ORIGINAL ARTICLE

# Identification of a variant hotspot in *MYBPC3* and of a novel *CSRP3* autosomal recessive alteration in a cohort of Polish patients with hypertrophic cardiomyopathy

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**KEY WORDS**

*CSRP3* human KO, hypertrophic cardiomyopathy, *MYBPC3* founder mutation, Polish population

**EDITORIAL**

by Walsh and Bezzina, see p. 76

**ABSTRACT**

**INTRODUCTION** Hypertrophic cardiomyopathy (HCM) is a heart disorder caused by autosomal dominant alterations affecting both sarcomeric genes and other nonsarcomeric loci in a minority of cases. However, in some patients, the occurrence of the causal pathogenic variant or variants in homozygosity, compound heterozygosity, or double heterozygosity has also been described. Most of the HCM pathogenic variants are missense and unique, but truncating mutations of the *MYBPC3* gene have been reported as founder pathogenic variants in populations from Finland, France, Japan, Iceland, Italy, and the Netherlands.

**OBJECTIVES** This study aimed to assess the genetic background of HCM in a cohort of Polish patients.

**PATIENTS AND METHODS** Twenty-nine Polish patients were analyzed by a next-generation sequencing panel including 404 cardiovascular genes.

**RESULTS** Pathogenic variants were found in 41% of the patients, with ultra-rare *MYBPC3* c.2541C>G (p.Tyr847Ter) mutation standing for a variant hotspot and correlating with a lower age at HCM diagnosis. Among the nonsarcomeric genes, the *CSRP3* mutation was found in a single case carrying the novel c.364C>T (p.Arg122Ter) variant in homozygosity. With this finding, the total number of known HCM cases with human *CSRP3* knockout cases has reached 3.

**CONCLUSIONS** This report expands the mutational spectrum and the inheritance pattern of HCM.

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**INTRODUCTION** Hypertrophic cardiomyopathy (HCM) is a cardiac disease characterized by left ventricular hypertrophy (thickness ≥15 mm) unexplained by secondary causes, and a nondilated left ventricle with preserved or increased ejection fraction. It is an important cause of sudden cardiac death, and its prevalence has been estimated at approximately 1/500 individuals in the adult population.[1] Hypertrophic cardiomyopathy is considered a disease of the cardiac sarcomere mainly caused by pathogenic variants in over 10 loci, with *MYBPC3*, *MYH7* and *TNNT2* accounting for approximately 50% of the HCM families and encoding cardiac myosin-binding protein C, β-myosin heavy chain, and cardiac troponin T, respectively.[2] Other inheritable causes of the disease include pathogenic variants in genes encoding proteins involved in calcium handling and for proteins of the Z-disk.[3] Most of the pathogenic variants found in HCM genes are missense and

## WHAT'S NEW?

Most of the DNA mutations leading to hypertrophic cardiomyopathy (HCM) are inherited with an autosomal dominant pattern and are unique or seen in a limited number of families. However, in some ethnic groups, founder mutations have been described. We report the analysis of 404 genes in a cohort of 29 Polish patients affected by HCM. Our results showed that an ultra-rare alteration in the *MYBPC3* gene, absent in databases from large-scale sequencing projects, was a mutation hotspot in the present Polish cohort and could represent a novel founder pathogenic variant. Moreover, we found that 1 patient was homozygous for a novel truncating mutation in the *CSRP3* gene, thus increasing the number of known human *CSRP3* knockout cases to 3. This finding also suggests that the autosomal recessive inheritance pattern could be more frequent in HCM than reported thus far.

unique or private within families.[2] These alterations are believed to have a dominant negative effect, acting as a "poison polypeptide" on sarcomere function.[4] The only exception is *MYBPC3*, in which about two-thirds of pathogenic variants are truncating and haploinsufficiency is postulated as a pathogenetic mechanism of the disease.[5] To date, at least 12 different truncating *MYBPC3* pathogenic mutations have been reported as founder variants in populations from Finland, France, Japan, Iceland, Italy, Spain, and the Netherlands.[5-8]

