Next Generation Sequencing and Linkage Analysis for the Molecular Diagnosis of a Novel Overlapping Syndrome Characterized by Hypertrophic Cardiomyopathy and Typical Electrical Instability of Brugada Syndrome

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Background: Familial hypertrophic cardiomyopathy (HCM) is an autosomal dominant inherited disorder; mutations in at least 20 genes have been associated. Brugada syndrome (BrS) is an autosomal dominant inherited disorder caused by mutations mainly in the *SCN5A* gene. A new clinical entity that consists of HCM, typical electrical instability of BrS and sudden death (SD), is described.

Methods and Results: The family was constituted by 7 members, 4 of who presented clinical features of HCM and electrical instability of BrS. The clinical presentation of proband was ventricular fibrillation. All members were clinically evaluated by physical examination, 12-lead electrocardiography, 2-dimensional echocardiography, stress test, electrocardiogram Holter, flecainide test, and electrophysiological study. An integrated linkage analysis and next generation sequencing (NGS) approach was used to identify the causative mutation. Linkage with the α -tropomyosin (*TPM1*) gene on chromosome 15q22 was identified. The NGS study identified a missense mutation within the *TPM1* gene (c.574G>A; p.E192K), exactly located in a binding domain with polycystin-2 protein. No other pathogenic mutations were identified.

Conclusions: This is the first report of an association between HCM and BrS, and the first to use a combined approach of linkage and NGS to identify a causative mutation in SD. The present study expands the clinical spectrum of disorders associated with the *TPM1* gene and may be useful to report novel mechanisms of electrical instability in HCM and BrS. (*Circ J* 2016; **80**: 938–949)

Key Words: Brugada syndrome; Hypertrophic cardiomyopathy; Next generation sequencing; Sudden death

Familial hypertrophic cardiomyopathy (HCM) is the most common genetic myocardial disease, with a prevalence of 1:500.¹ It is characterized by left ventricular hypertrophy (LVH) in the absence of other loading conditions.² Genetic studies have shown that HCM is caused by >450 different mutations in at least 20 genes encoding sarcomere or sarcomere-related proteins.¹ Patients with echocardiographically documented hypertrophy showed a significantly increased risk of developing malignant arrhythmias, which accounts for a substantial component of the mortality associated with cardiac hypertrophy.³ Mechanisms underlying these arrhythmias are multifactorial, but they stem, at least in part, from altered

electrical currents due to a prolongation of ventricular action potentials. The resulting delay in the recovery of excitability, a consistent feature of ventricular hypertrophy, predisposes to early and late afterdepolarizations. Hypertrophy is also associated with myocardial fibrosis, altered electrotonic coupling between cells, slowed conduction, and dispersion of refractoriness, all of which predispose to re-entrant mechanisms of arrhythmia. Together, these “electrical remodelling” responses underlie the propensity to arrhythmia, syncope, and sudden death (SD).⁴

The Brugada syndrome (BrS) is a cardiac disorder showing variable electrocardiographic features characteristic of right

Received June 23, 2015; revised manuscript received January 18, 2016; accepted February 3, 2016; released online March 9, 2016 Time for primary review: 20 days

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ISSN-1346-9843 doi:10.1253/circj.CJ-15-0685

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Figure 1. Pedigree structure of the family. (A) Family members are identified by generations and numbers. Square, male family member; circle, female member; symbols with a slash, deceased members; closed symbols, affected members; open symbols, unaffected members; red arrow, proband. (B) Basal ECG of the proband (II-4). (C) Transthoracic echocardiography of the proband. (D) ECG after flecainide administration in the proband (II-4).

bundle-branch block, persistent ST-segment elevation in the precordial leads (V1–V3) at rest, and sudden cardiac death.⁵ Inherited as autosomal dominant with incomplete penetrance disorder, symptoms tend to manifest at an average age of 40 years.⁶ BrS is responsible for 20% of SD in people under 50 years of age with apparently structurally normal hearts. It has a significant male preponderance, but its true prevalence (1–5 in 10,000 worldwide) is difficult to quantify, not only because of its transient signature electrocardiographic signs, but also for the failure to correctly identify cases of suspected sudden arrhythmic death and the lack of subsequent family screening.⁷

The genetic abnormalities that cause BrS have been linked to mutations in the ion channel gene, *SCN5A*, which encodes for the α -subunit of the cardiac sodium channel.⁸ Many others genes have been subsequently identified^{9,10} explaining, however, only 35% of reported cases, and indicating a marked genetic heterogeneity in the pathogenesis of the BrS.

As several genes are potentially implicated in the clinical phenotype described above, the combined use of linkage analysis and a large multi-gene disease-targeted panel based on next generation sequencing (NGS) technologies could aid in the identification of causative mutations.

Taking this approach, we obtained the molecular diagnosis of a new clinical entity characterized by clinical features of HCM and BrS and caused by a mutation in the α -tropomyosin (*TPM1*) gene.

Methods

Patients

The family was constituted by 7 members, 4 of whom presented with clinical features of HCM and BrS (Figure 1A). The clinical presentation of the proband was ventricular fibrillation (VF). All family members were clinically evaluated by a review of medical history, a complete physical examination, a 12-lead ECG, a 2-dimensional echocardiographic examination, stress test and an ECG Holter. HCM was diagnosed by unexplained thickening of the left ventricular myocardium, according to international guidelines.¹¹ All individuals underwent a flecainide test (2 mg/kg IV administered as a bolus over 10 min) and an invasive electrophysiological study (EFS), according to standard methods.

Informed written consent was obtained from each family member who agreed to participate to the study.

Target Region Panel Design

We designed 2 custom panels whose content was selected to focus on functional portions of the human genome with known involvement in sudden cardiac death.

In the first design, we attempted to target all exonic regions (with 100 base pair exon padding) of 61 genes by an in-solution target enrichment (Ion TargetSeq Custom Panel; Life Technologies). The design comprised 1,299 exons, targeting 418.6 kb (Table 1). In the second design, we utilized ultra-high multiplex PCR (Ion AmpliSeq Custom Panel, Life Technologies) to analyze those regions not covered in the first panel. The design comprises 334 regions, targeting 55.18 kb. Combining these 2 panels, we managed to achieve 98% region design coverage.

Samples Preparation

DNA was isolated from peripheral blood using a Flexigene Kit (Qiagen). Genomic DNA samples were quality-checked both on a DNA NanoDrop 1000, and with the Qubit2.0 fluorometer using the Quant-IT dsDNA BR Assay (Invitrogen).

