	0.318 (0.000)**	0.301 (0.000)**
Moderate MR	0.118 (0.154)	0.177 (0.033)*	0.163 (0.048)*	0.243 (0.003)**	0.326 (0.000)**	0.311 (0.000)**
Sarcomere mutation	-0.005 (0.954)	0.028 (0.737)	0.024 (0.770)	0.116 (0.163)	0.164 (0.048)*	0.160 (0.052)
Non-thick filament sarcomere gene mutation	-0.151 (0.069)	-0.108 (0.194)	-0.148 (0.073)	0.030 (0.715)	0.089 (0.285)	0.065 (0.433)

r, Pearson's correlation coefficient; eGFR, estimated glomerular Filtration Rate; BSA, body surface area; LV, left ventricle; EDD, end-diastolic diameter; ESD, end-systolic diameter; EDV, end-diastolic volume; ESV, end-systolic volume; CMR, cardiac magnetic resonance; LVMI, left ventricular mass index; EF, ejection fraction; LAVI, left atrial volume index; LAV, left atrial volume; LAD, left atrial dimension; Max-T, maximal wall thickness by CMR; LVOT, left ventricular outflow tract; PPG, peak pressure gradient; MR, mitral regurgitation; *p<0.05, **p<0.01

13B. Multivariate linear regression analysis showing the influence of other variables for indexed anterior mitral leaflet lengths

	Model 1				Model 2				Model 3			
Variables	B	p	95% CI		B	p	95% CI		B	p	95% CI	
			lower	upper			lower	upper			Lower	upper
Age	-0.001	0.825	-0.007	0.005	0.001	0.744	-0.005	0.007	-0.001	0.832	-0.007	0.005
LVMI-CMR	0.003	0.012	0.001	0.005	0.003	0.015	0.001	0.005	0.003	0.037	0.000	0.005
Indexed LV EDD	0.032	0.011	0.008	0.056	0.032	0.011	0.008	0.057	0.031	0.012	0.007	0.054
LAVI	-0.001	0.587	-0.006	0.003	-0.003	0.254	-0.008	0.002	0.001	0.770	-0.003	0.005
LVOT- PPG	0.001	0.285	-0.001	0.003	0.000	0.735	-0.002	0.002	0.002	0.020	0.000	0.004
MR grade	0.334	0.002	0.127	0.541								
Mild MR					0.080	0.003	0.169	0.780				
Moderate MR									0.766	0.001	0.354	1.177
Sarcomere mutation	0.051	0.419	-0.075	0.178	0.474	0.216	-0.048	0.208	0.025	0.689	-0.100	0.149
	Model 4				Model 5				Model 6			
Age	-0.001	0.645	-0.007	0.005	-8.543E-5	0.978	-0.006	0.006	-0.001	0.738	-0.007	0.005
LVMI-CMR	0.003	0.014	0.001	0.005	0.003	0.020	0.000	0.005	0.002	0.038	0.000	0.005
Indexed LV EDD	0.034	0.006	0.010	0.058	0.035	0.005	0.011	0.059	0.031	0.009	0.008	0.055
LAVI	-0.001	0.728	-0.005	0.004	-0.002	0.419	-0.007	0.003	0.001	0.674	-0.003	0.005
LVOT- PPG	0.001	0.246	-0.001	0.003	0.000	0.877	-0.002	0.002	0.002	0.015	0.000	0.004
MR grade	0.338	0.002	0.133	0.544								

Mild MR					0.457	0.004	0.150	0.763				
Moderate MR									0.779	0.000	0.376	1.181

Dependent variable, average indexed anterior mitral leaflet length; Adjusted R^2, 0.491 in Model 1, 0.483 in Model 2, 0.520 in Model 3, 0.483 in Model 4, 0.465 in Model 5, and 0.518 in Model 6; the level of significance at $p<0.05$; B, regression coefficient; CI, confidence interval