In most adolescents and adults, HCM is inherited as an autosomal dominant trait with a clinical outcome characterized by incomplete penetrance and variable expression. The disease phenotype can indeed be modulated by environmental factors, by the genetic context (including polymorphisms of the renin–angiotensin system [RAS]),[9] and by the occurrence of the causal pathogenic variant or variants in homozygosity, compound heterozygosity, or double heterozygosity.[10] Janin et al[11] have recently reported 2 unrelated kindreds with homozygous truncating variants in the cysteine and glycine-rich protein 3 (*CSRP3*) gene encoding a member of the CSRP family of muscle LIM protein (MLP).

It is likely that HCM individuals are underdiagnosed,[12] especially in countries where the availability of genetic testing based on next-generation sequencing (NGS) is limited. Considering the prevalence of HCM, the estimated number of HCM patients in Poland is approximately 78 000. Until now, only single reports describing genetic causes of HCM in Polish patients have been reported.[13-15] Therefore, the aim of the current study was to assess the genetic background of HCM in a cohort of patients from the south-eastern part of Poland, analyzed by an NGS panel including 404 genes known to harbor alterations affecting cardiovascular system function. Thanks to this approach, we could identify a *MYBPC3* truncating alteration that could represent a novel Polish founder *MYBPC3* pathogenic variant. Also, we detected a new patient with the homozygous *CSRP3* variant; thus, the number of known HCM cases with null homozygous alterations in this gene has increased to 3.

## PATIENTS AND METHODS

**Patients** Twenty-nine unrelated patients were selected from those attending an outpatient service dedicated to the diagnosis and management of HCM at the Institute of Cardiology, John Paul II Hospital (Kraków, Poland). Patients underwent clinical history taking, physical examination, electrocardiography (ECG), echocardiography, cardiopulmonary exercise test coupled with ambulatory ECG monitoring, and cardiac magnetic resonance imaging. Diagnostic criteria for HCM were defined in adults by a maximal left ventricular wall thickness of 15 mm or higher on echocardiography, or of 13 mm or higher in relatives, in the absence of loading conditions.[16] Family history of sudden cardiac death, syncope episodes, and the presence of nonsustained ventricular tachycardia were defined as described by O'Mahony et al.[17] Electrocardiographic abnormalities that were considered of clinical significance included abnormal Q waves (0.04 s or 25% depth of the R wave), left ventricular hypertrophy (voltage criteria), and marked repolarization changes (eg, T-wave inversion in at least 2 leads). Familial HCM cases were defined if at least 1 additional affected family member with HCM or 1 case of sudden cardiac death was present in the pedigree. When available, the relatives of index cases were recruited for genetic testing. All patients gave informed consent for the DNA analyses, and the study was approved by local ethics committees in accordance with the principles of the Declaration of Helsinki.

**Genetic analyses** Genomic DNA from peripheral blood was tested by NGS with the Ion AmpliSeq™ Cardiovascular Research Panel (ThermoFisher, Carlsbad, California, United States), including the 16 "core HCM genes" defined by the American College of Medical Genetics and Genomics (ACMG),[18] as well as other 388 genes known to harbor alterations affecting cardiovascular functioning (Supplementary material, *Table S1*). The Ion Chef System (ThermoFisher) was employed for the automated library and template preparation, as well as for chip loading. Sequencing reactions were carried out on the Ion S5 XL System (ThermoFisher). Sequencing reads were aligned on the GRCh37/hg19 reference sequence by the Torrent Suite Software v.5.4.0 (ThermoFisher). For every patient, the panel coverage as well as the mean and median read depth reached for each of 404 genes is given in Supplementary material, *Table S1*. The mapped reads were analyzed to determine the presence of DNA point variants by Variant Caller v5.4.0.46 plugin using "germline – low stringency" parameters (ThermoFisher). Variants' calls were scored and prioritized by the TGex software (LifeMap Sciences, Alameda, California, United States; http://tgex.genecards.org/), which ranks variants according to their association to the phenotype (ie,