For TargetSeq library preparation, 1 μ g gDNA was used as input for fragmentation according to the manufacturer's instructions. DNA fragmentation was assessed by running the

Table 1. Targetseq and Ampliseq Analysed Genes

Gene name	Chr.	Exons	NM_number	Location/function
ABCC9	12p12.1	36	NM_020297	ATP-sensitive potassium channel
ACTC1	15q14	6	NM_005159	Sarcomere
ACTN2	1q43	21	NM_001103	Z disk
AKAP9	7q21.2	51	NM_005751	Bind ion channels on sarcolemma
ANK2	4q25-q26	46	NM_001148	Bind ion channels on sarcolemma
BAG3	10q25.2-q26.2	4	NM_004281	Anti-apoptotic activity
CACNA1C	12p13.33	47	NM_001129844	Calcium ion channel
CACNB2	10p12.32	13	NM_201596	Calcium ion channel
CALR3	19p13.11	9	NM_145046	Calcium-binding protein
CASQ2	1p13.1	11	NM_001232	Calcium storage and transport
CAV3	3p25	3	NM_033337	Sarcolemma
CSRP3	11p15.1	5	NM_003476	Z disk
DES	2q35	9	NM_001927	Intermediate filament
DSC2	18q12.1	16	NM_024422	Desmosome complex
DSG2	18q12.1	15	NM_001943	Desmosome complex
DSP	6p24	24	NM_004415	Desmosome complex
EYA4	6q23	19	NM_172105	Transcription factor
FKTN	9q31.2	10	NM_006731	Golgi-resident proteins
GPD1L	3p22.3	8	NM_015141	Regulation cardiac Na ⁺ current
HCN4	15q24.1	8	NM_005477	Na ⁺ and K ⁺ transporter
JPH2	20q13.12	6	NM_020433	Junctional membrane complexes
JUP	17q21.2	15	NM_002230	Desmosome complex
KCNE1	21q22.2	3	NM_001127670	Potassium ion channel
KCNE2	21q22.11	2	NM_172201	Potassium ion channel
KCNE3	11q13.4	3	NM_005472	Potassium ion channel
KCNH2	7q36.1	15	NM_000238	Potassium ion channel
KCNJ2	17q24.3	2	NM_000891	Potassium ion channel
KCNQ1	11p15.5	16	NM_000218	Potassium ion channel
LDB3	10q23.2	13	NM_007078	Z disk
LMNA	1q22	12	NM_170707	Nuclear lamina
MYBPC3	11p11.2	34	NM_000256	Sarcomere
MYH6	14q11.2-q12	39	NM_002471	Sarcomere

(Table 1 continued the next page.)

Gene name	Chr.	Exons	NM_number	Location/function
<i>MYH7</i>	14q11.2	37	NM_000257	Sarcomere
<i>MYL2</i>	12q24.11	7	NM_000432	Sarcomere
<i>MYL3</i>	3p21.31	7	NM_000258	Sarcomere
<i>MYOZ2</i>	4q26-q27	6	NM_016599	Z disk
<i>NEXN</i>	1p31.1	13	NM_144573	Z disk
<i>PKP2</i>	12p11	14	NM_001005242	Desmosome complex
<i>PLN</i>	6q22.31	1	NM_002667	Cardiac Ca++ ATPase
<i>PRKAG2</i>	7q36.1	16	NM_016203	AMP activated protein kinase
<i>PSEN1</i>	14q24.3	12	NM_000021	Cardiac development
<i>PSEN2</i>	1q42.12	11	NM_000447	Cardiac development
<i>RBM20</i>	10q25.2	14	NM_001134363	Regulator of mRNA splicing
<i>RyR2</i>	1q42.1-q43	105	NM_001035	Sarcoplasmic reticulum
<i>SCN1B</i>	19q13.12	6	NM_199037	Sodium ion channel
<i>SCN3B</i>	11q24.1	7	NM_018400	Sodium ion channel
<i>SCN4B</i>	11q23.3	5	NM_174934	Sodium ion channel
<i>SCN5A</i>	3p22.2	28	NM_001160161	Sodium ion channel
<i>SDHA</i>	5p15.33	15	NM_004168	Oxidative phosphorylation
<i>SGCD</i>	5q33.2	8	NM_001128209	Dystrophin associated protein
<i>SNTA1</i>	20q11.2	8	NM_003098	Membrane scaffold proteins
<i>TCAP</i>	17q12	2	NM_003673	Z disk
<i>TGFB3</i>	14q24.3	7	NM_003239	Embryogenesis and cell differentiation
<i>TMEM43</i>	3p25.1	12	NM_024334	Nuclear envelope structure
<i>TMPO</i>	12q22	4	NM_003276	Nuclear envelope structure
<i>TNNC1</i>	3p21.1	6	NM_003280	Sarcomere
<i>TNNI3</i>	19q13.42	8	NM_000363	Sarcomere
<i>TNNI2</i>	1q32.1	15	NM_000364	Sarcomere
<i>TPM1</i>	15q22.2	9	NM_001018020	Sarcomere
<i>TTN</i>	2q31	311	NM_133378	Sarcomere
<i>VCL</i>	10q22.2	21	NM_014000	Z disk

Chr., chromosome.

samples on an Agilent High Sensitivity DNA chip on a Bioanalyzer 2100 (Agilent). Each sample was barcoded by ligation of IonXpress adapters (Life Technologies) according to protocol. Libraries were size-selected with an E-Gel system (Invitrogen) to an average length of ~330bp, purified and amplified. Before hybridization with target probes, 250 ng of DNA from 2 samples were combined. Hybridization, post-hybridization washes and elution of the enriched samples was performed according to the manufacturer's instructions. The enriched library was diluted, and clonally amplified by emulsion polymerase chain reaction; beads with amplicons were enriched, loaded onto an Ion-318 chip (Life Technologies) and sequenced on the Ion-PGM (Life Technologies). Average base pair coverage was >500× for each sample.

For the Ampliseq Library, 20 ng of gDNA were used to generate the amplicon libraries. Libraries were indexed using the IonXpress Barcode Adapter Kit (Life Technologies) and quantitated using the High Sensitivity DNA Chip on the Agilent BioAnalyzer (Agilent). Appropriate dilutions were performed based on amplicon concentration at the 130–210bp range. Twenty pmol/L of individual indexed amplicon libraries were pooled for emulsion PCR and all 4 samples were sequenced on the Ion Torrent PGM platform using the Ion-316v2 chip (Life Technologies). Average base pair coverage was >400× for each sample.

To analyze the data, 2 strategies were proposed: the use of proprietary bioinformatic tools and the use of a custom pipeline.

