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Genetic investigation of point mutations in sarcomeric genes in Malaysian patients with cardiomyopathy

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ABSTRACT: Cardiomyopathy comprises a diverse group of diseases affecting the myocardium. The genetic composition is one of the major disease-defining factors of cardiomyopathies, with globally more than 100 genes implicated in this pathogenesis. Most genetic studies were performed in the Western populations, with only limited data available for the Asian populations. In this study, 152 cardiomyopathy patients (104 dilated cardiomyopathy and 48 hypertrophic cardiomyopathy) were recruited. A total of 20 genetic mutations previously reported in Caucasian populations in *MYBPC3*, *MYH7*, *TNNT2* and *TPM1* genes were examined via Tetra Primer Amplification-Refractory Mutation System Polymerase Chain Reaction approach. Of all subjects, only one patient with hypertrophic cardiomyopathy was found as heterozygous carrier of a point mutation (c.1208G>A) in the *MYH7* gene. The remaining 19 mutations were not observed among the cardiomyopathy patients in this study. Our finding suggests a different genetic architecture of the Malaysian cardiomyopathy patients compared to the Caucasian populations. Therefore, a more comprehensive mutation study on the Malaysian cardiomyopathy patients is essential for better illustration of the genetic causes of cardiomyopathy in Malaysia.

KEYWORDS: cardiomyopathy, missense variant, mutation, *MYH7*, sarcomere

INTRODUCTION

Cardiomyopathy (CMP) can be best translated as "disease of the myocardium". It is defined by the American Heart Association (AHA) as a heterogeneous group of diseases of the myocardium associated with mechanical and/or electrical dysfunction due to a variety of aetiologies that frequently are genetic [1]. In clinical practice, majority cases of diseased heart muscle are ischemic in nature that is caused by coronary artery disease. The term cardiomyopathy is however reserved for patients with diseases of the myocardium other than ischemia. Hence, non-ischaemic cardiomyopathy is commonly employed to describe the assumed non-ischaemic aetiology of the disease [2]. The AHA definition emphasizes the exclusion of myocardial diseases secondary to coronary artery disease, systemic hypertension, and valvular and congenital heart disease [1]. Various diagnostic techniques are employed for the diagnostic workup of CMP patients, including 12-lead electrocardiogram, echocardiography, cardiac magnetic resonance imaging, coronary imaging and genetic testing [3, 4].

CMP can be classified into various subtypes. The two most common subtypes of CMP are dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) [2]. DCM is characterized by abnormally dilated and thinned cardiac chambers and a reduction in the ejection fraction. Cardiac rhythm disturbances such as atrioventricular block and atrial fibrillation are commonly associated with DCM [4]. The prevalence of DCM is reported as 1 in 2500 individuals [5]. HCM is characterized by cardiac myocyte hypertrophy, with normal or increased ejection fractions. The thickening of cardiac muscles, especially at the left ventricular outflow tract, can impair the blood flow from the left ventricle to the aorta. Complications commonly associated with HCM include arrhythmias and thromboembolic events [3]. The prevalence of HCM is reported as 1 in 500 individuals [6].

Phenotypical DCM can have various causes, including infectious pathogens, drugs, toxins, autoimmune and metabolic disorders, neuromuscular diseases and genetic mutations [4]. Idiopathic DCM, where an exact aetiology cannot be established, constitutes about 50% of DCM cases [7]. On the other hand, familial DCM is assumed to account for about 30% to 48% of all DCM cases, mainly through autosomal dominant transmission. In addition, familial DCM cases were also reported to transmit in X-linked, autosomal recessive and mitochondrial-linked patterns [5].

HCM is typically a monogenic disorder with autosomal dominant inheritance pattern. While autosomal recessive and X-linked HCM cases have been reported, the occurrences are rare. About 60% of HCM cases are caused by mutations in sarcomeric genes, whereas other causes such as mitochondrial dysfunction, neuromuscular factors and drugs account for 10% of the cases [3].

There are more than 20 causative genes reported for both DCM and HCM [5]. These genes can be

categorized into two major categories: cytoskeletal and sarcomeric [4, 5]. Cytoskeletal genes are crucial in cardiomyocytes for coordination and signalling, converting mechanical signals into chemical signals (mechano-chemical signal transduction), and regulating transcription and cell differentiation [8]. Cytoskeletal genes that were reported to be highly associated with CMP occurrence include dystrophin (*DMD*), desmin (*DES*), sarcoglycan delta (*SGCD*), lamin A/C (*LMNA*) and vinculin (*VCL*) [4, 5, 8].

Sarcomeres are contractile structures present in cardiomyocytes, consisting of various components such as actin, myosin and z-disc proteins that allow contraction and relaxation of the heart [9]. The prominent CMP-associated sarcomeric genes include titin (*TTN*), myosin heavy chain 7 (*MYH7*), myosin binding protein C3 (*MYBPC3*), tropomyosin 1 (*TPM1*) and troponin T2, cardiac type (*TNNT2*). Sarcomeric mutations play a role in both DCM and HCM by affecting the contraction of cardiomyocytes and the structure of the ventricles [10]. In DCM, mutations in sarcomeric genes result in reduced contractility of cardiomyocytes, which lead to increased ventricular volumes and reduced cardiac output. In contrast, sarcomeric mutations in HCM predominantly impair relaxation of cardiomyocytes through inhibition of actin-myosin interactions [9]. Various genetic studies have been conducted to examine the spectrum of CMP-associated sarcomeric mutations in different populations [11, 12]. The common sarcomeric mutations reported are *MYBPC3* c.1504C>T, *MYH7* c.1750G>C, *TNNT2* c.281G>A and *TPM1* c.574G>A [11].

While CMP mutations were studied extensively in the West, there are limited studies reporting CMP mutations in Asia, and none has been reported for the Malaysian population [13, 14]. In the present study, a total of 20 CMP-associated sarcomeric genetic mutations identified in a previous study were examined to report the prevalence of these sarcomeric mutations in the Malaysian CMP patients [11].

MATERIALS AND METHODS

Study cohort

A total of 152 clinically confirmed non-ischemic CMP patients were recruited between 2015 and 2018 from two medical centres, Universiti Malaya Medical Centre and the National Heart Institute of Malaysia. DCM patients were included in the study, if the ejection fraction was equal or less than 35% and the following causes for a reduced ejection fraction were ruled out: (a) tachycardia induced CMP, (b) peripartum CMP, (c) ischaemic CMP in the presence of significant coronary artery disease or a history of ischemic events, (d) toxin-induced CMP, (e) ethanol induced CMP, (f) hypertension induced CMP, (g) CMP due to valvular or congenital heart disease [15]. A diagnosis of HCM was accepted when patients fulfilled the AHA echocardiographic criteria for HCM in the absence of severe uncontrolled hypertension or aortic valve diseases [16]. The study was performed according to the Declaration of Helsinki and was approved by the Medical Ethics Committee of Universiti Malaya Medical Centre (MECID. NO: 20152-1016) and Ethics Committee of the National Heart Institute of Malaysia [IJNEC NO: IJNEC/08/2015(3)]. Written informed consent was obtained from all patients. The demographic and clinical details of patients, including clinical symptoms, comorbidities, imaging results (echocardiography, cardiac MRI and coronary angiography), family history, and treatment were collected.