HCM). Between the variants scored by TGex and matching with the HCM phenotype, only the ones meeting all the following parameters were filtered: 1) nonsynonymous exonic or ±10-bp intronic variants; 2) minor allele frequency (MAF) in the Genome Aggregation Database (GnomAD) of less than 0.01; 3) high quality of the call (ie, Q&R score = Coverage ≥20 × and GQ ≥50); and 4) at least 20% of reads showing the alternative allele (% Alt >20%). The resulting variants were confirmed by Sanger sequencing (polymerase chain reaction [PCR] primers are listed in Supplementary material, *Table S2*) and classified into 5 categories presented in the 2015 guidelines of the ACMG / Association for Molecular Pathology (AMP),[19] modelled by a Bayesian framework as previously described.[20] This approach allowed a better categorization of the DNA variants into 7 classes: 1) pathogenic; 2) likely pathogenic; 3) variants of unknown significance (VUS)–favoring pathogenic; 4) VUS; 5) VUS-favoring benign; 6) likely benign; and 7) benign. Data for this classification were obtained from the CardioVai (www.cardioclassifier.org), Cardio Classifier (www.cardiovai.engenome.com), Intervar (www.wintervar.wglab.org), and Varsome (www.varsome.com) systems, as well as from the ClinVar database (www.ncbi.nlm.nih.gov/clinvar). Regarding *MYH7*, the ACMG/AMP 2015 classification was adapted following the ClinGen's Inherited Cardiomyopathy Expert Panel.[21] Variants not yet reported in literature were referred to as "novel."

The presence of copy number alterations encompassing the *CSRP3* c.364C>T variant was investigated by a SYBR Green–based quantitative PCR on an ABI7900 HT Fast Real Time PCR System (ThermoFisher), with the following primer pair mapping in the *CSRP3* gene: FW-5'-TGGGAATTCTGGTTTGCTTTG-3' and Rv-5'-GAGGCATGTAAGATCCAGTGGTT-3'. The experiment included patient 58 as well as 4 unaffected control individuals. The reference gene, *TERT*, was simultaneously quantified in a separate tube for each specimen.

***MYBPC3* haplotype analysis** To test whether the carriers of the same *MYBPC3* variant share a common haplotype, a linkage analysis around the *MYBPC3* region on chromosome 11 was performed with 8 microsatellite markers upstream the gene (ie, D11S4109, D11SA1, D11S1784, D11S4165, D11S1395, and D11S1765), 6 single nucleotide polymorphisms within the gene (ie, rs1052373, rs11570078, rs2856650, rs3729989, rs11570051, and rs11570050), and 6 microsatellite markers downstream *MYBPC3* (ie, D11S905, D11S1763, D11S986, D11S4174, D11S4137, D11S1385, D11S1344, and D11S1252). These polymorphic markers cover about 19.8 Mb around *MYBPC3*.

**Statistical assessment of genotype–phenotype correlations** All statistical analyses were performed using the SPSS software package version 20.0 (IBM SPSS Statistics, Milan, Italy). For each patient, the clinical and molecular data were tabulated (FIGURE 1). Phenotype data were presented as continuous variables obtained from clinical data and instrumental measurements, and they were summarized using means and standard deviations. The mean values of age at diagnosis, maximal wall thickness, and left ventricular mass were compared using the *t* test for independent data between the subgroups of patients with different genotypes. Also, associations between sex, the genotype of 2 polymorphisms in the genes of the RAS, and clinical features were tested. The penetrance of HCM, according to age at diagnosis, was analyzed by the Kaplan–Meier method, and differences between cumulative hazard were evaluated with the log-rank test. Due to the relatively low number of patients, no adjustments were planned for multiple testing. Therefore, the analysis is exploratory and the results should be considered as hypothesis generating.

