Final Report

Project Title: Identification of Genes Causing Hereditary Cardiomyopathies in Thai Population

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Abstract

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Abstract:

Background and objective: Familial hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) are the hereditary cardiac disorder that can cause early morbidity and mortality in young people without any cardiovascular risk factors. These particular conditions are genetically heterogenous. To date, genetic information of HCM and DCM in Thai population is limited. We aimed to develop the effective high-throughput molecular strategy to detect the pathogenic variants of HCM and DCM in Thai patients, mainly to support clinical cardiovascular services and genetic counseling.

Methods: Ramathibodi inherited cardiac disease (RICD) chip was developed using next-generation sequencing (NGS)-based technology (Ion PGM™). The chip contains 72 genes causing various types of cardiomyopathies and sudden cardiac death with total 3,280 amplicons within 1,696 targets. Using genomic DNA from the patients' samples, all exon and their flanking splice junctions of the filtering gene targets for HCM and DCM was sequenced. Bioinformatic analysis was performed using minor allele frequency in 1,000-genome project and in-house exome database, protein prediction tools (SIFT and PolyPhen-2) and genetic evolution data crossing several animal species (PhastCons). Nucleotide variants obtained by NGS were classified their predicted pathogenicity based on American College of Medical Genetics and Genomics: known pathogenic, likely pathogenic, variant of unknown certain significance (VUS), likely benign and benign. The findings from NGS were subsequently confirmed by capillary sequencing.

Results: Nine HCM patients were enrolled for this study and two of them (22.22%) showed the pathogenic variants in *MYBPC3*, which was the common causative gene for this condition. One case (11.11%) revealed VUS in *TPM1*, which presented the possibility to be pathogenic by protein prediction analysis and evolution data, whereas the rest (66.67%) was unable to uncover the variants. For DCM, 17 patients were also enrolled. Three of them (17.65%) were identified for known pathogenic variants in *SCN5A*, a common gene for sudden cardiac death in Thai population. One of them (5.88%) showed a likely pathogenic variant in *TTN*, the common gene for DCM in global population. No variants were found in three cases (17.65%), while the others (58.82%) were characterized with VUS in various genes.

Conclusions: This is a preliminary study to demonstrate the molecular characterization of HCM and DCM in Thailand. NGS is proposed as an effective tool to detect pathogenic variants to facilitate risk stratification in patients and family members.

Keywords: Cardiomyopathy; hypertrophic; dilated; next-generation sequencing (NGS)

Executive summary

Hereditary cardiomyopathies are the group of disorders involving in the abnormal structure and function of the myocardium. The well-known diseases include hypertrophic and dilated cardiomyopathies that can result in sudden cardiac death and chronic congestive heart failure, respectively. More than half of these disorders are caused by monogenic origin and can be inherited in the family. To date, based on the studies in Caucasians, Japanese and Chinese, there are at least 14 genes causing hypertrophic cardiomyopathy and at least 40 genes for dilated type. Here we aim to identify the genes and mutations causing hereditary cardiomyopathies in Thai population using polymerase chain reaction and Next-generation-based amplicon sequencing technique. Genes and mutations obtained from this research will be helpful in the construction of genetic disease database in Thai population. This work aims to support clinical service in the identification of asymptomatic family members. Thus, new patients can be diagnosed earlier and receive appropriate prophylactic treatment before turning into either handicapped condition or sudden death, resulting in the improvement of standard medical care for Thai people.

Objectives:

Primary objective: To identify genes and mutations causing hereditary cardiomyopathies in Thai

Secondary objective: To develop mutation detection assay for hereditary cardiomyopathies using Next-generation sequencing-based technique

Research methodology:

Subjects

Subjects are recruited from the patients in Cardiology Clinic of Ramathibodi Hospital during 2013-2015. Informed consent was performed by the patients themselves the legal guidance/caregiver if the patient's age is less than 20 years old.