Blood collection and genomic DNA extraction

Venous blood was collected from the patients and a conventional phenol-chloroform extraction method was employed to extract genomic DNA from the whole blood samples [17]. The concentration and purity of the extracted DNA was determined with a Nanophotometer (Implen, Germany). The extracted genomic DNA was diluted and stored at −20 °C until use for the genetic study.

Tetra-primer amplification refractory mutation system polymerase chain reaction (T-ARMS PCR) genotyping assays

A total of 20 most dominant genetic mutations in four CMP-associated sarcomeric genes (*MYH7*, *MYBPC3*, *TPM1* and *TNNT2*) were identified from a previous publication conducted in a predominantly Caucasian population (Table 1). The selected mutations comprised of 13 pathogenic mutations, three likely pathogenic mutations and four mutations with uncertain significance based on Laboratory for Molecular Medicine Classification Criteria [11]. These mutations were examined in the Malaysian CMP patient cohort by individually designed T-ARMS PCR assays [18].

Batch Primer3 and Primer1 were used for primer design and the specificity of the primers was checked by Primer-Blast [19–21]. A total of 20 sets of primers were designed for the selected mutation points in four different sarcomeric genes: *MYH7*, *MYBPC3*, *TPM1*, and *TNNT2* (Table S1). The sizes of the internal control fragment ranged from 209 bp to 970 bp. The T-ARMS PCR assays were performed in a total volume of 10 µl containing 5 µl of 2X MyTaq™ PCR Master Mix (Bioline, UK), 0.2 µM of each inner and outer primers and 50 ng of template DNA. Thermocycling was performed in a Mastercycler Gradient Thermal Cycler (Eppendorf, Germany) by using a three-step PCR program as followed: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 65 °C (60 °C for rs199476315 and rs397516037) for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. T-ARMS PCR product of each reaction was electrophoresed on 1.5%

Table 1 Comparison of mutation frequency between Laboratory of Molecular Medicine, Oxford Molecular Genetics Laboratory and the Malaysian CMP cohort.

No	rs number (dbSNP)	cDNA change	Gene name	Gene symbol	Clinical significance	Change impact	Laboratory of Molecular Medicine, Boston, USA		Oxford Molecular Genetics Laboratory, Oxford, UK [32]		Malaysian CMP cohort in the present study	
							HCM, ($N = 2912$) n (%) [11]	DCM, ($N = 756$) n (%) [31]	HCM[a], ($N = 3200$) n (%)	DCM, ($N = 559$) n (%)	HCM, ($N = 48$) n (%)	DCM, ($N = 104$) n (%)
1	rs121913624	c.1208G>A	*Myosin Heavy Chain 7*	*MYH7*	Pathogenic	Missense	11(0.4)	0(0.0)	4(0.1)	0(0.0)	1(2.1)	0(0.0)
2	rs121913625	c.1357C>T	*Myosin Heavy Chain 7*	*MYH7*	Pathogenic	Missense	13(0.4)	0(0.0)	10(0.3)	0(0.0)	0(0.0)	0(0.0)
3	rs121913626	c.1750G>C	*Myosin Heavy Chain 7*	*MYH7*	Likely Pathogenic	Missense	22(0.8)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
4	rs121913627	c.1816G>A	*Myosin Heavy Chain 7*	*MYH7*	Pathogenic	Missense	9(0.3)	0(0.0)	13(0.4)	0(0.0)	0(0.0)	0(0.0)
5	rs371898076	c.1988G>A	*Myosin Heavy Chain 7*	*MYH7*	Pathogenic	Missense	20(0.7)	0(0.0)	17(0.5)	0(0.0)	0(0.0)	0(0.0)
6	rs121913641	c.2156G>A	*Myosin Heavy Chain 7*	*MYH7*	Pathogenic	Missense	11(0.4)	0(0.0)	1(0.03)	0(0.0)	0(0.0)	0(0.0)
7	rs121913630	c.2167C>T	*Myosin Heavy Chain 7*	*MYH7*	Pathogenic	Missense	9(0.3)	0(0.0)	4(0.1)	0(0.0)	0(0.0)	0(0.0)
8	rs3218716	c.2389G>A	*Myosin Heavy Chain 7*	*MYH7*	Pathogenic	Missense	12(0.4)	0(0.0)	24(0.8)	0(0.0)	0(0.0)	0(0.0)
9	rs121913631	c.2722C>G	*Myosin Heavy Chain 7*	*MYH7*	Pathogenic	Missense	16(0.5)	0(0.0)	5(0.2)	0(0.0)	0(0.0)	0(0.0)
10	rs145532615	c.2945T>C	*Myosin Heavy Chain 7*	*MYH7*	Unknown	Missense	11(0.4)	1(0.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
11	rs397516083	c.927–9G>A	*Myosin-Binding Protein C3*	*MYBPC3*	Likely Pathogenic	Intronic	25(0.9)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
12	rs200411226	c.1484G>A	*Myosin-Binding Protein C3*	*MYBPC3*	Unknown	Missense	10(0.3)	0(0.0)	4(0.1)	0(0.0)	0(0.0)	0(0.0)
13	rs375882485	c.1504C>T	*Myosin-Binding Protein C3*	*MYBPC3*	Pathogenic	Missense	45(1.5)	0(0.0)	59(1.8)	0(0.0)	0(0.0)	0(0.0)
14	rs397515982	c.2670G>A	*Myosin-Binding Protein C3*	*MYBPC3*	Pathogenic	Nonsense	7(0.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
15	rs387907267	c.2827C>T	*Myosin-Binding Protein C3*	*MYBPC3*	Pathogenic	Nonsense	7(0.2)	0(0.0)	11(0.3)	0(0.0)	0(0.0)	0(0.0)
16	rs193922380	c.2870C>G	*Myosin-Binding Protein C3*	*MYBPC3*	Unknown	Missense	9(0.3)	1(0.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
17	rs373746463	c.3330+5G>C	*Myosin-Binding Protein C3*	*MYBPC3*	Pathogenic	Intronic	10(0.3)	0(0.0)	6(0.2)	0(0.0)	0(0.0)	0(0.0)
18	rs397516037	c.3697C>T	*Myosin-Binding Protein C3*	*MYBPC3*	Pathogenic	Nonsense	9(0.3)	0(0.0)	4(0.1)	0(0.0)	0(0.0)	0(0.0)
19	rs121964857	c.832C>T	*Troponin T2, Cardiac Type*	*TNNT2*	Unknown	Missense	16(0.5)	1(0.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
20	rs199476315	c.574G>A	*Tropomyosin 1*	*TPM1*	Likely Pathogenic	Missense	13(0.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