**RESULTS AND DISCUSSION** In total, 12 of the 29 patients (41%) were found to carry at least 1 pathogenetic, likely pathogenetic, or VUS-favoring pathogenic alteration of the "core HCM genes," as classified by the 2015 ACMG/AMP guidelines and ClinVar database (TABLE 1 and FIGURE 1). Most of the changes were identified in the *MYBPC3*, *MYH7* and *TNNT2* genes. The genotype of the rs5186 and rs699 polymorphisms, mapping respectively in the angiotensin receptor type 1 (*AGTR1*) and angiotensinogen (*AGT*) genes, belonging to the RAS, was also recorded (FIGURE 1). Indeed, some studies have shown that rs5186 and rs699 may influence the clinical phenotype of HCM,[22] since the RAS regulates cardiac function, blood pressure, and electrolyte homeostasis.[23] However, in the present cohort, no significant correlation with the disease expression was found.

The identified mutational spectrum included 10 distinct substitutions: 6 missense, 3 nonsense, and 1 intronic change. Most of the missense variants were sarcomeric, and, among them, 3 mapped in *MYH7*, 2 in *MYBPC3*, and 1 in *TNNT2*. On the other hand, 2 of the nonsense alterations were found in *MYBPC3* and 1 in *CSRP3*. The GnomAD allele frequency of all the sarcomeric alterations was smaller than 0.01%, that is, the MAF threshold suggested for HCM pathogenic variants.[24]

A single variant was found in more than 1 patient (Supplementary material, *Figure S1A*). The *MYBPC3* c.2541C>G variant, resulting in the premature insertion of a stop codon in exon 24 (p.Tyr847Ter), was indeed identified in 4 unrelated cases, despite the fact that it is absent in the GnomAD. Carriers of the *MYBPC3* c.2541C>G showed a lower age at HCM diagnosis compared with carriers of other DNA variants, with a 50% probability of HCM diagnosis at 38 years for *MYBPC3* c.2541C>G carriers, in comparison with the age of 49 years for the carriers of other DNA variants ($P$ = 0.04; FIGURE 2).