Sanger Sequencing

TPM1 mutation was confirmed by direct sequencing, using primers designed by Primer Express software. Analysis was performed according to the ABI BigDye Terminator Cycle Sequencing protocol using a 3130xL automated sequencer (Applied Biosystems).

Results

Scene of the Crime: Clinical and Instrumental Characterization of the Family

The proband (II-4), a 22-year-old woman, was admitted to our hospital after successful resuscitation from VF cardiac arrest (**Figure 1A**). Results of examination and laboratory tests were normal. However, the 12-lead-ECG was highly suggestive for LVH; PQ and QT corrected (QTc) intervals were within normal limits (180 ms and 431 ms, respectively) (**Figure 1B**). Transthoracic echocardiography revealed severe left ventricular thickening of the interventricular septum (23 mm) without left ventricular outflow tract obstruction and with a normal systolic left ventricular function (**Figure 1C**). The patient underwent an EFS that revealed inducibility of polymorphic ventricular tachycardia (VT) with degeneration into VF (data not shown). Interestingly, intravenous flecainide caused ST-segment elevation in the inferior leads and coved-type ST-segment elevation in the right precordial leads, suggesting a link with a typical electrical instability of BrS (**Figure 1D**). According to the guidelines,¹¹ the patient underwent the

Table 2. Clinical Characteristics of the Patients Carrying Mutations in the <i>TPM1</i> Gene														
Individual	Gender	Age (years)	Clinical presentation	NYHA	Cardiac phenotype	LVH (mm)	EF	OTO	QTc (ms)	Flecainide infusion	VT/VF induction at EPS	8 years FU events	Mutation	
I:2	F	51	Asymptomatic	I	HCM	16	Normal	No	431	Unmasking Type I Brugada ECG	Positive	No	E192K	
II:2	M	28	Exercise dyspnea and palpitations	II	HCM	24	Normal	No	484	Unmasking Type I Brugada ECG	Positive	No	E192K	
II:3	M	25	Asymptomatic	I	HCM	20	Normal	No	414	Unmasking Type I Brugada ECG	Positive	No	E192K	
II:4	F	22	Aborted sudden death	I	HCM	20	Normal	No	431	Unmasking Type I Brugada ECG	Positive	VT	E192K	

ECG, electrocardiogram; EF, ejection fraction; EPS, electrophysiologic study; F, female; FU, follow up; HCM, hypertrophic cardiomyopathy; LVH, left ventricular hypertrophy; mm, maximum wall thickness; M, male; NYHA, New York Heart Association; OTO, outflow tract obstruction; QTc, corrected QT interval; VT, ventricular tachycardia; VF, ventricular fibrillation.

Figure 2. Microsatellite analysis demonstrating linkage of the *TPM1* locus on chromosome 15 and the flecainide test in affected subjects. Family members are identified by generations and numbers. Square, male family member; circle, female member; symbols with a slash, deceased members; closed symbols, affected members; open symbols, unaffected members; red arrow, proband; +, carrier of the heterozygous missense mutation; –, non carrier. During the administration of flecainide, the diagnostic type I Brugada ECG pattern appeared in the right precordial leads (V1–V3) of all affected subjects. Interestingly, the coved ST elevation also appeared in the inferior leads (DII, DIII, aVF).

Table 3. Markers for Linkage Analysis

Gene	Markers	Chr.
Titin (<i>TTN</i>)	D2S118, D2S335, D2S364, D2S2188	2q31
Troponin T type 2 (<i>TNNT2</i>)	D1S412, D1S413, D1S249, D1S2692	1q32.1
AMP-activated protein kinase γ 2 subunit (<i>PRKAG2</i>)	D7S661, D7S636, D7S483, D7S798	7q36.1
Frataxin (<i>FXN</i>)	D9S1817, D9S1874, D9S273, D9S175	9q21.11
Myosin binding protein C (<i>MYBPC3</i>)	D11S4116, D11S902, D11S4190, D11S915	11p11.2
Myosin light chain 2 (<i>MYL2</i>)	D12S1613, D12S1583, D12S1646, D12S79	12q24.11
Myosin heavy chain 6–7 (<i>MYH6</i> and <i>MYH7</i>)	D14S283, D14S990, D14S972, D14S275	14q12
α -cardiac actin (<i>ACTC1</i>)	D15S1007, D15S1040, D15S118, D15S1012	15q14
Tropomyosin α (<i>TPM1</i>)	D15S1033, D15S1036, D15S1011, D15S974, D15S997, D15S987, D15S1009, D15S1020, D15S3651, D15S213, D15S153, D15S988	15q22.1
Myotonic dystrophy protein kinase (<i>DMPK</i>)	D19S420, D19S903, D19S902, D19S904	19q13.3
Myosin light chain kinase 2 (<i>MYLK2</i>)	D20S912, D20S871, D20S195, D20S107	20q11.21
Troponin I type 3 (<i>TNNI3</i>)	D19S572, D19S418, D19S210	19q13.4
Cysteine and glycine-rich protein 3 (<i>CSRP3</i>)	D11S4116, D11S902, D11S4190, D11S915	11p15.1
Myosin light chain 3 (<i>MYL3</i>)	D3S3521, D3S3685, D3S1581, D3S1289	3p21.31
Sodium channel protein type 5 subunit α (<i>SCN5A</i>)	D3S1277, D3S3521, D3S3685, D3S1581	3p21.2
Galactosidase α (<i>GLA</i>)	DXS8077, DXS8020, DXS1106, DXS1059	Xq22

Chr., chromosome.

implantation of an automatic cardioverter defibrillator (AICD) as secondary prevention. The instrumental screening of the entire family displayed a severe left ventricular thickening of the interventricular septum in the mother (**Table 2; I-2**) and 2 brothers (**Table 2; II-2** and **II-3**) of the proband. Very intriguingly, all these subjects presented a positive response to flecainide test, and the EFS revealed inducibility of polymorphic VT with degeneration into VF (**Figure 2**). The flecainide tests in unaffected subjects were negative, as expected (data not shown).

As suggested by the guidelines¹¹ and because of the positive family history of SD in first-degree relatives, the AICD implantation was considered reasonable in primary prevention and all affected individuals underwent an AICD implantation. Interestingly, the AICD follow up revealed an intrafamilial variability in manifesting malignant arrhythmia. In fact, after 10 years of follow up, only the proband manifests sporadic episode of ventricular tachycardia terminated with antitachycardia pacing (ATP).