[a] HCM – $N = 3267$ (c.927-9G>A; c.1484G>A; c.1504C>T; c.2670G>A; c.2827C>T; c.2870C>G; c.3330+5G>C; c.3697C>T); $N = 3191$ (c.832C>T); $N = 1535$ (c.574G>A).
[b] DCM – $N = 405$ (c.927-9G>A; c.1484G>A; c.1504C>T; c.2670G>A; c.2827C>T; c.2870C>G; c.3330+5G>C; c.3697C>T); $N = 498$ (c.832C>T); $N = 355$ (c.574G>A).

(w/v) agarose gels stained with SafeView™ FireRed (Applied Biological Materials, Canada). Visualization of the amplified bands was performed in a UV transilluminator and gel images were captured with a CCD compact gel documentation system (Major Science, USA). The presence of the mutation was determined based on the electrophoretic patterns.

Mutant construction using modified combined overlap extension polymerase chain reaction (COE-PCR)

A modified COE-PCR was employed to generate mutant constructs that served as the positive typing controls for each of the designed T-ARMS assay [22]. A total of four primers were designed for each COE-PCR, two gene-specific primers were designed using Primer-Blast while two mutagenic primers were designed using Primer-X [19]. Primer-dimer and hairpin formation of the primers were validated by OligoAnalyzer tool (Table S2) [23].

The modified COE-PCR consisted of two primary PCR reactions and a secondary PCR reaction. The primary PCR reaction for each primer pair (one gene-specific outer primer and one mutagenic primer either as gene-specific forward-mutagenic reverse combination or a mutagenic forward-gene specific reverse combination) were carried out separately. PCR was performed in a total volume of 10 μl containing 5 μl of 2X MyTaq™ PCR Master Mix (Bioline), 0.2 μM of gene-specific primer, 0.2 μM of mutagenic primer and 50 ng of template DNA. Thermocycling of the primary reactions was performed at the same conditions as T-ARMS PCR. The amplified product was visualized on 1.5% (w/v) agarose gel and purified prior to the secondary PCR reaction.

The primary PCR products for each mutant were mixed at 1:1 ratio and served as the template for the secondary PCR reaction. PCR was performed in a total volume of 40 μl containing 20 μl of 2X MyTaq™ PCR Master Mix (Bioline), 0.2 μM of gene specific primers and 2 μl of primary PCR mixed template DNA. The full-length mutagenic DNA construct was excised and gel purified with QIAquick Gel Extraction Kit (Qiagen, Germany) according to the manufacturer's protocol. Sanger sequencing was performed to confirm the mutagenic state of the DNA constructs.

Genotype validation with Sanger sequencing

The genotyping results of T-ARMS PCR assays were validated by DNA-resequencing approach. Representative samples were selected and amplified by gene-specific primer pairs used in COE-PCR that flank the respective mutation points prior to Sanger sequencing [19]. PCR amplification was performed in a total volume of 50 μl containing 25 μl of 2X MyTaq™ PCR Master Mix (Bioline), 0.2 μM of each outer primer and 250 ng of template DNA in a thermal cycler. PCR products were purified using QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's protocol. The purified PCR products were subjected to Sanger sequencing by outer primers (either forward or reverse). The DNA sequences were aligned and compared with reference gene sequences using Sequencher version 5.3 (Gene Codes Corporation, USA). The state of the mutation was identified based on electropherogram and compared to the results by T-ARMS PCR assays.

Table 2 Demographic details of cardiomyopathy patients in Malaysia ($N = 152$).

Clinical parameter	DCM ($N = 104$)	HCM ($N = 48$)
Age at diagnosis (years)	49 ± 16	54 ± 16
Gender, n (%)		
Male	76 (73.1%)	28 (58.3%)
Female	28 (26.9%)	20 (41.7%)
Ethnicity, n (%)		
Malay	53 (51.0%)	18 (37.5%)
Chinese	37 (35.6%)	19 (39.6%)
Indian	10 (9.6%)	8 (16.7%)
Others	4 (3.8%)	3 (6.3%)

Statistical analysis

Data tabulation and descriptive statistics calculations (mean and standard deviation) were performed using commercial software (Microsoft Excel, USA). G*Power software (version 3.1.9.7) was used to perform a priori analysis (sample size calculation) and post-hoc analysis (statistical power calculation) [24]. The following conditions were used for a priori analysis: 2-tailed test, d (effect size) = 0.05, α (significant level) = 0.05 and statistical power = 0.8. For post-hoc analysis, the following conditions were used: 2-tailed test, d (effect size) = 0.5, α (significant level) = 0.05, sample size group 1 (CMP samples) = 152.

RESULTS

Demographic and echocardiographic characteristics of CMP patients

The demographic and echocardiographic details of 152 CMP patients were collected and tabulated for the two major subtypes of CMP: 104 cases of DCM and 48 cases of HCM (Table 2 and Table 3). The mean age at diagnosis for HCM patients was slightly higher when compared to DCM patients (54 ± 16 years vs. 49 ± 16 years). Male gender predominated in both DCM and HCM groups (68.4% of overall cases; 73.1% DCM and 58.3% HCM). For ethnicities, Malay ethnicity had the highest number of cases in both overall and DCM (46.7% overall; 51.0% DCM and 37.5% HCM). Chinese ethnicity ranked the second highest overall and highest HCM cases (36.8% overall; 35.6% DCM and 39.6% HCM). While Indian ethnicity ranked the third highest overall cases (11.8% overall;

Table 3 Echocardiographic details of cardiomyopathy patients in Malaysia ($N = 122$).

Echocardiographic parameter†	DCM ($N = 88$)	HCM ($N = 34$)
IVSd (mm ± SD)	9.8 ± 2.4	17.1 ± 4.9
LVPWd (mm ± SD)	9.5 ± 1.9	12.7 ± 3.9
LVIDd (mm ± SD)	64.0 ± 8.0	44.8 ± 11.5
LVIDs (mm ± SD)	60.4 ± 45.8	27.2 ± 12.9
LAD (mm ± SD)	43.1 ± 8.6	41.0 ± 8.3
LVEF (% ± SD)	24.9 ± 11.1	67.9 ± 15.8

† IVSd, interventricular septal diameter at end-diastole; LVPWd, left ventricular posterior wall thickness at end-diastole; LVIDd, left ventricular internal diameter at end-diastole; LVIDs, left ventricular internal diameter at end-systole; LAD, left atrial diameter; LVEF, left ventricular ejection fraction.