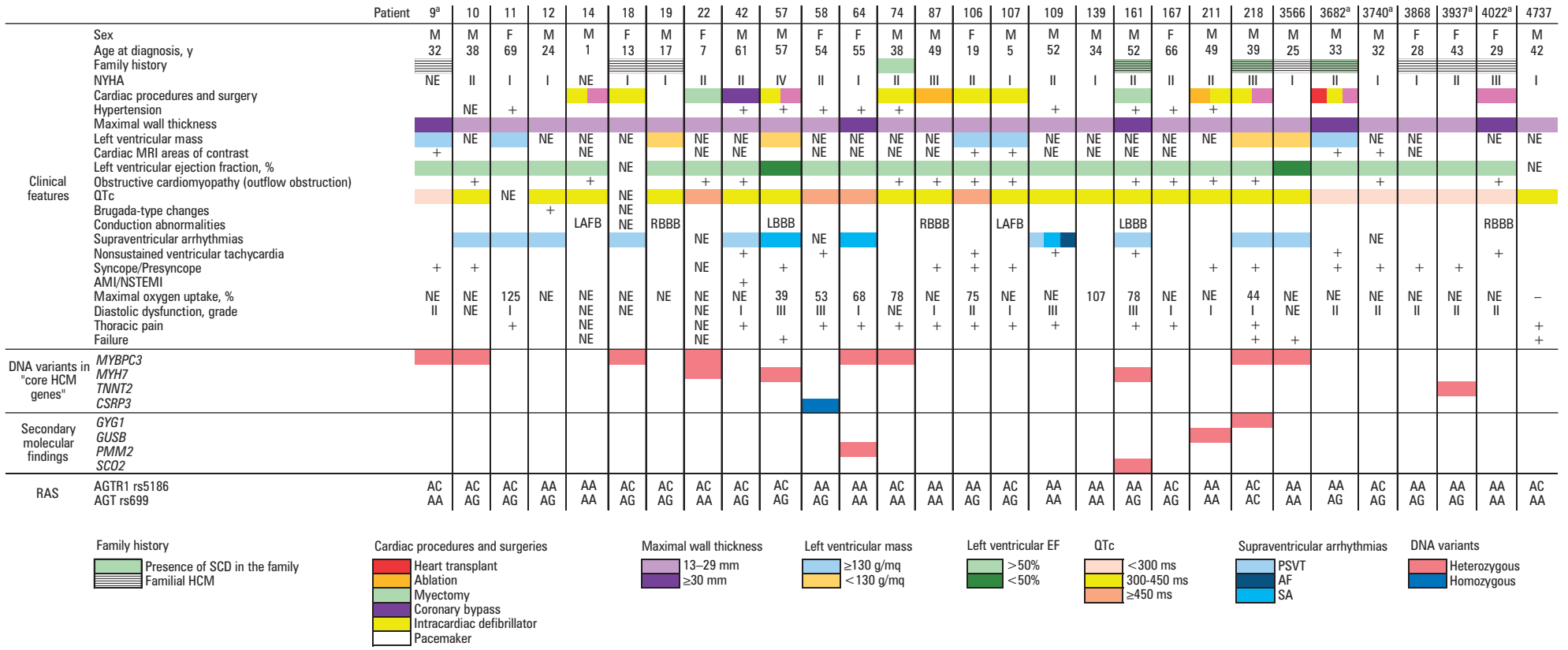


**FIGURE 1** Clinical and molecular data of the 29 Polish cases affected by hypertrophic cardiomyopathy. The x-axis shows the 29 patients. The y-axis shows the clinical parameters (top), "core HCM genes" (middle), and other genes found mutated (bottom). The last 2 rows show the genotype of the rs5186 and rs699 polymorphisms, mapping respectively in the *AGTR1* and *AGT* genes of the renin–angiotensin system. The color code employed in the figure is shown at the bottom.

**a** Patient on drug treatment

Abbreviations: +, present; AF, atrial fibrillation; AMI, acute myocardial infarction; EF, ejection fraction; F, female; LAFB, left anterior fascicular block; LBBB, left bundle branch block; M, male; MRI, magnetic resonance imaging; NE, not evaluated; NSTEMI, non–ST-segment elevation myocardial infarction; NYHA, New York Heart Association; PSVT, paroxysmal supraventricular tachycardia; RAS, renin–angiotensin system; RBBB, right bundle branch block; SA, sinus arrest

**TABLE 1** Genetic alterations identified in 12 of the 29 Polish patients affected by hypertrophic cardiomyopathy (for references 51–67, see Supplementary material) (continued on the next page)