IV. DISCUSSION

In this study, we investigated the clinical and genetic characteristics according to phenotype, and the differences between apical and non-apical HCM. Our study has strength in two aspects. The first strength is that relatively higher number of cases with apical HCM were enrolled, and the second one is that whole genome sequencing of mtDNA as well as extensive sarcomere and hypertrophy inducing non-sarcomere and mitochondria related nuclear gene analysis was performed using NGS. Thirdly, genetic relevance to various phenotypic expressions, such as apical hypertrophy, mitral leaflet length, diastolic function, myocardial fibrosis and inflammation, was evaluated with novel rare variant analysis. Apical HCM has been shown with worldwide prevalence of 3-14% of HCM patients, whereas higher prevalence with 15-38% has been reported in Asian population, which means that apical HCM is an important issue in Asian HCM population.[38, 49-53] In our study, 49% (73/149) patients were apical HCM, although which is higher than to the prevalence of non-Asian population[50, 51], when considering the 45% prevalence in unselected total registry of our hospital and application of same reported diagnostic criteria, selection bios might be weak. Apical HCM is a specific variant of HCM, and it is unclear why the prevalence is different between Asian and non-Asian population. The conflicting data about the mechanism and prognosis of apical HCM means the distinct characteristics of apical HCM.[38, 53, 54] Despite some efforts to reveal genetic characteristics of apical HCM, to date, sufficient genetic data has not been established. Most of these studies identified the presence of sarcomere gene mutation.[49, 50, 55] However, there has been no established results that targeted non-sarcomere gene or mtDNA variants in apical HCM. So, we investigated whether mtDNA variant as well as non-classical sarcomere and hypertrophy inducing nuclear gene variants contribute development of apical HCM.

1. Sarcomere gene mutations

Since *MYH7*, the first gene to be associated with HCM, had been identified in 1989, further seven sarcomere genes have been identified as pathologic variants

associate with HCM.[1, 56] More than 1,500 individual mutations have been identified among ≥11 causative genes.[7] Eighty percentageof pathogenic genetic mutations are detected in *MYH7* and *MYBPC3* genes, which encode the heavy chain of β–myosin and C-binding protein of myosin.[57] The troponin complex associated genes such as *TNNT2*, *TNNI3* and *TPM1* also relatively common genes in which mutations are detected.[58] However, one recent study revealed that approximately 40% of HCM probands had a non-familial subtype.[59] In our study, the prevalence of sarcomere mutations was 36%[8] and *MYH7* and *MYBPC3* was also major gene which is consistent with the previous reports. Among sarcomere gene mutation positive patients, the prevalence of *MYH7* and *MYBPC3* mutation was 59% (20/34) in non-apical HCM and 37% (7/19) in apical HCM, which suggests different contribution to apical HCM. There was one dilated HCM patient, who had mutation at *MYPN* sarcomere gene. Myopalladin *(MYPN)* is a protein located in the Z-line and I-band, and its mutation had been detected in patients with dilated and restrictive cardiomyopathy previously as well as HCM.[12, 60, 61]

Sarcomere gene mutation-positive group was younger and had less hypertension and less male compared with sarcomere gene mutation-negative group, which means that sarcomere gene mutation roles as a disease-causing contributors. Moreover, sarcomere gene mutation-positive group had a higher LAVI and RVSP (all $p<0.05$), and showed a higher tendency of diastolic dysfunction ($p=0.098$) compared with sarcomere gene mutation-negative group. One recent study also revealed that sarcomere gene mutation-positive group was characterized by younger age, larger maximal LV wall thickness and asymmetric septal hypertrophy compared with sarcomere gene mutation-negative group.[62]

Although sarcomere gene mutations were detected with significantly lower prevalence in apical HCM (26.0%) compared to non-apical HCM (44.7%), sarcomere mutation does count for much in pathogenic mutation of apical HCM. Several reports had identified the sarcomere mutations with the various prevalence of 13-47% in apical HCM. [49, 50, 55, 63] In our study, 26% of patients revealed sarcomere gene mutation in apical HCM. The result of sarcomere mutation analysis

was consistent with previous reports in both apical HCM and non-apical HCM, and our results reminded us the sarcomere gene mutation as major contributors of HCM.