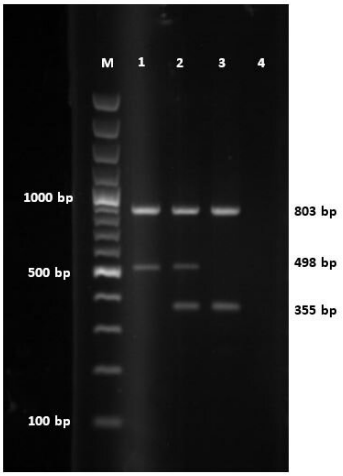

Fig. 1 Electrophoretic patterns of T-ARMS PCR assay for *MYH7* c.1208G>A on a 1.5% (w/v) agarose gel. M: 100 bp plus DNA marker (Thermo Fisher Scientific); Lane 1: homozygous G; Lane 2: heterozygous G/A; Lane 3: homozygous A; Lane 4: Non-template control (NTC).

9.6% DCM and 16.7% HCM) followed by others (4.6% overall; 3.8% DCM and 6.3% HCM). In keeping with the echocardiographic phenotype, HCM patients had higher Interventricular Septal Wall thickness at end-Diastole (IVSD), Left Ventricular Posterior Wall thickness at end-Diastole (LVPWd) and Ejection Fractions (EF) (67.9%). DCM patients had higher Left Ventricular Internal Diameter at end-Diastole and at end-Systole (LVIDd and LVIDs), larger left atrial size (LAD) and lower EF (24.9%).

T-ARMS PCR genotyping and confirmation with PCR-resequencing

Out of the 20 mutation points examined, only one mutation point (*MYH7* c.1208G>A) was observed in one HCM patient (HC19) in our cohort, who was a heterozygous carrier of the mutation (Fig. 1). All other

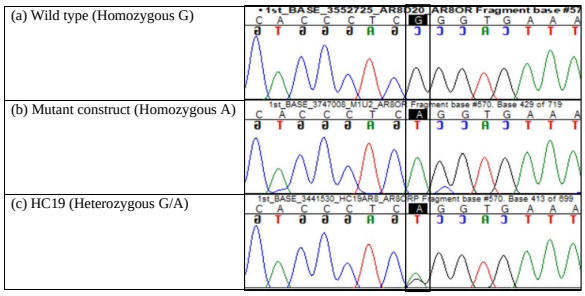

Fig. 2 Electropherograms of Sanger sequencing for the validation of *MYH7* c.1208G>A typing results. The position of *MYH7* c.1208 is indicated by the box. (a) wild type (homozygous G); (b) Mutant construct (homozygous A); (c) HC19 patient (heterozygous G/A carrier).

typed mutation points were homozygous wild type for all CMP patients. Mutant DNAs were constructed for each T-ARMS PCR genotyping assay using COE-PCR with a new set of outer and mutagenic primers (Table S2). The constructed mutants served as a homozygous mutant control for each genotyping assay, confirming the specificity of the mutant-specific primer in the genotyping assay. The genotyping result by T-ARMS PCR assays was consistent with the observation on electropherograms via Sanger sequencing, including the *MYH7* c.1208G>A mutation (Fig. 2). The selected representative samples with homozygous wild-type genotype showed a single peak for G signal on the electropherograms. On the other hand, the electropherogram of the mutant carrier exhibited a mixed peak of G and A signals while the mutant construct showed a single peak of A signal.

Case presentation of *MYH7* c.1208G>A mutant carrier

The heterozygous *MYH7* c.1208G>A mutation carrier (HC19) identified in the present study was first admitted to Universiti Malaya Medical Centre in 2011 at the age of 52 years. He was diagnosed with hypertrophic cardiomyopathy with left ventricular outflow tract obstruction (HOCM) and was clinically in New York Heart Association Class 3. There was absence of family history of sudden cardiac death for the case. He was in atrial fibrillation. The echocardiogram demonstrated severe concentric left ventricular hypertrophy with an ejection fraction of 58%. Multiple treatments had been prescribed to him during the disease course, which included beta blockers, spironolactone, and loop diuretics. In addition, he had recurrent hospitalizations for decompensated heart failure. An implantable cardioverter/defibrillator (ICD) was implanted as the patient had documented episodes of non-sustained ventricular tachycardia. The patient passed away in 2018 due to cardiogenic shock and multiorgan dys-

function.

Sample size and statistical power

A priori analysis was performed prior to the genotyping study to determine the ideal sample size with medium effect size ($d = 0.05$). Based on the calculated results, a total of 64 CMP subjects were needed to achieve 0.8 statistical power. The post-hoc analysis revealed that the current sample size ($N = 152$) had a statistical power of 0.9914.

DISCUSSION

CMP genetics are diverse, whereby more than 100 genes have been reported in causing various types of CMP, including DCM and HCM [25]. *MYH7*, *MYBPC3*, *TPM1* and *TNNT2* genes are a group of genes involved in the formation of sarcomeres in cardiomyocytes, which is the basic unit of force production in striated muscles, especially cardiac muscle cells. There are three major components in a sarcomere, i.e., thick filament (A-band), thin filament (I-band) and Z-discs (Z-band). *MYH7* and *MYBPC3* are involved in the formation of myosin (thick filament) while *TPM1* and *TNNT2* are instrumental in the formation of actin (thin filament) [26]. The *MYH7* gene has been extensively reported as one of the CMP-causing genes, especially in HCM, accounting for 14% of all cases [27]. Located on chromosome 14 and comprising of 40 exons, *MYH7* produces cardiac β-myosin heavy chain (β-MHC), which consists of intrinsic ATPase that generates energy for contracting sarcomeres [28]. *MYBPC3* mutations are mainly present in HCM, comprising 24% of all cases [27]. The gene is located on chromosome 11 and consists of 37 exons. *MYBPC3* encodes a crucial regulatory protein (cMyBP-C) that controls the interaction of the myosin head domain with the surface of thick filament, actin and titin through molecular tethering [28]. The *TPM1* gene is located on chromosome 15 and consists of 15 exons. The TPM1 proteins regulate the stability of actin and the concentration of Ca^{2+} when performing muscle contraction together with TNNT2 [29]. *TNNT2* is located on chromosome 1 and consists of 17 exons. Similar to the function of TPM1, TNNT2 is a component of the troponin complex that permits actin-myosin interaction and regulates the concentration of Ca^{2+} in sarcomere contraction [30].