On the Hill of the Assassin: Molecular Analysis

Genetic counselling of the family showed a strong familiarity for SD of maternal origin, with no history of SD or syncope present from a paternal origin. Because of the overlap between HCM and BrS, we carried out segregation analysis of microsatellite markers in order to confirm or exclude putative linkage to several but more frequently candidate chromosomal regions linked to both diseases. Four microsatellite markers, 2 centromeric and 2 telomeric, were used to test the presence of linkage for each candidate locus, as reported in **Table 3**. Segregation analysis excluded evidence of linkage for all loci except for the *TPM1* locus on chromosome 15 (**Figure 2**). Successively, the linkage analysis of the *TPM1* locus was deepened using further 8 microsatellite markers (**Table 3, Figure 2**). All together, these markers, mapped on the *TPM1* locus, generated a positive logarithm of odds score value (value=0.426).

In order to characterize the genetic defect in the *TPM1* gene, to clarify the pro-arrhythmic intrafamilial variability and because of the overlapping phenotype (HCM and BrS), we

expanded genetic analysis to all known genes causing cardiomyopathies and channelopathies. For this purpose, DNA was analysed using 2 multi gene panels in which the coding regions of 61 genes were screened (**Table 1**). Each sample showed a mean coverage of 500 $\times$, with a mean uniformity of coverage 90% for the target regions. A total of 313 variants were identified by the single nucleotide polymorphism (SNP) calling procedure in the II-2 sample, 270 of which were previously reported (in dbSNP v136) and 43 were novel variants. Moreover, 297 variants (282 known, 15 novel) were identified in the II-3 sample, and 386 (346 known, 40 novel) in the II-4 sample (**Figure 3**).

Twenty-six variants are shared in the 3 samples, 25 of which fall into 15 genes, and 1 intergenic. Seven distinct variants were mapped into the gene *CALR3*, 2 into *CACNB2*, *SCN3B*, *TTN* and *VCL*, and 1 each into *ACTC1*, *DES*, *KCNH2*, *KCNJ2*, *MYH7*, *PKP2*, *RYR2*, *SCN5A*, *SDHA*, and *TPM1*. The transcript context of each of the 26 variants was estimated on all the alternative splicing variants that the involved genes can encode. Hence, the same variant can be classified multiple times and in different manners depending on the splicing isoform. We obtained 44 variants classified as intronic, 19 as synonymous, 10 as non-synonymous, and 1 falling in the 3'UTR. Only one variant in *TTN* (S21716L) and 1 found in *TPM1* (E192K) were missense.

The missense mutation (c.574G>A) (**Table 4**), which segregates with disease in the family, was identified within the coding sequence of the *TPM1* gene with either the built-in or the in-house pipeline analysis (**Figure 4A**). All the individuals carrying the mutation developed a pathological phenotype, suggesting complete penetrance of the genetic defect. The mutation was confirmed by direct Sanger sequencing (**Figure 4B**) and resulted to be absent in healthy family individuals (**Figure 4C; II-1** and **II-5**).

SNPs as Partners in Crime

Although the mutation showed a complete penetrance, only the proband displayed arrhythmias. As previously described,¹² SNPs can interact with mutations modulating cellular and clinical manifestation of cardiomyopathies and channelopathies.

With this background and in order to explain the variable expression of the disease in terms of arrhythmic events, we isolated all identified rare variants exclusively present in the proband analysed genes. Five of these were non synonymous (3 in the *TTN* gene, 2 in *TMEM43* gene), and between them, only the *TMEM43* variations (rs4685076 and rs2340917) showed a possible functional effect following in silico analysis (see [Supplementary Section](#)). *TMEM43* is a nuclear inner membrane protein; the 2 analyzed variants occur in 2 distinct consecutive exons that are part of a large protein region in the perinuclear space. The rs4685076 variant, by abolishing an exonic splicing enhancer, could impair the splicing of the exon containing it, leading to the retention of the 24 residues encoded by the domain. Similarly, the rs2340917, by creating an exonic splicing silencer, could lead to the inclusion of the mature mRNA of the exon containing it. Further speculations are difficult, because no known protein coding alternative splicing variants are currently reported in the Ensembl database.

Crime Weapon: Molecular Docking Showed That E192K in TPM1 Could Alter Interactions With a Patch of Positively Charged Residues of PKD2

In order to explain the molecular basis of the Brugada phenomenon secondary to flecainide administration in *TPM1*-mutated patients, we explored literature data on *TPM1* function and protein interaction. As previously described, *TPM1* inter-

acts with polycystin-2 protein (PKD2),¹³ an intracellular calcium channel expressed in renal epithelial cells and in cardiomyocytes, acting as modulator of intracellular calcium and cardiac function.^{14,15} Because potentially causative variants of BrS are also found in the calcium channel genes and PKD2 interacts with different sarcomeric proteins regulating calcium signalling,¹⁵ we investigated in silico the functional effect of E192K mutation on *TPM1*-PKD2 interaction. The modelling and docking procedures are described in the [Supplementary Section](#). We first modelled the 3-dimensional structure of the wild-type tropomyosin protein using the *Sus scrofa* tropomyosin to model the human tropomyosin-coiled coil homodimer. The E192 residue of each monomer is involved in electrostatic interactions with residues K189 and E192 of the opposite chain. We then verified the possibility of reconstructing the coiled coil using docking procedures. The best docking solution overlaps quite well with the modelled coiled coil, having a root mean squared deviation of 1.17Å. The residue E192 in the best docking solution is involved in electrostatic interactions with K189, E192 and K198 of the opposite monomer.

We then assessed the impact of the E192K mutation on the formation of the tropomyosin coiled coil, predicting a slight destabilizing effect ($\Delta\Delta G + 0.7$ kJ/mol) and the loss of the 2 electrostatic interactions involving residue 192, while showing 4 new interactions (with residues E187, E194, E195, and E196). None of the top 20 ranked docking solutions can be