Inclusion criteria

- Hypertrophic cardiomyopathy

Total twenty-six unrelated Thai patients were diagnosed as HCM based on medical history, physical examination, 12-lead electrocardiogram, echocardiogram or magnetic resonance cardiac imaging, and other special tests if necessary. The diagnostic criteria for HCM was defined according to 2011 ACCF/AHA Guideline for the Diagnosis and Treatment of Hypertrophic American Heart Association Task Force on Practice Guidelines Cardiomyopathy: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines

- Dilated cardiomyopathy

All clinical cases of age over 15 years presented with heart failure and underwent coronary angiography in Ramathibodi Hospital were recruited. Only the patients with normal coronary angiography were included. Subsequently, echocardiography of each patient was reviewed. Patients diagnosed with either definite or suspected DCM were enrolled for the study. The criteria of DCM consist of 2 criteria: 1) the left ventricular

ejection fraction <0•45 (>2 SD) and/or fractional shortening (FS) <25% (>2 SD), as ascertained by echocardiography radionuclide scanning or angiography, 2) left ventricular end-diastolic diameter (LVEDD) >117% of the predicted value corrected for age and body surface area (3), which corresponds to 2 SD of the predicted normal limit +5%. Patients with definite DCM were fulfilled in both criteria. Suspect DCM patients include patients with clinical heart failure and LVEF <45% or FS < 25% without LVEDD from medical record. Exclusion criteria were coronary heart disease, history of chronic excess of alcohol consumption (>40 g/day for female and >80 g/day for males for more than 5 years), acute viral myocarditis, thyroid disorder, HIV, arrhythmias, systemic diseases, pericardial diseases, congenital heart disease and cor pulmonale.

Exclusion criteria

- Having identifiable causes such as congenital malformation, muscular dystrophy, coronary artery disease, hypertensive heart disease, hypothyroidism, hyperthyroidism, flank evidence of myocarditis, autoimmune disease, pertinent evidence of substance abuse and so on.

- Withdrawing informed consents

Sample collection

Ten millilires of peripheral venous blood were taken from cubital vein of each patient by well-trained nurse staffs using EDTA as an anticoagulant. Subsequent DNA extraction was done for further Next-Generation amplicon sequencing

Amplicon sequencing

Multiplexed primer pool of the entire coding sequence of the candidate genes, 14 genes for HCM and 27 genes for DCM, was designed as custom panel from the manufacturer. Target gene list for HCM and DCM was shown in tables 1 and 2. Using genomic DNA from the submitted specimen, all exons and/or their flanking splice junctions of gene regions were sequenced. Next generation sequencing was performed by Ion Proton™ semiconductor sequencer (Thermo Fisher Scientific, Inc.). The DNA sequences were assembled, aligned against reference gene sequences based on human genome build GRCh37/UCSC hg19, and analyzed for sequence variants.

Table 1. Target gene list for hypertrophic cardiomyopathy and prevalence

Gene symbol	Protein Name	OMIM	% of HCM Caused by the Mutations in This Gene	Pattern of Inheritance
MYH7	Myosin heavy chain, cardiac muscle beta isoform	160760 192600	40%	AD
MYBPC3	Myosin-binding protein C, cardiac-type	600958	40%	AD
TNNT2	Troponin T, cardiac muscle	115195	5%	AD
TNNI3	Troponin I, cardiac muscle	191044	5%	AD
TPM1	Tropomyosin 1 alpha chain	115196 191010	2%	AD
MYL2	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform	160781 608758	Unknown	AD
MYL3	Myosin light polypeptide 3	160790 608751	1%	AD
ACTC1	Actin, alpha cardiac muscle 1	102540	Unknown	AD
SRP3	Cysteine and glycine-rich protein 3, muscle LIM protein	600824	Unknown	AD
TTN	Titin	188840	Unknown	AD
ACTN2	Alpha-actinin-2	160760 192600	Unknown	AD
MYH6	Myosin heavy chain, cardiac muscle alpha isoform	160710	Unknown	AD

Gene symbol	Protein Name	OMIM	% of HCM Caused by the Mutations in This Gene	Pattern of Inheritance
TCAP	Telothonin	604488	Unknown	AD
TNNC1	Troponin C, slow skeletal and cardiac muscles	191040	Unknown	AD

Table 2. Target gene list for DCM and prevalence

Gene symbol	Protein Name	OMIM	% Caused by Mutations in This Gene	Pattern of Inheritance
ACTC1	Actin, alpha cardiac muscle 1	102540	<1%	AD
DES	Desmin	125660	<1%	AD
LMNA	Lamin-A/C	150330	7%-8%	AD
SGCD	Delta-sarcoglycan	601411	Unknown	AD
MYH7	Myosin-7	160760	5%-8%	AD
TNNT2	Troponin T, cardiac muscle	191045	2%-4%	AD
TPM1	Tropomyosin alpha-1 chain	191010	Unknown	AD
TTN	Titin	188840	Unknown	AD
VCL	Vinculin	193065	Unknown	AD
MYBPC3	Myosin-binding protein C, cardiac-type	600958	Unknown	AD
PLN	Cardiac phospholamban	172405	Unknown	AD
LDB3	LIM domain-binding protein 3	605906	Unknown	AD
ACTN2	Alpha-actinin-2	102573	Unknown	AD