A total of 20 most dominant mutations (reported > 7 times) in a predominantly Caucasian cohort (Caucasian = 62%; African American = 7%; Asian = 6%) were selected for investigation in this study [11]. These mutations present within *MYH7*, *MYBPC3*, *TNNT2* and *TPM1* genes, which form the contractile structures present within cardiomyocytes. These genes are prominent sarcomeric genes that are associated to CMP development and manifestation, in which 60% of HCM cases and 10% of DCM cases, respectively, were reported to carry mutations in these genes. Among the selected mutation points, 18 were present in the exonic region and two mutation points were present in the intronic region. All the mutation points were genotyped by T-ARMS assays for the Malaysian CMP subjects.

In the present study, only one heterozygous mutation carrier (*MYH7* c.1208G>A) was observed among the 152 Malaysian CMP patients, constituting 0.7% of the overall cohort or 2.1% of the HCM cohort. In comparison to the combined mutation frequency data of 7340 CMP patients obtained from the Laboratory of Molecular Medicine (LMM) and Oxford Molecular Genetics Laboratory (OMGL) (Table 1), the *MYH7* c.1208G>A mutation point had a lower frequency in LMM HCM cohort (0.4%) and the OMGL HCM cohort (0.1%) [11, 31]. The frequencies of the other 19 mutation points in the reference HCM cohorts ranged between 0.2% to 1.5% in the LMM HCM cohort and from 0% to 1.8% in the OMGL HCM cohort. A total of seven mutation points (*MYH7* c.1750G>C and c.2945T>C, *MYBPC3* c.927-9G>A, c.2670G>A and c.2870C>G, *TNNT2* c.832C>T and *TPM1* c.574G>A) were not found in the OMGL HCM cohort, which is consistent with the absence of those mutation points in the Malaysian cohort. The study at hand suggests that Malaysian CMP patients may have different causal mutations when compared to Caucasian populations due to unique genetic architectures within each population. Apart from that, these mutation points were found predominantly in HCM cases compared to DCM cases. None of the mutations was observed among the OMGL DCM cohort and only three mutation points (*MYH7* c.2945T>C, *MYBPC3* c.2870C>G and *TNNT2* c.832C>T) were found among the LMM DCM cohort [31, 32]. This might be due to the fact that sarcomeric mutations are the main factor for HCM manifestations, being responsible for 40% to 60% of all HCM cases. As for DCM, the manifestation are genetically more heterogenous, whereby sarcomeric mutations only accounted for 10% of all DCM cases [33].

Despite limited CMP mutation data in the Asian cohort compared to the Caucasian cohort, several previous studies have highlighted the presence of these sarcomeric mutation points in Asian cohorts. In a Chinese cohort, five *MYH7* gene mutations (c.1357C>T, c.1750G>C, c.1816G>A, c.1988G>A and c.2389G>A) were observed among 102 HCM patients [34]. *MYH7* c.1988G>A was reported with the highest frequency (3.9%) in the Chinese cohort. In a Vietnamese study, two *MYH7* gene mutations (c.1750G>C and c.2156G>A) and two *MYBPC3* gene mutations (c. 1484G>A and c.1504C>T) were observed in 104 HCM patients [35]. *MYH7* c.2156G>A and *MYBPC3* c.1504C>T were each observed in three Vietnamese patients, constituting 2.9% of the cohort. On the other hand, *MYH7* c.1357C>T and *MYBPC3* c.2870C>G were each observed in one Japanese

HCM patient, which constituted 0.8% among the 112 Japanese patients [13].

There is a plethora of mutations present within the CMP-associated genes. About 300 mutations in the *MYH7* gene have been reported in CMP patients [28, 36]. Generally, *MYH7* gene mutations affect energy production in cardiac cells. They are associated with increased ventricular wall thickness, early-onset, and more severe phenotypes, a poorer prognosis and a higher rate of premature death [37]. The *MYH7* c.1208G>A is a G to A missense mutation in exon 13 that results in the substitution of arginine to glutamine (R403Q). A functional study reported that c.1208G>A reduces actin motility in a rabbit model resulting in diminished kinetics, forces and output of myofibrils [38]. However, this mutation point in a mouse model resulted in "gain of function" in α-cardiac myosin, with improved kinetics of actin filaments [39]. This may be due to different types of myosin heavy chain isoform. Generally, humans mostly express β-cardiac myosin, which results in impaired sarcomeric function in the presence of R403Q mutation of the *MYH7* gene. In addition, it is reported that R403Q mutations may affect the stability of interacting heads motif (IHM) in myosin and increasing the number of myosin heads, causing hypercontractility in HCM patients [40]. Further functional studies are needed to elucidate the signalling pathways of the *MYH7* gene, particularly in the development of CMP.

CONCLUSION

Among the 20 mutation points studied, only one mutation carrier (*MYH7* c.1208G>A) was identified in a HCM patient among a Malaysian CMP patient cohort. However, the findings of this study might be limited by the low minor allele frequencies (< 0.1%) of the studied mutation points. Therefore, a more comprehensive mutation study on the Malaysian CMP patients should be conducted with a larger sample size with an equal subject proportion of different ethnicities to illustrate the mutational landscape of CMP in Malaysia.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2023.008.

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Appendix A. Supplementary data

Table S1 T-ARMS PCR primers for the genotyping of 20 selected sarcomeric mutation points.