Table 4. In Silico Analysis of the TPM1 Mutation

Chr	chr15
Start	63353922
End	63353922
Ref	G
Alt	A
Func	Exonic
Gene	TPM1
ExonicFunc	Nonsynonymous SNV
AACChange	TPM1:NM_001018008:exon5:c.G466A:p.E156K TPM1:NM_001301289:exon5:c.G466A:p.E156K TPM1:NM_001018004:exon6:c.G574A:p.E192K TPM1:NM_001018005:exon6:c.G574A:p.E192K TPM1:NM_001018007:exon6:c.G574A:p.E192K TPM1:NM_001301244:exon6:c.G574A:p.E192K
1000G ALL	—
1000G AFR	—
1000G AMR	—
1000G EAS	—
1000G EUR	—
1000G SAS	—
ExAC Freq	—
ExAC AFR	—
ExAC AMR	—
ExAC EAS	—
ExAC FIN	—
ExAC NFE	—
ExAC OTH	—
ExAC SAS	—
ESP6500si ALL	—
ESP6500si AA	—
ESP6500si EA	—
CG46	—
NCI60	—
dbSNP	rs199476315
COSMIC ID	COSM963661 COSM963660
COSMIC DIS	1
ClinVar SIG	Untested Probable-pathogenic Pathogenic Pathogenic
ClinVar DIS	Not_provided Familial_hypertrophic_cardiomyopathy_3 Left_ventricular_noncompaction_9 Cardiomyopathy
ClinVar STATUS	Nnot mult Single Single
ClinVar ID	RCV000024578.1 RCV000036627.3 RCV000054795.23 RCV000159372.1
GWAS DIS	—
GWAS OR	—
GWAS BETA	—
GWAS PUBMED	—
GWAS SNP	—
GWAS P	—
SIFT score	0
SIFT pred	D

(Table 4 continued the next column.)

Polyphen2 HDIV score	0.527
Polyphen2 HDIV pred	P
Polyphen2 HVAR score	0.184
Polyphen2 HVAR pred	B
LRT score	0.000
LRT pred	D
MutationTaster score	1.000
MutationTaster pred	D
MutationAssessor score	3.055
MutationAssessor pred	M
FATHMM score	−1.52
FATHMM pred	D
RadialSVM score	1.047
RadialSVM pred	D
LR score	0.939
LR pred	D
VEST3 score	0.967
CADD raw	3.235
CADD phred	16.84
GERP++ RS	6.17
PhyloP46way placental	2.941
PhyloP100way vertebrate	9.869
SiPhy 29way logOdds	19.868

AACChange, Amino acid change; 1000G ALL, 1000 Genome all; 1000G AFR, 1000 Genome african; 1000G AMR, 1000 Genome american; 1000G EAS, 1000 Genome East Asian; 1000G EUR, 1000 Genome europe; 1000G SAS, 1000 Genome South Asian; ExAC Freq, Exome Aggregation Consortium Frequencies; ExAC AFR, Exome Aggregation Consortium African; ExAC AMR, Exome Aggregation Consortium American; ExAC EAS, Exome Aggregation Consortium East Asian; ExAC FIN, Exome Aggregation Consortium Finnish; ExAC NFE, Exome Aggregation Consortium Non-Finnish European; ExAC OTH, Exome Aggregation Consortium Other; ExAC SAS, Exome Aggregation Consortium South Asian; ESP6500siALL, exome sequencing project si all; ESP6500siAA, exome sequencing project si African American; ESP6500siEA, exome sequencing project si European American; CG46, Complete Genomic 46 unrelated subjects; NCI60, NCI-60 tumor cell line; dbSNP, Single nucleotide polymorphism database; COSMIC ID, catalogue of somatic mutations in Cancer number; COSMIC DIS, REMOVE this line; ClinVar SIG, significance of the variant in clinical variant database; ClinVar DIS, disease associated in clinical variant database; ClinVar STATUS, REMOVE this line; ClinVar ID, variant identification number in Clinical Variant database; GWAS DIS, Genome Wide Association Studies disease; GWAS OR, Genome Wide Association Studies odds ratio; GWAS BETA, Genome Wide Association Studies beta coefficient; GWAS PUBMED, Genome Wide Association Studies pubmed link; GWAS SNP, Genome Wide Association Studies Single nucleotide polymorphism; GWAS P, Genome Wide Association Studies pubmed P-value; SIFT score, score of variant effect on protein from SIFT algorithm; SIFT pred, prediction of variant effect on protein from SIFT algorithm; D=damaging; Polyphen2 HDIV score, score of variant effect on protein from Polyphen 2 algorithm considering Mendelian disease variants vs. divergence from close mammalian homologs of human proteins; Polyphen2 HDIV pred, prediction of variant effect from Polyphen 2 algorithm considering Mendelian disease variants vs. divergence from close mammalian homologs of human proteins; P=possibly damaging; Polyphen2 HVAR score, score of variant effect on protein from Polyphen 2 algorithm considering all human variants associated with some disease (except cancer mutations) or loss of activity/function vs. common (minor allele frequency >1%) human polymorphism with no reported association with a disease of other effect; Polyphen2 HVAR pred, prediction of variant effect on protein from Polyphen 2 algorithm considering all human variants associated with some disease (except cancer mutations) or loss of activity/function vs. common (minor allele frequency >1%) human polymorphism with no reported association with a disease of other effect; B=benign; LRT score, Likelihood Ratio Test score; LRT pred, Likelihood Ratio Test prediction; D=deleterious; MutationTaster score, Mutation Taster algorithm; MutationTaster pred, Mutation Taster algorithm prediction; D=disease causing; MutationAssessor score, Mutation Assessor algorithm score; MutationAssessor pred, Mutation Assessor algorithm prediction; M=predicted functional; FATHMM score, Functional Analysis through Hidden Markov Models algorithm score; FATHMM pred, Functional Analysis through Hidden Markov Models algorithm prediction; D=damaging; RadialSVM score, RadialSVM algorithm score; RadialSVM pred, RadialSVM algorithm prediction; D=damaging; LR score, LR algorithm score; LR pred, LR algorithm prediction; D=deleterious; VEST3 score, VEST3 algorithm score; CADD raw, Combined Annotation Dependent Depletion raw value; CADD phred, Combined Annotation Dependent Depletion phred-like scores; GERP++RS, GERP++ algorithm RS score; PhyloP46way placental, PhyloP46way algorithm placental score; PhyloP46way vertebrate, PhyloP46way algorithm vertebrate score; SyPhy29way logOdds, SyPhy29way algorithm log Odds score.

Figure 4. Next generation sequencing (NGS), Sanger sequencing and molecular docking. **(A)** c.574G>A mutation in the *TPM1* gene identified by NGS sequencing in the affected sample. **(B)** Mutation confirmation of the variant by Sanger sequencing on the same DNA. **(C)** No nucleotide variation was detected in the control DNA. **(D)** Molecular docking of the PKD2-TPM1 interaction. **(Left)** Ribbon representation of the modelled coiled coil interaction between PKD2 (red) and the wild-type TPM1 (green). Highlighted are the E192 TPM1 residue, and the arginine residues of PKD2 with which it can establish electrostatic interactions. **(Right)** Ribbon representation of the modelled coiled coil interaction between PKD2 (red) and the E192K TPM1 mutant (yellow). Highlighted are the K192 and the 3 PKD2 arginine residues that can form destabilizing interactions.

well superimposed to the native coiled coil structure; all have a (usually large) positive energy and the pattern of electrostatic interactions is disrupted in all examined dockings. These results suggest that the E192K mutation can have a profound impact on the establishment of the correct tropomyosin homodimeric coiled coil quaternary structure.