| Patient | Gene | Location | Ref | Alt | RefSeq | Nucleotide | AA | Coding impact | Zygosity | dbSNP | GnomAD-Exomes allele frequency, % | | ClinVar | ACMG/AMP 2015 | | ClinVar conditions | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | European Non-Finnish | Total | | Classification | Activeted rules | | |
| Pt 9 | *MYBPC3* | 11:47369975 | C | T | NM_000256.3 | c.772G>A | Glu258Lys | Missense | Het | rs397516074 | 0.003 | 0.001 | P, LP | P | PP2, PP3, PP5, PM1, PM2, PS4 | HCM | Reported in unrelated patients with HCM (Niimura et al[34]; Richard et al[35]; Nanni et al[36]; Van Driest et al[37]; Song et al[38]; Murphy et al[39]) |
| Pt 10 | *MYBPC3* | 11:47359003 | G | C | NM_000256.3a | c.2541C>Ga | Tyr847Tera | Nonsense | Het | rs397515974 | 0 | 0 | P, LP | P | PP3, PP5, PM1, PM2, PS1, PVS1 | HCM | Reported in individuals with HCM (Van Driest et al[37]; Berge et al[40]; Kapplinger et al[41]; Viswanathan et al[42]; Zhao et al[43]) |
| Pt 18 | *MYBPC3* | 11:47369403 | C | T | NM_000256.3 | c.821+5G>A | – | Intronic | Het | rs397516077 | 0 | 0 | P, LP | P | PP3, PP5, PM1, PM2, PS1, PVS1 | HCM | Reported in association with HCM (Carrier et al[44]; Millat et al[45]) |
| Pt 22 | *MYBPC3* | 11:47359003 | G | C | NM_000256.3a | c.2541C>Ga | Tyr847Tera | Nonsense | Het | rs397515974 | 0 | 0 | P, LP | P | PP3, PP5, PM1, PM2, PS1, PVS1 | HCM | Reported in individuals with HCM (Van Driest et al[37]; Berge et al[40]; Kapplinger et al[41]; Viswanathan et al[42]; Zhao et al[43]) |
| | *MYH7* | 14:23891518 | T | C | NM_000257.3 | c.3116A>G | Glu1039Gly | Missense | Het | rs199573700 | 0.004 | 0.01 | VUS | VUS-3B | PP2, PP3, PM2 | HCM | Novel but present in ClinVar |
| Pt 57 | *MYH7* | 14:23886875 | A | C | NM_000257.3 | c.4190T>G | Leu1397Arg | Missense | Het | – | 0 | 0 | – | P | PP3, PP5, PM1, PM2, PS1, PVS1 | – | Novel |
| Pt 58 | *CSRP3* | 11:19207813 | G | A | NM_003476.4 | c.364C>T | Arg122Ter | Nonsense | Hom | rs902082118 | 0 | 0.001 | LP | P | PP2, PP3, PM2, PVS1 | Not assessed | Novel |
| Pt 64 | *MYBPC3* | 11:47364270 | G | A | NM_000256.3 | c.1483C>T | Arg495Trp | Missense | Het | rs397515905 | 0 | 0 | P/VUS | LP | PP2, PP3, PM1, PM2, PM5 | HCM | Reported in at least 4 individuals with HCM (Garcia-Castro et al[46]; Rodriguez-Garcia[47]; Coto[48]; Martin[49]) |
| Pt 74 | *MYBPC3* | 11:47359003 | G | C | NM_000256.3a | c.2541C>Ga | Tyr847Tera | Nonsense | Het | rs397515974 | 0 | 0 | P, LP | P | PP3, PP5, PM1, PM2, PS1, PVS1 | HCM | Reported in individuals with HCM (Van Driest et al[37]; Berge et al[40]; Kapplinger et al[41]; Viswanathan et al[42]; Zhao et al[43]) |
| Pt 161 | *MYH7* | 14:23901922 | C | T | NM_000257.3 | c.428G>A | Arg143Gln | Missense | Het | rs397516209 | 0.0009 | 0.0004 | LP | P | PP1, PP3, PM2, PM5, PS4 | HCM | Reported in association with HCM (Kimura et al[50]; Van Driest et al[37]; Song et al[38]; Coto et al[48]; Marsiglia et al[51]) |

**TABLE 1** Genetic alterations identified in 12 of the 29 Polish patients affected by hypertrophic cardiomyopathy (for references 51–67, see Supplementary material) (continued from the previous page)

| Patient | *Gene* | Location | Ref | Alt | RefSeq | Nucleotide | AA | Coding impact | Zygosity | dbSNP | GnomAD-Exomes allele frequency, % | | ClinVar | ACMG/AMP 2015 | | ClinVar conditions | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | European Non-Finnish | Total | | Classification | Activeted rules | | |
| Pt 3566 | *MYBPC3* | 11:47359003 | G | C | NM_000256.3a | c.2541C>Ga | Tyr847Tera | Nonsense | Het | rs397515974 | 0 | 0 | P | P | PP3, PP5, PM1, PM2, PS1, PVS1 | HCM | Reported in individuals with HCM (Van Driest et al[37]; Berge et al[40]; Kapplinger et al[41]; Viswanathan et al[42]; Zhao et al[43]) |
| Pt 3937 | *TNNT2* | 1:201334426 | G | A | NM_000364.3 | c.304C>T | Arg102Trp | Missense | Het | rs397516456 | 0.001 | 0.0004 | P | LP | PP3, PP5, PM1, PM2 | HCM | Reported in association with HCM (Koga et al[52]; Moolman et al[53]; Moolman-Smook et al[54]; Palm et al[55]; Van Driest et al[56]; Revera et al[57]; Ho et al[58]; Ripoll-Vera et al[59]). Some authors suggested that it is associated with a higher risk of sudden death (Moolman et al[55]; Moolman-Smook et al[54]; Ripoll-Vera et al[59]) |