Set	Gene	cDNA change	Primer	Primer sequence (5′ to 3′)	T_m (°C)	Fragment size
1	*MYH7*	c.1208G>A	Outer Forward	TGTCAAGTCATGGAGGGCCATGTCTGC	64	Common Fragment: 803 bp
			Outer Reverse	TCCCAACTCACATCGAAGATCTCGAAGCC	63	
			Inner Forward (Allele A)	AAGGGGCTGTGCCACCCGCA	68	Allele G: 498 bp
			Inner Reverse (Allele G)	TGGTGACGTACTCATTGCCCACTTTCAACC	64	Allele A: 355 bp
2	*MYH7*	c.1357C>T	Outer Forward	GCCAAGGCAGTGTATGAGAGGATGT	61	Common Fragment: 209 bp
			Outer Reverse	CCACAGCTGGCTCTAAGCAAATAGC	60	
			Inner Forward (Allele T)	ACCCTGGAGACCAAGCAGCAAT	61	Allele T: 156 bp
			Inner Reverse (Allele C)	TGTCCAGGACTCCTATGAAGTACTGTCG	61	Allele C: 103 bp
3	*MYH7*	c.1750G>C	Outer Forward	GGGGAATATAAAAATAGAAGGGGCTGA	57	Common Fragment: 725 bp
			Outer Reverse	CAGCCAGCCAATGATGTTGTAGTC	58	
			Inner Forward (Allele G)	ACCCAACATGGCACCTCCACTAG	61	Allele A: 471 bp
			Inner Reverse (Allele A)	TGGATTCTGCTCTATGGTCAATATACTGGT	59	Allele G: 307 bp
4	*MYH7*	c.1816G>A	Outer Forward	GACACTCAGTGATGCTCTCTCCTGCTTC	62	Common Fragment: 453 bp
			Outer Reverse	ACCTTGGGGTACAACCTGACATTGAAGT	62	
			Inner Forward (Allele A)	CAAGGATCCTCTCAATGAGACTGGCA	60	Allele A: 209 bp
			Inner Reverse (Allele G)	TTGAGGGAAGACTTCTGATACAAGCCAAC	60	Allele G: 299 bp
5	*MYH7*	c.1988G>A	Outer Forward	GGACCATTTTCACTCTGTCTTCTCTTCCC	60	Common Fragment: 657 bp
			Outer Reverse	GGGGGATTAAGTGGTTCTGCAGGTAAA	60	
			Inner Forward (Allele G)	TCTGAACAAGCTGATGACCAACTTTCG	59	Allele G: 398 bp
			Inner Reverse (Allele A)	AAAGTGGGGATGGGTGGCGT	62	Allele A: 306 bp
6	*MYH7*	c.2156G>A	Outer Forward	TTCCTGCTATCAATGAACTGTCCCT	58	Common Fragment: 531 bp
			Outer Reverse	TGGCTACCACCTATGATAATAGGTGTGA	59	
			Inner Forward (Allele T)	GCATCCTCTACGGGGACTTACG	59	Allele C: 230 bp
			Inner Reverse (Allele C)	GCACCCTCATACCCACCTCTTCT	61	Allele T: 346 bp
7	*MYH7*	c.2167C>T	Outer Forward	GCAGAGGTGGGTATGAGGGTGCACCAGA	67	Common Fragment: 289 bp
			Outer Reverse	TCTCCTTTCCTCACCTTGGTGTGGCCAA	65	
			Inner Forward (Allele C)	TGACTCTGGACACTTCCCTCCTCAGGTCTC	65	Allele C: 164 bp
			Inner Reverse (Allele T)	GGGATGGCCGCTGGGTTCAGGATTCA	67	Allele T: 181 bp
8	*MYH7*	c.2389G>A	Outer Forward	TGAAGGCCCGAATGTTCCACTGG	62	Common Fragment: 478 bp
			Outer Reverse	CGTACCCCTCCCTAGTCATGGCCAA	64	
			Inner Forward (Allele C)	TTCCAGCAGCTTTTTGTACTCCATTCTTGC	61	Allele C: 176 bp
			Inner Reverse (Allele T)	CCAGTCCCGAGGTGTGCGCA	65	Allele T: 352 bp
9	*MYH7*	c.2722C>G	Outer Forward	GTGCCTCTCCTTCCCTCTACCTGCAAGA	65	Common Fragment: 330 bp
			Outer Reverse	CTGCCCTTACCTTGTTCTCT GTTGCGTG	63	
			Inner Forward (Allele G)	AGATGCTGAGGAGCGCTGTGATCTGG	64	Allele G: 236 bp
			Inner Reverse (Allele C)	GGCCTCCAGCTGAATCTTGTTTTTGATGAG	61	Allele C: 149 bp
10	*MYH7*	c.2945T>C	Outer Forward	AAGACAACCTGGCAGATGCTGAG	59	Common Fragment: 650 bp
			Outer Reverse	GGCACAGATAGACATGGCATATCTAGG	58	
			Inner Forward (Allele T)	GCAGGTGAAAAACCTGACAGAGGATAT	59	Allele C: 466 bp
			Inner Reverse (Allele C)	ATGATCTCATCCAGCCCAGACG	59	Allele T: 233 bp
11	*MYBPC3*	c.927–9G>A	Outer Forward	GTGGCTACAGCTCCTTGGTC	58	Common Fragment: 329 bp
			Outer Reverse	CTATGCCCTCTCCTCTCCTG	56	
			Inner Forward (Allele A)	GGCCACAGCCTAGACTTCA	57	Allele G: 102 bp
			Inner Reverse (Allele G)	GCTTCGAGTCCCTGTGTACC	58	Allele A: 265 bp
12	*MYBPC3*	c. 1484G>A	Outer Forward	GTGTCCGCAGCTTTCCTGCCACTTCC	66	Common Fragment: 692 bp
			Outer Reverse	TGCTCCCCTACAGGGCTAGGTGGGGT	69	
			Inner Forward (Allele A)	GGCTGAAGGACGGGGTGGAGCTGACACA	69	Allele G: 500 bp
			Inner Reverse (Allele G)	TCTTGAACCGGTATTTGAAGGTCTCCTACC	61	Allele A: 250 bp
13	*MYBPC3*	c.1504C>T	Outer Forward	CCTGCTCCCCTACAGGGCTAGGT	64	Common Fragment: 520 bp
			Outer Reverse	CTGGGACCTGAGGATGTGGGAA	61	
			Inner Forward (Allele G)	TCTGCCCGTCCTTCTTGAAACG	60	Allele G: 339 bp
			Inner Reverse (Allele A)	CCGGGAGGAGACCTTCAAATCCT	61	Allele A: 226 bp

Table S1 (Continued ...)