Gene symbol	Protein Name	OMIM	% Caused by Mutations in This Gene	Pattern of Inheritance
CSRP3	Cysteine and glycine-rich protein 3	600824	Unknown	AD
MYH6	Myosin-6	160710	Unknown	AD
ABCC9	ATP-binding cassette transporter sub-family C member 9	601439	Unknown	AD
TNNC1	Troponin C, slow skeletal and cardiac muscles	191040	Unknown	AD
TCAP	Telethonin	604488	Unknown	AD
SCN5A	Sodium channel protein type 5 subunit alpha	600163	2%-4%	AD
EYA4	Eyes absent homolog 4	603550	Unknown	AD
TMPO	Thymopoietin	188380	Unknown	AD
PSEN1	Presenilin-1	104311	<1%	AD
PSEN2	Presenilin-2	600759	<1%	AD
FCMD	Fukutin	607440	Unknown	AD
DMD	Dystrophin	300377	Unknown	XL

Gene symbol	Protein Name	OMIM	% Caused by Mutations in This Gene	Pattern of Inheritance
TAZ	Tafazzin	30094	Unknown	XL
TNNI3	Troponin I, cardiac muscle	191044	Unknown	AR

Notes: AD, autosomal dominant; XL, X-linked; AR, autosomal recessive

Bioinformatic analysis

For quality filtering, variants that have read depths >10x coverage in single allele and >20x coverage in homozygous were selected. Each of selected variants has threshold quality score >Q40. Candidate variants of certain genes were determined using Minor Allele Frequency (MAF) $\leq$ 0.05 in the East Asian (EAS) population from 1000 Genomes Project phase III and Thai Exome database (202 exomes provided by the Center for Medical Genomics in the collaboration between Faculty of Medicine Ramathibodi Hospital, Mahidol University and the Thailand Center of Excellence for Life Science). Variant discovery analysis was performed with Ion Reporter™ Software 5.0 (Thermo Fisher Scientific, Inc.) and SNP & Variation Suite Version 8.3.4 (Golden Helix, Inc.). The interpretation of sequence variants based on HGMD professional 2015.2 release (The Human Gene Mutation Database, the Institute of Medical Genetics in Cardiff, UK), ClinVar database (National Center for Biotechnology Information, U.S. National Library of Medicine) and OMIM® database, updated 24 January 2016 (Online Mendelian Inheritance in Man®, Johns Hopkins University, USA). Data obtained from NGS were subsequently analyzed to detect the nonsynonymous deleterious mutations, protein prediction analysis and evolution analysis. Protein prediction analysis using online SIFT and Polyphen-2 softwares. Protein evolutionary conservation was analysed by PhyLoP and PhastCons databases. Variant analysis was relied on the crietria provided by the American College of Medical Genetics and Genomics (ACMG)(Tables 3 and 4). Pathogenic classification was established according to ACMG recommendation as pathogenic, likely pathogenic, variant of unknown significance, likely benign and benign (Table 5) (1).

Targeted re-sequencing

Only pathogenic and likely pathogenic variants were molecularly confirmed by Sanger sequencing.

Table 3. Criteria for classifying pathogenic variants (Modified from Richards et al., 2015)

Evidence for pathogenicity	Category
Very strong	PVS1 null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease
Strong	PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change PS2 De novo (both maternity and paternity confirmed) in a patient with the disease and no family history PS3 Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product PS4 The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls
Moderate	PM1 Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation PM2 Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium PM3 For recessive disorders, detected in *trans* with a pathogenic variant PM4 Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants PM5 Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before

	PM6 Assumed de novo, but without confirmation of paternity and maternity
Supporting	PP1 Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease PP2 Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.) PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology PP5 Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation

Table 4. Criteria for classifying benign variants (Modified from Richards et al., 2015)

Evidence of benign impact	Category
Stand-alone	BA1 Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
Strong	BS1 Allele frequency is greater than expected for disorder BS2 Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age BS3 Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing BS4 Lack of segregation in affected members of a family
Supporting	BP1 Missense variant in a gene for which primarily truncating variants are known to cause disease BP2 Observed in *trans* with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in *cis* with a pathogenic variant in any inheritance pattern BP3 In-frame deletions/insertions in a repetitive region without a known function BP4 Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.) BP5 Variant found in a case with an alternate molecular basis for disease BP6 Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation BP7 A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor

	the creation of a new splice site AND the nucleotide is not highly conserved

Table 5. Rules for combining criteria to classify sequence variants (Modified from Richards et al., 2015)