We tested whether the E192K tropomyosin mutant can establish a successful interaction with the polycystin-2 (PKD2) gene product. Interaction between TPM1 and PKD2 was experimentally verified¹³ and ascribed to the 821–878 region of PKD2 and to the 152–196 region of TPM1. The E192 residue therefore falls within this region. PKD2 is a large transmembrane protein in which the region 838–926 is reported as coiled coil in the UniProt entry (UniProt AC Q13563). This suggests the formation of a heterodimeric coiled coil interaction between PKD2 and the TPM1 interaction domain. PKD2 was modelled using the C-terminal coiled coil domain of the human transient receptor potential channel subfamily P member 2 (TRPP2), a member of the polycystin family. The model covers the PKD2 residues from 833 to 895, corresponding to a large portion of the TPM1 interacting region. The best docking solution between PKD2 and the wild type TPM1 has the lowest energy (−73.57 kJ/mol) and the largest number of electrostatic interactions (n=39), two of which involve residue E192 (with R845 and R848 of PKD2) (**Figure 4D Left**). When the modelled structure of the TPM1 E192K mutant was subjected to docking with the PKD2 model, the best solution has an estimated energy of −30.7 kJ/mol and a larger number of electrostatic interactions when compared with the best solution obtained using the native TPM1 model (57 vs. 39), but also a larger number of unfavourable electrostatic interactions (53 vs. 31). In particular, residue 192 is involved in 3 unfavourable interactions, with a patch of positively charged residues of PKD2 (R844, R845 and R848) that were instead stabilizing the interaction in the native TPM1 (**Figure 4D Right**). These data support the hypothesis that the E192K mutation in TPM1 might alter interactions with a patch of positively charged residues of PKD2. The lack of TPM1 interaction with PKD2 protein, as described for other sarcomeric proteins, could result in perturbation of calcium ion flux.

Discussion

From a genetic point of view, this is the first report combining linkage analysis and NGS for the identification of a genetic defect causing SD. The identification of causative genes or those that act synergistically to contribute to an increase in the risk of a disease is one of the aims of medical genetics. Genetic testing in patients with HCM provides prognostic and diagnostic benefits and can substantially assist the clinical management of index cases and family members. Detection of a mutation in an index case increases the probability of diagnosis of uncertain cases in the same family without diagnostic clinical features. Genetic analysis has prognostic value in certain index cases, but generally, there are significant obstacles to accurate genetic prognostication. These obstacles include: (1) the existence of many ‘private’ mutations with unknown functional characteristics; (2) the variable disease severity, seen even among patients carrying identical mutations; and (3) the genetic and allelic heterogeneity.

Role of NGS in Detecting Mutations Causing Overlapping Phenotypes

Both diseases, HCM and BrS, are a clinically and genetically heterogeneous group of pathologies; over 450 mutations in 20

sarcomere-related and myofilament-related genes have been identified in HCM.^{1,16} Mutations in the genes for myosin-heavy chain (*MYH7*), myosin-binding protein C (*MYBPC3*), and cardiac troponin I (*TNNI3*) and troponin T (*TNNT2*) account for the majority of reported genotyped cases.^{17–25} Regarding BrS mutations, the first was described in 1998 in the *SCN5A* gene. Since then, more than 350 pathogenic mutations have been identified in 16 genes encoding mainly subunits of cardiac sodium, potassium, and calcium channels, or genes involved in the trafficking/regulation of these channels.²⁶ To complicate the genetic diagnosis of HCM and BrS is the allelic heterogeneity. Mutations in a variety of sarcomeric genes lead to pleiotropic cardiac phenotypes ranging from HCM to dilated cardiomyopathy (DCM); each *TNNI3* mutation can be associated with diverse morphologies, and with both hypertrophic and restrictive cardiomyopathy, within the same family.^{27,28}

Pathogenic mutations in the *SCN5A* gene have been associated with several diseases, such as Long QT Syndrome (LQTS), Atrial Fibrillation (AF), Sick Sinus Syndrome (SSS), Progressive Conduction Cardiac Disease (PCCD) and Sudden Infant Death Syndrome (SIDS).^{29,30} Despite most of these diseases belonging to the group of channelopathies without structural heart alterations, *SCN5A* mutations have also been reported in association with DCM.³¹ In this scenario, novel technical approaches, such as NGS, promise to transform clinical practice. The major current application of NGS in diagnostics is through disease-targeted tests using single genes or multi-gene disease-targeted panels. The technology is convenient when applied to disorders for which both allelic and locus heterogeneity are substantial, because it dramatically increases clinical sensitivity allowing an analysis that would have been impossible to do using the traditional Sanger sequencing method. By this genotyping method, it is possible to analyse a very large number of genes simultaneously and even the entire genome quickly, and at a reasonable cost. However, the amount of data generated by this method is considerable and this makes the process of interpretation very difficult. To overcome these difficulties, our approach was to combine a classical linkage analysis with NGS technologies to guide the analysis and interpretation of NGS-identified variations. This approach was recently described to be a successful strategy for the identification of causative mutations.³² Here, it allowed us to rapidly obtain the molecular diagnosis of the present overlapping syndrome characterized by clinical features of HCM and BrS caused by mutation in the α -tropomyosin (*TPM1*) gene.

TPM1 is a member of the tropomyosin family of highly conserved, widely distributed actin-binding proteins. Defects in the *TPM1* gene are the cause of familial HCM type 3²⁸ (CMH3 [MIM:115196]), DCM type 1Y (CMD1Y [MIM:611878])³³ and left ventricular non-compaction 9 (LVNC9 [MIM:611878]).³⁴ Several mutations in *TPM1* have been reported as causative of HCM.

The described E192K mutation occurs in exon 6 and segregates in all affected members. It consists in a missense mutation inducing a non-conservative change (*Glu* to *Lys*) within a highly conserved region ranging from amino acid 166 to 207, and considered essential for the binding to actin,^{13,35} in addition to lying near the calcium-dependent troponin-T binding domain. Mutations occurring within the tropomyosin-binding domain of cardiac troponin T and altering the charge of the residue have been already reported as causative of HCM, because critical for cooperative actin binding and regulatory function.³⁶

The E192K mutation was first described by Probst et al³⁴ in a family with LVNC, but has never been associated with the

Brugada phenomenon. Our data confirm that the mutation, seen as associated with the 2 clinically distinct entities, HCM and LVNC, confers a malignant phenotype with complete penetrance and variable expressivity.