2. Rare variant contribution to phenotype of HCM

Our study is novel and first to analyze extensive nuclear genes and mtDNA genes with well-established rare variants analysis by SKAT. Contributions of nuclear gene and mtDNA gene, especially for mitochondrial function, are novel idea for HCM geno-phenotyping. Recently, R. Walsh et al. tried to determine the contribution of non-sarcomere genes to HCM.[64] They detected significantly HCM-associated rare variants in three non-sarcomere genes; *CSRP3* (Z-disc related protein), *FHL1* (X-linked, desmosome related protein) and *PLN* (calcium signaling related protein). Among them, FHL1 mutation has been shown associated with HCM in another recent report.[65] However, the novel variants in these non-sarcomere genes were rarely interpretable, and they could not identify the pathogenic role of non-sarcomere rare variants in HCM. In our study, we identified the association between rare variants and phenotype of HCM and clinical presentation such as diastolic dysfunction, and LA remodeling. Further studies such as family screening and animal study are needed to evaluate whether the rare variants work as pathogenic factors.

3. Apical HCM and non-sarcomere variant, focusing on mitochondrial genes

In this study, apical HCM was predominantly associated with mitochondrial genes and mt-DNA variants compared with non-apical HCM. Rare variants of two mitochondrial related nuclear gene (*PCCB, COQ4*) and three mtDNA gene (*MT-tRNA (s(AGY), MT-ND4 (12S), MT-RNR1*) were related to apical HCM compared to non-apical HCM. VCL gene was only one sarcomere gene associated with apical HCM. VCL is a cytoskeletal protein associated with cell-cell and cell-matrix junctions. Although VCL gene has been detected in patients with either dilated cardiomyopathy or HCM,[66-68] their pathogenicity has not been established in cardiomyopathy. PCCB, COQ4 and *MT-ND4* genes are known as coding electron transport chain. *MT-tRNA* and *MT-12S rRNA* (*MT-RNR1*) were associated to aminoglycoside inducing hearing loss. *MT-tRNA (s(AGY))* would also related to

MERRF syndrome or MELAS syndrome. In these mutations, sensory neural hearing loss and neurologic deficit and diabetes are main component of systemic involvement. COQ4 is related to biosynthesis of CoQ10, the deficiency could make various clinical conditions such as encephalomyopathy, isolated myopathy, cerebellar ataxia, and nephrotic syndrome, is reported to cause HCM.[69] *MT-ND4* is coding to electric transfer system protein NADH dehydrogenase in complex I, it could make several mitochondrial disease related to systemic sign and also related to HCM. PCCB is related to propionic academia and important enzyme called propionyl-CoA carboxylase of beta subunit. All these significant variant were not reported to development of specifies as apical HCM, according to our analysis results, these mitochondria related gene variants might be specifically more related to apical HCM. Apical HCM was more related to mitochondrial related gene variants, especially mtDNA mutation, which suggests one of entities of metabolic disease. It also suggests that apical HCM would have heterogeneous genetic cause such as classical sarcomere related mutation and mitochondrial functional related metabolic origin. Our finding also explains why sarcomere gene mutation is lower in apical HCM. Therefore, individualized genetic counselling is needed in apical HCM and treatment strategy also needs to be different from classical sarcomere gene mutation-positive HCM patients. Although now sarcomere gene mutation based ATP inhibitor was introduced and now on clinical trial,[70] our results suggest medical treatment should be individualized based on genetic study. Subset of apical HCM with mitochondrial related variants would be benefited by targeted metabolism altering treatment such as coenzyme Q10, L-carnitine, antioxidants, carnitine palmitoyl trasferase inhibitor (Perhexiline) or ranolazine. In our study, systemic involvement score was not different between apical HCM and non-apical HCM, which suggests apical HCM would not be classified into classical mitochondrial cardiomyopathy. LVOT obstruction or mid-LV obstruction was related with mainly sarcomere gene variants, which supports that dynamic obstruction is classical phenotype of HCM.[71]