Classification	Criteria
Pathogenic	(i) 1 Very strong (PVS1) *AND* (a)≥1 Strong (PS1–PS4) *OR* (b)≥2 Moderate (PM1–PM6) *OR* (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) *OR* (d)≥2 Supporting (PP1–PP5) (ii) ≥2 Strong (PS1–PS4) *OR* (iii) 1 Strong (PS1–PS4) *AND* (a)≥3 Moderate (PM1–PM6) *OR* (b)2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR* (c)1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Likely pathogenic	(i) 1 Very strong (PVS1) *AND* 1 moderate (PM1– PM6) *OR* (ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR* (iii)1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR* (iv) ≥3 Moderate (PM1–PM6) *OR* (v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR* (vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1-PP5)
Likely benign	(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) *OR* (ii) ≥2 Supporting (BP1–BP7)
Benign	(i) 1 Stand-alone (BA1) *OR* (ii) ≥2 Strong (BS1–BS4)
Variant of unknown significance	(i)Other criteria shown above are not met OR (ii) the crietria for benign and paythogenic are contradictory

Results:

Overview of the subjects enrolled to HCM genetic study

During 2013-2015, 26 patients were registered in HCM database at Division of Cardiology, Department of Medicine, Ramathibodi Hospital. Unfornutalely, 14 cases were excluded from the study due to the stricted exclusion criteria and the final diagnosis of some cases were changed after intensive clinical review. Therefore, only 12 cases were fitted with the inclusion criteria. However, only 9 patients were enrolled to the study since the other three were failed for getting contact (Figure 1).

Overview of the subjects enrolled DCM genetic study

DCM cases were uncovered from all people receiving coronary angiography at Ramathibodi Hospital during 2013-2015. There were 120 cases fitted with clinical criteria for DCM, described as normal coronary findings with LVEF less than 45%. Of them, 93 cases were found the acquired causes; therefore, they were excluded from the study. Hence, 27 cases were eligible for the diagnosis of non-acquired DCM, including both idiopathic and familial causes. Nevertheless, only 17 cases were enrolled to the study since the rest were unable to get contact (Figure 2).