Set	Gene	cDNA change	Primer	Primer sequence (5′ to 3′)	T_m (°C)	Fragment size
14	*MYBPC3*	c.2670G>A	Outer Forward	TTCCTTAAAGTGGCCAAAAGCCAGCTGA	62	Common Fragment: 535 bp
			Outer Reverse	CCATCGCGCCTGGCCTTAAATATGTTT	62	
			Inner Forward (Allele G)	ACACCACGGTCTCCCTCAAGGGG	65	Allele G: 356 bp
			Inner Reverse (Allele A)	CCACGCGCTCTGGGGGCAGT	68	Allele A: 222 bp
15	*MYBPC3*	c.2827C>T	Outer Forward	GGTGACACAGCCTGTGGCCTTGCCTCC	69	Common Fragment: 399 bp
			Outer Reverse	TGCCTGGCCACCCTCTCTGCACTTTTTCC	67	
			Inner Forward (Allele T)	ACGGGGGCCCGGCTGCTTTGCT	71	Allele C: 289 bp
			Inner Reverse (Allele C)	CTGCCATATTGTGTGCCCGCACGCG	67	Allele T: 157 bp
16	*MYBPC3*	c.2870C>G	Outer Forward	AGTGGGCAGTGGGAGTGGGGTGTCAGTG	69	Common Fragment: 370 bp
			Outer Reverse	CAACCCCTCCTGTCTCTGCCCAGCGTTC	68	
			Inner Forward (Allele C)	CAATATGGCAGGGCCTGGAGCCCCTGTAAC	67	Allele C:198 bp
			Inner Reverse (Allele G)	CTGCACTGTCACCGGCTCCGTGGAGC	69	Allele G:228 bp
17	*MYBPC3*	c.3330+5G>C	Outer Forward	CCCCGGCCTCAGGTGACCTGG	67	Common Fragment: 970 bp
			Outer Reverse	GCCCCAGCCCCTGGTTGGAAGAAT	66	
			Inner Forward (Allele G)	GCAGAAAGCCGACAAGAAGACCATGGTCAG	64	Allele G: 443 bp
			Inner Reverse (Allele C)	GGGGACCCCAGACCCTGCGG	68	Allele C: 577 bp
18	*MYBPC3*	c.3697C>T	Outer Forward	GCATAGTCAGGGACTCTCGT	56	Common Fragment:364 bp
			Outer Reverse	GAGGACAACGGAGCAAAG	54	
			Inner Forward (Allele C)	CCTGGACCTGGGAGAATAC	54	Allele C: 218 bp
			Inner Reverse (Allele T)	TCTCCAGAGTCAACACTCCATA	55	Allele T: 186 bp
19	*TNNT2*	c.832C>T	Outer Forward	CAGGGAACACTCTGTCCCCCTC	61	Common Fragment: 759 bp
			Outer Reverse	CTTCAGCCCACAGGTTTCCTTCTC	60	
			Inner Forward (Allele G)	CGGTGACTTTAGCCTTCCCTCG	60	Allele T: 516 bp
			Inner Reverse (Allele A)	TTCCCCTGCAGCTCCAAGAACT	61	Allele C: 287 bp
20	*TPM1*	c.574G>A	Outer Forward	CAGTGTTCCTGGAAAACCTAAACAT	56	Common Fragment: 469 bp
			Outer Reverse	TATCCACTTGGCACTTGGTTTAAAT	55	
			Inner Forward (Allele G)	CGAAAACATTAGCAAATGTGACG	53	Allele G: 314 bp
			Inner Reverse (Allele A)	TCACAGTTTTCAATTCTTCTTCAAGATT	54	Allele A: 206 bp

Table S2 Primers for DNA mutant construction using COE-PCR.

Set	Gene	cDNA change	Primer	Primer sequence (5′ to 3′)	T_m (°C)	Fragment size (bp)
1	*MYH7*	c.1208G>A	Outer Forward	AAGTTCAAGCTGAAGCAGCG	56	971
			Outer Reverse	CCTTCCCATGTCTGGTCCAC	58	
			Mutagenic Forward	CTGTGCCACCCTC**a**GGTGAAAGTGGG	65	
			Mutagenic Reverse	CCCACTTTCACC**t**GAGGGTGGCACAG	65	
2	*MYH7*	c.1357C>T	Outer Forward	CCTACCTCATGGGGCTGAAC	58	829
			Outer Reverse	TCGATGAGGTCAATGCAGGC	58	
			Mutagenic Forward	GAGACCAAGCAGCCA**t**GCCAGTACTTCATAG	63	
			Mutagenic Reverse	CTATGAAGTACTGGC**a**TGGCTGCTTGGTCTC	63	
3	*MYH7*	c.1750G>C	Outer Forward	GGGGAATATAAAAATAGAAGGGGCTGA	57	918
			Outer Reverse	GAAGTGGTGGGGTGTAGCAA	57	
			Mutagenic Forward	GATCCACTATGC**c**CGCATCGTGGACTAC	64	
			Mutagenic Reverse	GTAGTCCACGATGC**g**GGCATAGTGGATC	64	
4	*MYH7*	c.1816G>A	Outer Forward	TGCATGATGACCTCCCACAC	57	847
			Outer Reverse	TGTGGCTGCCTCAGTTTCTT	57	
			Mutagenic Forward	CTCAATGAGACTGTC**a**TGGGCTTGTATCAG	60	
			Mutagenic Reverse	CTGATACAAGCCCA**t**GACAGTCTCATTGAG	60	
5	*MYH7*	c.1988G>A	Outer Forward	GGCTCGTCCTTTCAGACTGT	57	814
			Outer Reverse	TGGGGGATTAAGTGGTTCTGC	57	
			Mutagenic Forward	GATGACCAACTTGC**a**CTCCACCCATCCC	65	
			Mutagenic Reverse	GGGATGGGTGGAG**t**GCAAGTTGGTCATC	65	
6	*MYH7*	c.2156G>A	Outer Forward	ATGTTCCACTGGGAGGGGTA	58	991
			Outer Reverse	ACCTCATCAGTGGCCCTACA	58	
			Mutagenic Forward	CATACCCACCTCTGC**t**GGAAGTCCCCGTAG	66	
			Mutagenic Reverse	CTACGGGGACTTCC**a**GCAGAGGTGGGTATG	66	
7	*MYH7*	c.2167C>T	Outer Forward	CTTCTTGCCACAGGGGTGAT	58	815
			Outer Reverse	ACCTACGTTCCAGCAGCTTT	57	
			Mutagenic Forward	CCTCCTCAGGTAT**t**GCATCCTGAACCC	62	
			Mutagenic Reverse	GGGTTCAGGATGC**a**ATACCTGAGGAGG	62	
8	*MYH7*	c.2389G>A	Outer Forward	GCGTGTGAACTCCTCCTTCA	57	840
			Outer Reverse	CAAGTTTGGCCACACCAAGG	57	
			Mutagenic Forward	GTACTCCATTCTGG**t**GAGCACACCTCGG	64	
			Mutagenic Reverse	CCGAGGTGTGCTC**a**CCAGAATGGAGTAC	64	
9	*MYH7*	c.2722C>G	Outer Forward	AAGGGCAGAGGGAAAAGAGC	58	887
			Outer Reverse	TCACCTGCCGACCAAGAATC	57	
			Mutagenic Forward	GAGCGCTGTGATCAG**g**TGATCAAAAACAAG	61	
			Mutagenic Reverse	CTTGTTTTTGATCA**c**CTGATCACAGCGCTC	61	
10	*MYH7*	c.2945T>C	Outer Forward	TGCCTCTCCTTCCCTCTACC	58	828
			Outer Reverse	ACATCCTCTAACCCTACCCCC	58	
			Mutagenic Forward	GAAAAACCTGACAGAGGAGA**c**GGCTGGGCTGGATGAGATC	68	
			Mutagenic Reverse	GATCTCATCCAGCCCAGCC**g**TCTCCTCTGTCAGGTTTTTC	68	
11	*MYBPC3*	c.927–9G>A	Outer Forward	AGGTGAGTGCCCAACATAGC	57	892
			Outer Reverse	GCCAGGCTCTTTTCTTTGCC	57	
			Mutagenic Forward	CACAGCCTAGACTGC**a**GGACACAGGGACTC	66	
			Mutagenic Reverse	GAGTCCCTGTGTCC**t**GCAGTCTAGGCTGTG	66	
12	*MYBPC3*	c. 1484G>A	Outer Forward	GGATTATCATGGGGCGCACT	58	817
			Outer Reverse	GGTCACCTCAGCATCGTCAT	57	
			Mutagenic Forward	GTGGAGCTGACCC**a**GGAGGAGACCTTC	65	
			Mutagenic Reverse	GAAGGTCTCCTCC**t**GGGTCAGCTCCAC	65	
13	*MYBPC3*	c.1504C>T	Outer Forward	CCATGTTTGCCTGTGATGGT	56	838
			Outer Reverse	TTTGAGTCCATCGGTGCCAA	57	
			Mutagenic Forward	CCTTCTTGAACC**a**GTATTTGAAGGTC	56	
			Mutagenic Reverse	GACCTTCAAATAC**t**GGTTCAAGAAGG	56	
14	*MYBPC3*	c.2670G>A	Outer Forward	TTCCTTAAAGTGGCCAAAAGCCAGCTGA	62	535
			Outer Reverse	CCATCGCGCCTGGCCTTAAATATGTTT	62	
			Mutagenic Forward	CTCCCTCAAGTG**a**CGGCCCCCAGAG	67	
			Mutagenic Reverse	CTCTGGGGGCCG**t**CACTTGAGGGAG	67	