a The *MYBPC3* variant hotspot

Abbreviations: AA, amino acid; ACMG/AMP, American College of Medical Genetics and Genomics and the Association for Molecular Pathology; Alt, alternative; HCM, hypertrophic cardiomyopathy; Het, heterozygous; Hom, homozygous; LP, likely pathogenic; P, pathogenic; Ref, reference; Pt, patient; VUS-3B, variant of unknown significance–favoring pathogenic

The *MYBPC3* c.2541C>G mutation was the only identified variant in cases 10, 74, and 3566, while it was associated with another sarcomeric alteration (ie, the *MYH7* c.3116A>G,p.Glu1039Gly) in patient 22. The allele frequency of the *MYH7* c.3116A>G was very close to the 0.01% threshold, and we infer that this variant could be a modifier allele or a low penetrance variant contributing to the severity of HCM expression in patient 22. Among the 4 carriers of the *MYBPC3* c.2541C>G, patient 22 indeed manifested the earliest age at diagnosis, the need for septal myectomy at the age of 41 years, and the presence of a long QTc interval (FIGURE 1). The segregation of *MYBPC3* c.2541C>G and of *MYH7* c.3116A>G was tested in 4 relatives of case 22: a 50-year-old sister affected by HCM and 3 children aged 9, 12, and 16 years, who are currently asymptomatic. All of them were found to carry *MYBPC3* c.2541C>G in heterozygosity, while they had the wild-type *MYH7* c.3116A variant (Supplementary material, *Figure S1A*).

The *MYBPC3* c.2541C>G variant was also detected in heterozygosity in a 19-year-old daughter of patient 74 (Supplementary material, *Figure S1A*). On clinical evaluation, she did not display any chronic cardiac or noncardiac disease. Her echocardiogram showed normal left ventricular (left ventricular end-diastolic diameter, 43 mm) and atrial size (left atrial appendage, 17 $cm^2$; right atrial appendage, 13.5 $cm^2$), as well as normal thickness of intraventricular septum (end-diastolic diameter, 10 mm). Also, left ventricular systolic function was normal (70%), and she did not show any valvular abnormalities, signs of systolic anterior motion, or left ventricular outflow obstruction. Therefore, at present, the children of case 22 and the daughter of case 74 are asymptomatic carriers of c.2541C>G and possibly would show later onset of the disease due to an age-related penetrance.

To investigate whether carriers of the *MYBPC3* c.2541C>G variant could share a common ancestor, we performed a markers analysis. A haplotype, spanning about 5 Mb, was found to segregate with the c.2541G variant allele in the family of patient 22. The alleles of the c.2541G-haplotype were also present in all the *MYBPC3* c.2541C>G carriers (TABLE 2), even if we could not reconstruct their phase. This finding supports the hypothesis that the *MYBPC3* c.2541C>G variant could have a founder role in the Polish population. In support of this hypothesis, the *MYBPC3* c.2541C>G variant has been recently found in another Polish patient with HCM, not related to any of the cases reported herein.[25]

Case 58 was found to carry the truncating *CSRP3* c.364C>T (p.Arg122Ter) variant in homozygosity (Supplementary material, *Figure S1B*), as confirmed by SYBR Green–based quantitative PCR on ABI7900 HT Fast Real Time PCR System (ThermoFisher) (Supplementary material, *Figure S1C*). Patient 58 was a 56-year-old woman diagnosed with HCM at the age of 54