Regarding the variable expressivity of the disease in terms of arrhythmic events, only the proband manifested episodes of VT. In our study, we failed to find a genetic explanation of this variability using the identified SNPs as partner in crime. However, this variability cannot be explained solely by the genetic defects involved or the type of mutation found. Disease manifestation is likely to be influenced by sex, other genetic factors, polymorphisms and modifier genes, and environmental factors such as lifestyle, degree of physical exercise and blood pressure.

From a clinical point of view, the present study describes, for the first time, an association between HCM, SD and flecainide-induced ST-segment elevation in the right precordial and inferior leads. This association was observed in all affected members carrying the c.574G>A *TPM1* mutation. Our data support and expand the previous evidence that the Brugada phenomenon can be demonstrated under sodium channel blocker provocation in patients with LQT3 syndrome, ARVD/C with structural epi-endocardial heterogeneity, and in typical BrS without structural heart disease. In the present study, the genetic screening excluded mutations or SNPs in genes most frequently associated with BrS. However, even if several pathogenic mutations in 16 genes have been associated with BrS, all these genes account only for 35% of total cases. It is noteworthy that the Brugada phenomenon observed in the proband could be due to mutations/SNPs present inside genes that are still unknown to be associated with BrS. Nevertheless, it is likely that Brugada phenomenon could be generated by alterations in the action potentials not directly connected to the genetic defect responsible for the syndrome. Thus, the lack of interaction between sarcomeric proteins and proteins regulating ion channel activity will likely trigger the Brugada phenomenon.

In the present study, the altered interaction between TPM1 and PKD2 proteins, demonstrated by molecular docking, might influence the calcium ion flux thus altering the transmembrane electric potential. This effect becomes more evident on ECG when a sodium channel blocking drug is used. In this scenario, the flecainide/ajmaline-induced Brugada phenomenon might represent a marker of electrical instability that might be useful to unmask and stratify subjects carrying mutations at high risk to develop arrhythmia and SD.³⁰ Moreover, our study confirms the previous evidence that sodium channel blocker drug-induced ST-segment elevation in peripheral leads is an independent predictor for a malignant arrhythmic events.

Recently, Haruki et al.³⁷ reported that oral flecainide administration may be considered a useful therapy to improve left ventricular pressure gradient and symptoms in patients with obstructive HCM, particularly for those with disopyramide-induced vagolytic side effects. Although the authors did not observed any flecainide-induced Brugada phenomenon or pro-arrhythmic events in treated patients, their data must be viewed with caution given the small number of patients enrolled and in light of our data. Therefore, further studies are needed to assess the safety of flecainide therapy in patients with symptomatic obstructive HCM, and the role of the flecainide test in unmasking and stratify HCM subjects at high risk to develop SD.

Conclusions

There are 3 main features of our study: (1) the discovery of the disease-causing variant of a novel overlapping syndrome characterized by HCM, typical electrical instability of BrS and SD; (2) these results were obtained combining together linkage and target resequencing analysis; and (3) the ajmaline or flecainide test, if confirmed in larger cohort of patients with cardiomyopathies, might be useful to unmask and stratify subjects at high risk of developing arrhythmia.

Finally, the multidisciplinary approach used in the study is an example of molecular medicine. Combining the expertise of a cardiologist, geneticist, molecular biologist and a specialist in bioinformatics has been shown to be fundamental in obtaining a rapid characterization of the genetic defect, and a molecular explanation of the clinical evidence with possible important future clinical implications.

Used appropriately, genetic analysis can have an important role in the prognosis, genetic counselling and clinical management of patients with HCM and their families.

Acknowledgments

We thank “Federazione Medico Sportiva Italiana (FMSI)” for their contribution (BIOGE project).

Disclosures

No conflicts of interest are declared.

References

1. Towbin JA. Inherited cardiomyopathies. *Circ J* 2014; **78**: 2347–2356.
2. Doolan A, Nguyen L, Semsarian C. Hypertrophic cardiomyopathy: From “heart tumour” to a complex molecular genetic disorder. *Heart Lung Circ* 2004; **13**: 15–25.
3. Brown DW, Giles WH, Croft JB. Left ventricular hypertrophy as a predictor of coronary heart disease mortality and the effect of hypertension. *Am Heart J* 2000; **140**: 848–856.
4. Hill JA. Hypertrophic reprogramming of the left ventricle: Translation to the ECG. *J Electrocardiol* 2012; **45**: 624–629.
5. Wilde AA, Antzelevitch C, Borggrefe M, Brugada J, Brugada R, Brugada P, et al. Proposed diagnostic criteria for the Brugada syndrome: Consensus report. *Circulation* 2002; **106**: 2514–2519.
6. Atarashi H, Ogawa S, Harumi K, Sugimoto T, Inoue H, Murayama M, et al. Three-year follow-up of patients with right bundle branch block and ST segment elevation in the right precordial leads. *J Am Coll Cardiol* 2001; **37**: 1916–1920.
7. Hermida J, Lemoine J, Aoun F, Jarry G, Rey J, Quiret J. Prevalence of the Brugada syndrome in an apparently healthy population. *Am J Cardiol* 2000; **86**: 91–94.
8. Antzelevitch C, Pollevick G, Cordeiro J, Casis O, Sanguinetti MC, Aizawa Y, et al. Loss-of-function mutations in the cardiac calcium channel underlies a new clinical entity characterized by ST segment elevation, short QT intervals, and sudden cardiac death. *Circulation* 2007; **115**: 442–449.
9. Crotti L, Marcou CA, Tester DJ, Castelletti S, Giudicessi JR, Torchio M, et al. Spectrum and prevalence of mutations involving BrS1-through BrS12-susceptibility genes in a cohort of unrelated patients referred for Brugada syndrome genetic testing: Implications for genetic testing. *J Am Coll Cardiol* 2012; **60**: 1410–1418.
10. Brugada P, Brugada J, Roy D. Brugada syndrome 1992–2012: 20 years of scientific excitement, and more. *Eur Heart J* 2013; **34**: 3610–3615.
11. Elliott PM, Anastakis A, Borger MA, Borggrefe M, Cecchi F, Charron P, et al. ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy: The task force for the diagnosis and management of hypertrophic cardiomyopathy of the European Society of Cardiology (ESC). *Eur Heart J* 2014; **35**: 2733–2779.
12. Cheng J, Morales A, Siegfried JD, Li D, Norton N, Song J, et al. SCN5A rare variants in familial dilated cardiomyopathy decrease peak sodium current depending on the common polymorphism H558R and common splice variant Q1077del. *Clin Transl Sci* 2010; **3**: 287–294.
13. Li Q, Dai Y, Guo L, Liu Y, Hao C, Wu G, et al. Polycystin-2 associ-