Table S2 (Continued ...)

Set	Gene	cDNA change	Primer	Primer sequence (5′ to 3′)	T_m (°C)	Fragment size (bp)
15	*MYBPC3*	c.2827C>T	Outer Forward	GGTGACACAGCCTGTGGCCTTGCCTCC	69	399
			Outer Reverse	TGCCTGGCCACCCTCTCTGCACTTTTTCC	67	
			Mutagenic Forward	GGCTGCTTTTC**t**GAGTGCGGGCAC	65	
			Mutagenic Reverse	GTGCCCGCACTC**a**GAAAAGCAGCC	65	
16	*MYBPC3*	c.2870C>G	Outer Forward	GATTCCTATCAGAGGAGTGGGC	57	400
			Outer Reverse	CATGGACGATGGCTCCAACC	59	
			Mutagenic Forward	GAGCCCCTGTTA**g**CACCACGGAGC	66	
			Mutagenic Reverse	GCTCCGTGGTG**c**TAACAGGGGCTC	66	
17	*MYBPC3*	c.3330+5G>C	Outer Forward	GTGGACTGATGTCTCAGGGC	58	1012
			Outer Reverse	TGGCCGCTCTGTCACTAAAG	57	
			Mutagenic Forward	GAAGACCATGGTGA**c**CCCAGGGTCTGGG	67	
			Mutagenic Reverse	CCCAGACCCTGGG**g**TCACCATGGTCTTC	67	
18	*MYBPC3*	c.3697C>T	Outer Forward	GACGTTGAGCAGTCCTCTCC	58	802
			Outer Reverse	CGGTTGTACCTGCAACACAG	56	
			Mutagenic Forward	GCATGTTCAGCAAG**t**AGGGAGTGTTGACTC	62	
			Mutagenic Reverse	GAGTCAACACTCCCT**a**CTTGCTGAACATGC	62	
19	*TNNT2*	c.832C>T	Outer Forward	CTGGCCTTCCTTCCTTCCTC	58	901
			Outer Reverse	CTTCCTCCTCTGCTTCAGCC	58	
			Mutagenic Forward	CTTTAGCCTTCCCGC**a**GGTCTTGGAGCTGC	67	
			Mutagenic Reverse	GCAGCTCCAAGACC**t**GCGGGAAGGCTAAAG	67	
20	*TPM1*	c.574G>A	Outer Forward	ACTGATGGCTCGTGTGGTTT	57	876
			Outer Reverse	CAAAGAACTCGCCAGCTCAG	56	
			Mutagenic Forward	GCAAATGTGCC**a**AGCTTGAAGAAG	58	
			Mutagenic Reverse	CTTCTTCAAGCT**t**GGCACATTTGC	58	

[a] Bolded, underlined and small-lettered nucleotide in the mutagenic primer's sequence indicates mutagenic point for the COE-PCR reaction.

Table S3 Comparison of mutation frequency of 20 selected mutation points between ethnicity and CMP subtypes (DCM and HCM).

No	rs number (dbSNP)	Gene	Gene name symbol	DCM ($N = 104$)				HCM ($N = 48$)			
				Malay (n=53) (%)	Chinese (n=37) (%)	Indian (n=10) (%)	Other (n=4) (%)	Malay (n=18) (%)	Chinese (n=19) (%)	Indian (n=8) (%)	Other (n=3) (%)
1	rs121913624	*Myosin Heavy Chain 7*	*MYH7*	0.0	0.0	0.0	0.0	5.6	0.0	0.0	0.0
2	rs121913625	*Myosin Heavy Chain 7*	*MYH7*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	rs121913626	*Myosin Heavy Chain 7*	*MYH7*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	rs121913627	*Myosin Heavy Chain 7*	*MYH7*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	rs371898076	*Myosin Heavy Chain 7*	*MYH7*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	rs121913641	*Myosin Heavy Chain 7*	*MYH7*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	rs121913630	*Myosin Heavy Chain 7*	*MYH7*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	rs3218716	*Myosin Heavy Chain 7*	*MYH7*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	rs121913631	*Myosin Heavy Chain 7*	*MYH7*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	rs145532615	*Myosin Heavy Chain 7*	*MYH7*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	rs397516083	*Myosin-Binding Protein C3*	*MYBPC3*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	rs200411226	*Myosin-Binding Protein C3*	*MYBPC3*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13	rs375882485	*Myosin-Binding Protein C3*	*MYBPC3*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14	rs397515982	*Myosin-Binding Protein C3*	*MYBPC3*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	rs387907267	*Myosin-Binding Protein C3*	*MYBPC3*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	rs193922380	*Myosin-Binding Protein C3*	*MYBPC3*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17	rs373746463	*Myosin-Binding Protein C3*	*MYBPC3*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18	rs397516037	*Myosin-Binding Protein C3*	*MYBPC3*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	rs121964857	*Troponin T2, Cardiac Type*	*TNNT2*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20	rs199476315	*Tropomyosin 1*	*TPM1*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0