Figure 1. Overview of the subjects enrolled to HCM genetic study

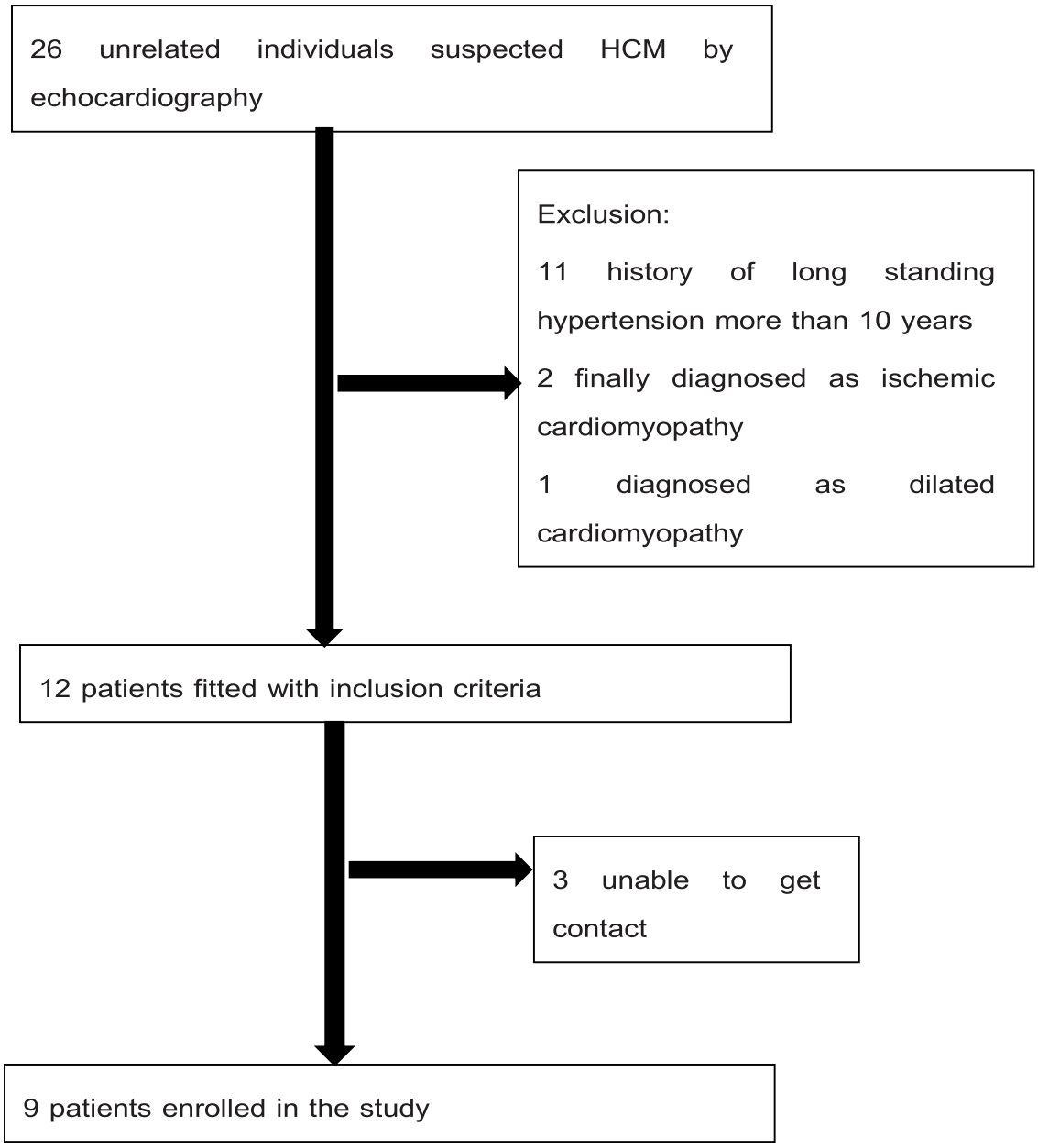
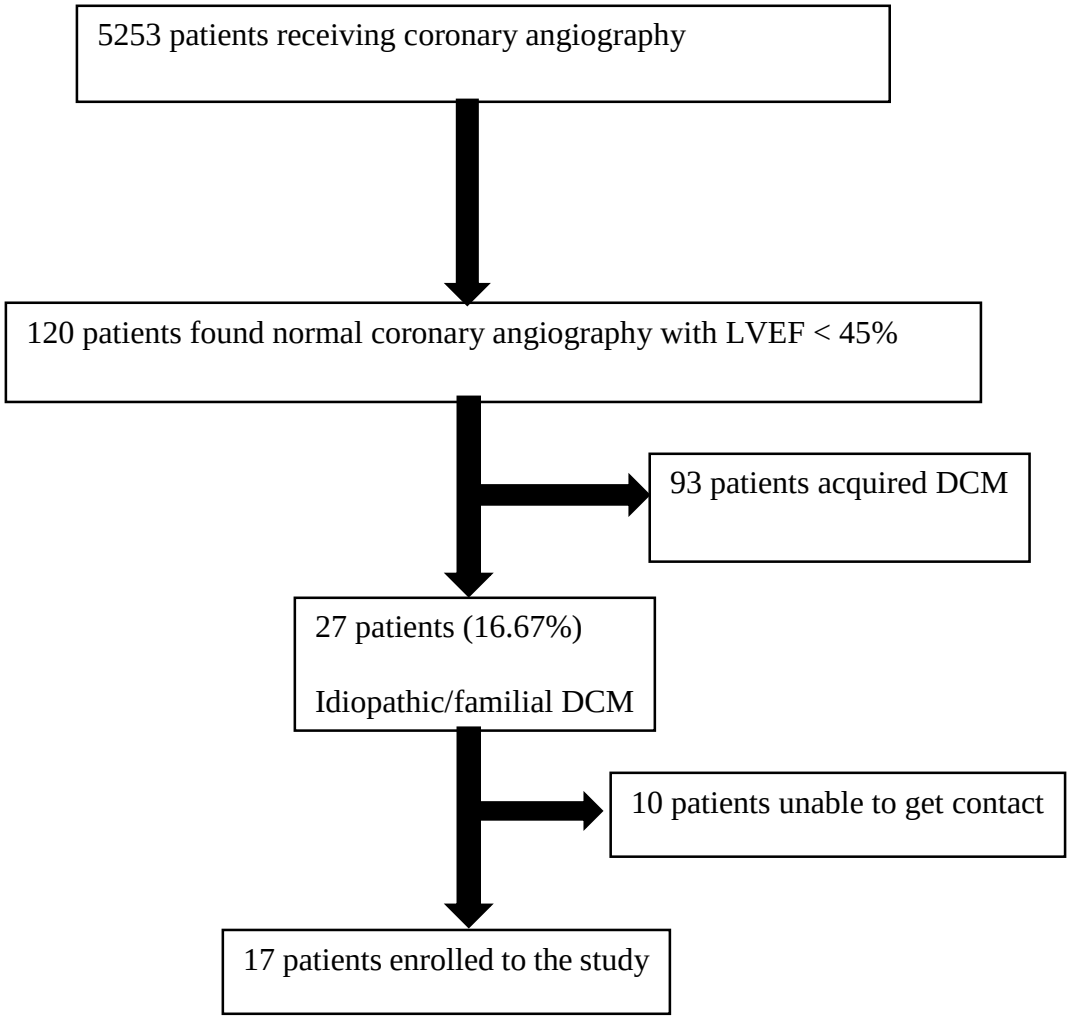