4. LA enlargement, diastolic function and MV geometry according to genetic

characteristics

Major clinical presentation of HCM is diastolic dysfunction due to disturbed LV filling. LA remodeling reflects the degree of diastolic dysfunction. Although there have been not established evidence of genetic test for predicting the clinical outcome, several studies demonstrated that some mutations were associated with disease severity or/and adverse clinical outcome. Some studies reported that the presence of certain sarcomere gene mutations was related with severe phenotypes in HCM.[62, 72] There have been some reports that pathogenic sarcomere mutations are related with increased adverse outcome in HCM.[13, 14, 62] However, there had been rare studies the clinical progress according to the presence of non-sarcomere gene or mtDNA gene mutation in HCM. In our study, LA size was increased in sarcomere gene mutation group, which suggests LA remodeling is independently related to genetic factors. Although LA size is mainly determined by longstanding hemodynamic load and electrical remodeling due to atrial fibrillation, recent studies showed that genetic factor also contributes to LA size.[15, 73] In the Framingham Cohorts, rare sarcomere variants were related to increase LA dimension.[15] In our study, rare variants of specific mitochondrial related genes, both mtDNA and mitochondria related nuclear DNA (*MT-16S/RNR3, MT-ND2, MT-C/Y, P/ATT, COA5, MYOZ2*), were significantly related to LA enlargement. Moreover, one sarcomere gene (*MYPN*) was also associated with enlarged LAVI. These relations were significant even after adjustment for sex, age, history of hypertension, diabetes and eGFR, which supports genetic role for LA remodeling in HCM. Moreover, rare variants of several mitochondrial related genes (*SLC25A20, COX15, SCO2, MT-16S, MT-G*) and one non-sarcomere gene (*FHL1*) and one sarcomere gene (*MYL3*) were significantly related to diastolic dysfunction. Mitochondrial dysfunction might be related to longstanding diastolic dysfunction which causes LA dilatation. K. Unno et al. reported that mitochondria showed functional impairment and morphological disorganization in the LV of HCM patients, and its variation and disorganization were related with impaired myocardial contractile and relaxation

reserves.[74]

LAVI was not significantly different between obstructive HCM and non-obstructive HCM, while however LAVI was significantly larger in non-apical HCM than apical HCM. It would be form worse diastolic function or higher LV filling pressure in non-HCM group, genetic factors also contribute to LA remodeling as supported by higher LAVI in sarcomere gene-positive group and results of rare variant analysis. Interesting finding is mitochondria related rare variant can contribute LA enlargement, which might suggest mitochondrial dysfunction can cause energy depletion and cause diastolic dysfunction or directly influence LA myocardium. Further study using genetically modified mouse model would be needed to confirm the causality of this mitochondrial related gene for LA remodeling.

In LV geometry, mitral leaflet elongation was a unique finding of HCM, and related to LV geometry and sarcomere gene mutations, especially in non-apical HCM. Previous reports have demonstrated that abnormal elongation of AML was associated with sarcomere gene mutation in patients without left ventricular hypertrophy.[75, 76] Moreover, Captur et al reported that AML adjusted with body surface area still persisted as a predictor of the presence of sarcomere gene mutation in subclinical HCM,[77] which is consistent with our data results. However, in our study their correlation significantly attenuated after controlling for LAVI, which suggests LV and LA geometry more strongly affects leaflet length.

5. Limitations

First, validation between genetic deficiency and microstructural or functional disturbance has not been performed such as checking mitochondrial function in cases with mitochondrial gene variants, which warrant further basic study. Secondly, because whole genetic screening test was not performed in mutation-positive family members, we could not see genetic penetrance in this study.