- ates with tropomyosin-1, an actin microfilament component. *J Mol Biol* 2003; **325**: 949–962.
14. Xu C, Rossetti S, Jiang L, Harris PC, Brown-Glaberman U, Wandinger-Ness A, et al. Human ADPKD primary cyst epithelial cells with a novel, single codon deletion in the PKD1 gene exhibit defective ciliary polycystin localization and loss of flow-induced Ca²⁺ signaling. *Am J Physiol Renal Physiol* 2007; **292**: F930–F945.
 15. Paavola J, Schliffke S, Rossetti S, Kuo IY, Yuan S, Sun Z, et al. Polycystin-2 mutations lead to impaired calcium cycling in the heart and predispose to dilated cardiomyopathy. *J Mol Cell Cardiol* 2013; **58**: 199–208.
 16. Roma-Rodrigues C, Fernandes AR. Genetics of hypertrophic cardiomyopathy: Advances and pitfalls in molecular diagnosis and therapy. *Appl Clin Genet* 2014; **7**: 195–208.
 17. Fatkin D, Graham RM. Molecular mechanisms of inherited cardiomyopathies. *Physiol Rev* 2002; **82**: 945–980.
 18. Varnava AM, Elliott PM, Mahon N, Davies MJ, McKenna WJ. Relation between myocyte disarray and outcome in hypertrophic cardiomyopathy. *Am J Cardiol* 2001; **88**: 275–279.
 19. Charron P, Komajda M. Molecular genetics in hypertrophic cardiomyopathy: Towards individualized management of the disease. *Expert Rev Mol Diagn* 2006; **6**: 65–78.
 20. Ho CY, Seidman CE. A contemporary approach to hypertrophic cardiomyopathy. *Circulation* 2006; **113**: e858–e862, doi:10.1161/CIRCULATIONAHA.105.591982.
 21. Woo A, Rakowski H, Liew JC, Zhao MS, Liew CC, Parker TG, et al. Mutations of the beta myosin heavy chain gene in hypertrophic cardiomyopathy: Critical functional sites determine prognosis. *Heart* 2003; **89**: 1179–1185.
 22. Niimura H, Patton KK, McKenna WJ, Soultis J, Maron BJ, Seidman JG, et al. Sarcomere protein gene mutations in hypertrophic cardiomyopathy of the elderly. *Circulation* 2002; **105**: 446–451.
 23. Moolman JC, Corfield VA, Posen B, Ngumbela K, Seidman C, Brink PA, et al. Sudden death due to troponin T mutations. *J Am Coll Cardiol* 1997; **29**: 549–555.
 24. Varnava AM, Elliott PM, Baboonian C, Davison F, Davies MJ, McKenna WJ. Hypertrophic cardiomyopathy: Histopathological features of sudden death in cardiac troponin T disease. *Circulation* 2001; **104**: 1380–1384.
 25. Kimura A, Harada H, Park JE, Nishi H, Satoh M, Takahashi M. Mutations in the cardiac troponin I gene associated with hypertrophic cardiomyopathy. *Nat Genet* 1997; **16**: 379–382.
 26. Antzelevitch C, Brugada P, Borggrefe M, Brugada J. Brugada syndrome: Report of the second consensus conference, endorsed by the Heart Rhythm Society and the European Heart Rhythm Association. *Circulation* 2005; **111**: 659–670.
 27. Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, Calkins H, et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies: This document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). *Heart Rhythm* 2011; **8**: 1308–1339.
 28. Keren A, Syrris P, McKenna WJ. Hypertrophic cardiomyopathy: The genetic determinants of clinical disease expression. *Nat Clin Pract Cardiovasc Med* 2008; **5**: 158–168.
 29. Campuzano O, Allegue C, Iglesias A, Brugada R. Genetic basis of Brugada syndrome. *J Genet Syndr Gene Ther* 2013; **4**: 7, doi:10.4172/2157-7412.1000167.
 30. Kanters JK, Yuan L, Hedley PL, Stoevring B, Jons C, Bloch Thomsen PE, et al. Flecainide provocation reveals concealed Brugada syndrome in a long QT syndrome family with a novel L1786Q mutation in SCN5A. *Circ J* 2014; **78**: 1136–1143.
 31. McNally EM, Golbus JR, Puckelwartz MJ. Genetic mutations and mechanisms in dilated cardiomyopathy. *J Clin Invest* 2013; **123**: 19–26.
 32. Lopes LR, Sirris P, Guttman OP, O'Mahony C, Tang HC, Dalageorgou C, et al. Novel genotype phenotype associations demonstrated by high-throughput sequencing in patients with hypertrophic cardiomyopathy. *Heart* 2014; **0**: 1–8.
 33. Olson TM, Kishimoto NY, Whitby FG, Michels VV. Mutations that alter the surface charge of alpha-tropomyosin are associated with dilated cardiomyopathy. *J Molec Cell Cardiol* 2001; **33**: 723–732.
 34. Probst S, Oechslin E, Schuler P, Greutmann M, Boye P, Knirsch W, et al. Sarcomere gene mutations in isolated left ventricular noncompaction cardiomyopathy do not predict clinical phenotype. *Circ Cardiovasc Genet* 2011; **4**: 367–374.
 35. White SP, Cohen C, Phillips GN Jr. Structure of co-crystals of tropomyosin and troponin. *Nature* 1987; **325**: 826–828.
 36. Palm T, Graboski S, Hitchcock-DeGregori SE, Greenfield NJ. Disease-causing mutations in cardiac troponin T: Identification of a critical tropomyosin-binding region. *Biophys J* 2001; **81**: 2827–2837.
 37. Haruki S, Minami Y, Suzuki A, Hagiwara N. Effects of flecainide on left ventricular pressure gradient and symptoms in obstructive hypertrophic cardiomyopathy: A comparison of flecainide and disopyramide. *Heart Vessels* 2015; **30**: 604–610.

Supplementary Files

Supplementary File 1

Methods

Results

Please find supplementary file(s);
<http://dx.doi.org/10.1253/circj.CJ-15-0685>