Figure 2. Overview of the subjects enrolled to DCM genetic study



Genetic findings in HCM

Relied on target gene list of HCM, NGS results revealed 2 cases (22.22%; ID #3 and #6) exhibited likely pathogenic variants in *MYBPC3* gene. One of nine (11.11%; ID #4) exhibited VUS in *TPM1*, whereas the others (66.67%) revealed negative finding (Table 6).

Genetic findings in DCM

Of 17 cases, three (17.64%; ID #12, 14 and #22) exhibited known pathogenic variants in *SCN5A*, whereas one (5.88%; ID #26) revealed likely pathogenic variant in *TTN*. No variants found in 4 cases (23.53%; ID #10, #13, #15 and #21). The others' genetic findings were classified as VUS and likely benign variant (Table 7).

Table 6. Summary of genetic variants found in subjects affected by HCM

ID	Sex /Age (yrs)	Gene	References	mRNA position	Amino acid variant	Zygosity	MAF	Classification
#1	M/65							No variants found
#2	M/57							No variants found
#3	M/49	MYBPC3	NM_000256.3	c.1522C>T	p.Gln508*	Het	0	Likely pathogenic
#4	M/27	TPM1	NM_000366.5	c.343G>A	p.Glu115Lys	Het	0	VUS
#5	F/58							No variants found
#6	F/35	MYBPC3	NM_000256.3	c.3624_3624delC	p.Lys1209Arg	Het	0	Likely pathogenic
#7	F/47							No variants found
#8	M/72							No variants found
#9	M/49							No variants found

Notes: Hom = homozygous; het = heterozygous; hem = hemizygous

Table 7. Summary of genetic variants found in subjects affected by DCM

ID	Sex	Gene	References	mRNA position	Amino acid variant	Zygosity	MAF	Classification
#10	M/75							No variants found
#11	M/43	PSEN2	NM_000447.2	c.640G>T	p.Val214Leu	Het	0	VUS
		LDB3	NM_007078.2	c.493C>T	p.Arg165Trp	Het	0	VUS
		MYH6	NM_002471.3	c.5410C>A	p.Gln1804Lys	Het	0	VUS
#12	F/45	SCN5A	NM_000335.4	c.3575G>A	p.Arg1192Gln	Het	0.011	Pathogenic
		DMD	NM_000109.3	c.3448A>G	p.Lys1150Glu	Hem	0	VUS
		MYBPC3	NM_000256.3	c.2992C>G	p.Gln998Glu	Het	0.007	VUS
#13	M/65							No variants found
#14	M/37	SCN5A	NM_000335.4	c.3575G>A	p.Arg1192Gln	Het	0.011	Pathogenic
		MYH6	NM_002471.3	c.1132G>A	p.Gly378Ser	Het	0.002	VUS
		TTN	NM_003319.4	c.7931C>T	p.Thr2644Ile	Het	0	VUS

#15	F/66							No variants found
#16	F/54	TTN	NM_003319.4	c.10753C>T	p.Arg3585Cys	Het	0	VUS
#17	M/36	DMD	NM_000109.3	c.2072C>G	p.Ala691Gly	Het	0.001	VUS
#18	M/31	ACTN2	NM_001103.2	c.1162T>A	p.Trp388Arg	Het	0	VUS
#19	M/67	MYH6	NM_002471.3	c.5410C>A	p.Gln1804Lys	Het	0	VUS
#20	M/54	MYH6	NM_002471.3	c.2774A>G	p.Asn925Ser	Het	0	VUS
#21	M/70							No variants found
#22	M/38	SCN5A	NM_000335.4	c.3575G>A	p.Arg1192Gln	Het	0.011	Pathogenic
		ACTN2	NM_001103.2	c.2425G>A	p.Val809Ile	Het	0	VUS
		VCL	NM_003373.3	c.2521G>C	p.Asp841His	Het	0	VUS
#23	M/46	MYH7	NM_000257.2	c.3157C>T	p.Arg1053Trp	Het	0	VUS
#24	M/24	LDB3	NM_007078.2	c.752A>G	p.Lys251Arg	Het	0.011	Likely benign
#25	M/28	TNNC1	NM_003280.2	c.421G>A	p.Asp141Asn	Het	0	VUS
#26	F/59	TTN	NM_003319.4	c.71731C>T	p.arg23911*	Het	0	Likely pathogenic