V. CONCLUSION

In this study, the prevalence of sarcomere gene mutation was lower in apical HCM group compared with non-apical HCM group. Instead, apical HCM was more

related to rare variants of mitochondrial related gene, and it could explain the lower prevalence of known pathogenic gene compared with non-apical HCM. Several mitochondrial, non-sarcomere gene and mtDNA gene variants were related to diastolic function and LA size, which suggests genetic test based risk stratification would be possible. However, further studies should be performed to confirm the association between rare variants and pathogenesis of disease using animal HCM model to verify the effect of target gene inhibitor. Nevertherless, this study results could open genetic characteristics based approach might enable individualized risk stratification and targeted therapy.

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APPENDICES

Supplementary 1. 83 nuclear genes which were designed for comprehensive HCM specific panel

S1A. 32 sarcomere genes

*ACTC1	ACTN2	ANKRD1	BAG3	CASQ2	CAV3	CRYAB	CRSP3
JPH2	LDB3	*MYBPC3	MYH6	*MYH7	*MYL2	*MYL3	MYLK2
MYO6	MYOM1	MYOZ2	MYPN	NEXN	OBSCN	PLN	RYR2
TCAP	TNNC1	TNNC2	*TNNI3	*TNNT2	*TPM1	TTN	VCL

*8 validated sarcomere genes which are linked with HCM

S1B. 6 hypertrophy inducing non-sarcomere genes

FHL1	GAA	LAMP2	PRKAG2	PTPN11	TTR

*Presence of GLA mutation was evaluated in outside of our lab

S1C. 44 mitochondrial genes

NDUFS2	NDUFV2	SCO2	COX15	TMEM70.	AGK
MRPL3	MRPL44	MRPS22	ELAC2	AARS2	YARS2
TSFM	COQ2	COQ4	COQ9	SLC25A3	SLC25A4
NDUFA2	NDUFA10	NDUFA11	NDUFAF1	ACAD9	SDHD
SURF1	COA5	GTPBP3	MTO1	SLC22A5	CPT2
SLC25A20	ACADVL	ECHS1	NDUFS4	NDUFS8	FOXRED1
COX6B1	COX10	COA6	LRPPRC	COX14	TRMT5
PCCB	HADHB				

Supplemental 2. Comparison between obstructive HCM and non-obstructive HCM.

	Obstructive HCM (n=38)	Non-obstructive HCM (n=111)	P value
Age, years	59.1±13.3	58.7±12.7	0.862
Women, n (%)	15 (40)	28 (25)	0.102
Hypertension, n (%)	24 (63)	59 (53)	0.345
Diabetes, n (%)	8 (21)	20 (18)	0.640
Body surface area, m^2	1.72±0.21	1.79±0.19	0.063
AF, n (%)	1 (3)	14 (13)	0.062
FHx of SCD-1st, n (%)	2 (5)	7 (6)	0.581
FHx of SCD-2nd, n (%)	2 (5)	7 (6)	0.581
Syncope, n (%)	2 (5)	4 (4)	0.484
NSVT, n (%) (n=63)	4 (21)	8 (17)	0.459
5-year SCD risk, % (n=63)	2.97±1.83	2.14±1.12	0.034
Echocardiography			
LVEDD, mm	43.2±4.3	46.9±4.3	<0.001
LVESD, mm	26.9±3.2	30.1±4.2	<0.001
LV ejection fraction, %	69.3±5.9	67.1±7.7	0.101
LAV, mL	66.3±34.3	67.7±33.6	0.829
LAVI, mL/m^2	39.3±22.5	38.3±18.9	0.793
MR grade	0.57±0.42	0.46±0.26	0.134
S', cm/s	6.4±1.7	6.9±1.7	0.160
E/e'	18.0±6.4	13.3±4.3	<0.001
RVSP, mmHg	26.0±8.5	26.8±8.2	0.628
Maximal thickness, mm	20.4±3.7	18.4±3.3	0.002
CMR-index (n=54)			
LVEDV, ml	132.7±24.4	137.4±24.8	0.554
LVESV, ml	41.2±11.8	50.7±15.9	0.055