Notes: Hom = homozygous; het = heterozygous; hem = hemizygous

Discussion

Genetics of HCM

The level of evidence to offer genetic testing to patients affected by HCM is class A (2-5). However, it is a big surprise that the mutation detection frequency amongst HCM is significantly lower compared to the study in other populations. Mainly, genetic study of HCM yields more than 90% if the phenotype is correct. The hypothesis for this phenomenon includes 1) genetics of HCM in Thais may be in the other genes outside the panel, 2) the pathogenic variants in Thais cannot be detected by NGS, e.g., large deletion/duplication and deep intronic mutation, and 3) the phenotype screened by cardiac imaging criteria in our institute may not be accurate. However, of 2 cases with likely pathogenic variants, all of them elicits the causative gene in *MYBPC3* which is the most common found in Asian and global population (6-11). One of them reveals the nonsense mutation and the other one shows the frameshift deletion mutation. Both mutations are characterized by very strong criteria for variant classification provided by ACMG since they are both null variants in a gene where LOF is a known mechanism for HCM. *MYBPC3* function is to encode cardiac myosin-binding protein C which is arrayed transversely in sarcomere A-bands and binds myosin heavy chain in thick filaments and titin in elastic filaments (12-14). When this protein is phosphorylated, the cardiomyocyte contraction appears to be modulated (15).

In addition, there is a case exhibited VUS in *TPM1*. This variant is required for subsequent prove of its pathogenicity. In the limited chance for functional study, the

other technique to facilitate the confirmation of hypothesis is to create the familial co-segregation analysis. Nevertheless, this variant is novel and exhibits zero minor allele frequency. The protein prediction analysis and evolution database also reveals high probability for protein damage and conservation among species, respectively. Therefore, this VUS has the increased chance to be the certain pathogenic variant if familial co-segregation analysis is fitted in. TPM1 encodes the tropomyosin-alpha in which the mutation in this gene comprises 2% of patients affected by HCM (7, 10, 11, 16).

Genetics of DCM

Mainly, the level of evidence to offer genetic testing to patients affected by DCM is class B since the chance of the mutations to be found among this population is not as high as detected in HCM (2-4, 17, 18). Non-acquired DCM is estimated to be 50% of all patients presented with this particular disorder but only half of them can uncover the mutations. The most difficult part is to review the history of the previous related disease such as viral myocarditis, drug or toxin that usually leads to the either recall bias or being not able to remember. However, the overall findings of pathogenic and likely pathogenic variants in our study is approximately 24%, which is lower than previous global characterization. Three of them (17.64%) revealed the known pathogenic variant in *SCN5A* which is higher than in global prevalence which is only 2-4%. Interestingly, all of this variants in *SCN5A* has been reported as causative mutations in Brugada syndrome and sudden cardiac death in global population (19, 20). Due to the high frequency of Brugada syndrome in Thai kindred, it is hypothesized that *SCN5A*-

induced Brugada syndrome and DCM may be correlated. *SCN5A* encodes the voltage-gated sodium channel type 5 and its mutation results in the diversity of phenotype, i.e., DCM type 1E, Brugada syndrome type 1, long QT syndrome type 3, sick sinus syndrome type 1, familial ventricular fibrillation type 1, complete heart block type 1A and familial atrial fibrillation type 10 (21, 22). Most of them, except sick sinus syndrome, is inherited by autosomal dominant manner, whereas, sick sinus syndrome is defined as autosomal recessive inheritance. It is interesting to explore whether the affected case of *SCN5A*-related Brugada syndrome is fatal due to isolated arrhythmia or arrhythmia as the consequence due to cardiomyopathy. However, the minor allele frequency of these *SCN5A* variants is approximately 1% in 1-k genome database. Therefore, we propose to perform target testing of this variant in Thai population to obtain the certain allele frequency data.

One case in our study revealed the likely pathogenic variant in *TTN*. This particular variant is nonsense; therefore, it is proposed to be included in a very strong criterion for variant classification recommended by ACMG. *TTN* encodes Titin or Connectin which is a huge muscle protein expressed in cardiomyocytes and skeletal muscles that spans half of the sarcomere from Z line to M line (23). This particular protein contributes a key role in muscle assembly, force transmission at the Z line, and maintenance of resting tension in the I band region. The phenotype expressed after the mutation is heterogeneous. i.e., DCM type 1G, HCM type 9, limb-girdle muscular dystrophy type 2J, early-onset myopathy with fatal cardiomyopathy, proximal myopathy

with early respiratory muscle involvement, and tardive tibial muscular dystrophy (23-26). Nevertheless, the most common mutation found in idiopathic/familial DCM is LMNA, encoding Lamin A/C, is not found in our study (27).

Clinical implication of genetic study for HCM and DCM

Only few genetic disorders are consensus for presymptomatic diagnosis, aiming for prevention of morbidity and mortality, that is cardiovascular and cancer genetic disorders. Best clinical practice to approach people affected by HCM and DCM is to take family history through 3 generations to identify the other family members at risk. In the non-genomic era, only clinical surveillance by transthoracic echocardiography is offered to the family which is certainly tailor made relied on the family history (2, 4, 5, 22, 28-30). Currently, this work aims to support clinical cardiovascular genetic services. When the first index case of the family is characterized for the causative mutation, all of the first-degree relatives and extended members at risk can be requested for target mutation testing as inherited. Thus, new patients can be diagnosed earlier and receive appropriate prophylactic treatment before turning into either handicapped condition or sudden death, resulting in the improvement of standard medical care for Thai people. NGS-based mutation detection assay is proposed to be the effective tool to screen a number of genes simultaneously in a short period of time and the cost is definitely economical compared to developed Sanger's sequencing of numerous single gene testing basis.

Limitation of the study

The main problem of this study is the number of sample size is lower than previously expected, which is the common problem in the study of rare monogenic disorders. Hence, this study is established as a preliminary report and proposed to continue the subsequent prospective study, aiming to obtain the national genomic data for HCM and DCM in Thai population.

Conclusion

Here we developed Next-Generation sequencing-based assay to identify causing hereditary cardiomyopathies in Thai patients. Amongst patients affected by HCM, likely pathogenic variants were found in *MYBPC3* for 22.22% and 11.11% were identified as VUS in *TPM1*. Mutation detection rate in our study is much lower than global studies which is approximately 90%. For DCM study, 17.65% were identified the mutations in SCN5A which has been reported as the causative gene for Brugada syndrome, implied that the phenotype expressed by *SCN5A* mutation is heterogeneous or overlapped. The other 5.88% were identified in *TTN*, whereas the most common global prevalence such as *LMNA* was negative. The technology and genetic findings from this study is aimed to support clinical cardiovascular genetic service in Thailand, indeed.

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Outputs

1. International Journal Publication: manuscript in preparation to Journal of Human Genetics
2. Application: development of Ramathibodi Inherited Cardiac Disease (RICD) chip for NGS-based assay, provided the service by the Center of Medical Genomics and Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University
3. Others e.g. national journal publication, proceeding, international conference, book chapter, patent

- Oral presentation in the 11th Asia-Pacific Conference on Human Genetics, 16th-19th September 2015, Hanoi, Vietnam: Trachoo O, Panthan B, Jittorntam P, Phusanti S, Mukdadilok A, Srisukh S, Sae-Chew P, Charoenyingwattana A, Pasomsub E, Vathesatogkit P, Chantratita W, Tangcharoen T. Next-generation sequencing as a tool for molecular diagnosis of hypertrophic and dilated cardiomyopathies in Thai patients. Ann Transl Med 2015;3(S2):AB138.

Appendix

1. Abstract ที่ได้รับคัดเลือกให้นำเสนอเป็น oral presentation ที่ 11th Asia Pacific Conference on Human Genetics, 16th-19th September 2015, Hanoi, Vietnam
2. Review article เรื่อง Hypertrophic cardiomyopathy: from genes to bedside ตีพิมพ์ในรามาธิบดีเวชสาร Rama Med J 2014; 37: 153-160
3. Case study เรื่อง โรคหัวใจที่ถ่ายทอดทางพันธุกรรม ตีพิมพ์ในรามาธิบดีเวชสาร Rama Med J 2014; 37: 229-231
4. แผ่นพับให้ความรู้ผู้ป่วยและประชาชนเรื่องโรคหัวใจที่ถ่ายทอดทางพันธุกรรม