LVEF-CMR, %	69.3±6.2	63.7±8.1	0.029
LV mass, g	149.5±50.4	145.9±45.5	0.763
LV mass index, g/m^2	87.4±30.5	79.5±23.9	0.333
LV M/V ratio	1.14±0.35	1.07±0.34	0.537
Presence of LGE, n (%)	8 (27)	22 (73)	0.432
%LGE mass	7.77±8.65	5.61±8.20	0.418
Number LGE segment	4.0±5.0	2.4±2.7	0.138
Native T1, ms	1052.9±47.2	1028.5±46.4	0.117
ECV, %	30.2±5.4	30.2±4.1	0.994
T2, ms	56.9±3.9	55.1±3.1	0.108
AML-lengths			
AML –PLX, mm	32.0±4.8	32.1±4.2	0.918
AML-3CH, mm	28.0±3.2	28.4±3.7	0.518
AML-average, mm	29.9±3.5	30.3±3.5	0.567
iAML-PLX, mm/m^2	18.9±3.3	18.0±2.5	0.172
iAML-3CH, mm/m^2	16.5±2.4	16.0±2.1	0.251
iAML-average, mm/m^2	17.6±2.6	17.0±2.0	0.231

ABSTRACT (IN KOREAN)

비후성 심근증에서 근섬유분절 및 미토콘드리아 관련 유전자 변이의 다양한 기여

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정혜문

배경: 비후성 심근증은 유전 질환으로서 60%까지 병적인 핵 유전자 돌연변이가 관찰된다. 지금까지 유전적인 관심사는 주로 근섬유분절 유전자에 제한되어 있었으나, 미토콘드리아 DNA 또는 미토콘드리아 관련 핵 유전자 또한 에너지 대사 및 비후에 기여한다. 따라서 우리는 비후성 심근증의 분류에 따른 근섬유분절 및 미토콘드리아 관련 유전적인 특징을 규명하고자 하였다.

재료 및 방법: 149명의 환자가 연속적으로 연구에 등록되어 유전자 검사 및 경흉부 심초음파를 시행하였다. 82개의 핵 DNA (32개의 근섬유분절 유전자, 6개의 심근 비후와 관련된 비근섬유분절 유전자 및 44개의 미토콘드리아 관련 유전자)와 미토콘드리아 DNA 전체 유전자를 포함한 비후성 심근증 특이 패널에 대하여 차세대 염기서열 분석을 시행하였다. 비후성 심근증 환자는 두 가지 타입으로 분류되었다: 비심첨부 비후성 심근증 (76명)과 심첨부 비후성 심근증 (73명). 비후성

심근증의 분류에 영향을 미치는 적은 변이 (rare variant)의 연관성을 분석하기 위해 Sequence Kernal Association Test (SKAT) 분석을 시행하였다. 적은 변이 (rare variant)는 최근 database에서 minor allele<5.9%로 정의하였다.

결과: 149명의 환자 중 알려진 병적인 근섬유분절 변이는 35.6% (53/149)에서 발견되었고, 비심첨부 비후성 심근증 (34/76, 44.7%)에서 심첨부 비후성 심근증 (19/73, 26.0%) (p=0.026)에 비하여 유의하게 높은 빈도로 관찰되었다. 2개의 미토콘드리아 관련 핵DNA, 3개의 미토콘드리아 DNA와 1개의 근섬유분절 핵DNA의 적은 변이가 심첨부 비후성 심근증과 유의한 관련성을 보였다 (모든 p<0.05). 좌심실 유출로 폐쇄형 비후성 심근증은 5개의 근섬유분절 핵DNA와 1개의 미토콘드리아 관련 핵DNA의 적은 변이와 유의하게 관련성을 보였다 (모든 p<0.05).

결론: 심첨부 비후성 심근증은 고전적인 근섬유분절 변이 뿐만 아니라 미토콘드리아 관련 핵DNA 및 미토콘드리아 DNA의 적은 변이와 관련이 있었다. 생화학적 배경에 따른 개별화된 접근이 개별화된 위험도 평가 및 표적 치료를 가능하게 할 것이다.

핵심되는 말: 비후성 심근증, 미토콘드리아, 유전적 검사