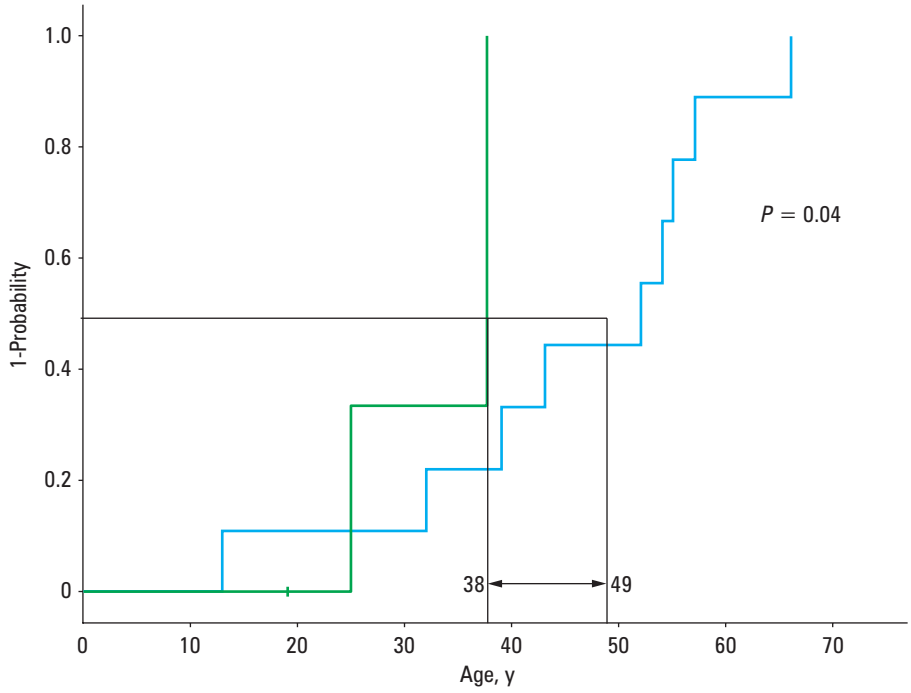


**FIGURE 2** Penetrance of hypertrophic cardiomyopathy, according to age at diagnosis, in *MYBPC3* c.2541C>G carriers (blue) and in carriers of other DNA variants (green). Plot represents 1 minus the probability of not having a diagnosis of hypertrophic cardiomyopathy (HCM) at each age. This probability is estimated by the Kaplan–Meier method. Patient 22 was not included in this analysis due to being a double-variant carrier.

**TABLE 2** Haplotype analysis in cases carrying the *MYBPC3* c.2541C>G variant

| Marker | Genomic position start (Hg19) | c.2541G-haplotype[a] | Pt 10 | Pt 74 | Daughter of Pt 74 | Pt 3566 |
|---|---|---|---|---|---|---|
| D11S1252 | 11:46446790 | 153* | 153*-153 | 153*-153 | 153*-153 | 153*-161 |
| *MYBPC3* start | 11:47353396 | | | | | |
| rs1052373 | 11:47354787 | A* | A*-G | A*-G | A*-G | A*-G |
| c.2541C>G | 11:47359003 | G* | G*-C | G*-C | G*-C | G*-C |
| rs11570078 | 11:47365014 | G* | G*-G | G*-A | G*-G | G*-G |
| rs2856650 | 11:47365199 | C* | C*-T | C*-C | C*-T | C*-T |
| rs3729989 | 11:47370041 | A* | A*-A | A*-G | A*-A | A*-A |
| rs11570051 | 11:47371442 | T* | T*-C | T*-C | T*-C | T*-C |
| rs11570050 | 11:47371485 | C* | C*-del | C*-C | C*-del | C*-del |
| *MYBPC3* end | 11:47374253 | | | | | |
| D11S4109 | 11:47601406 | 153* | 153*-167 | 153*-171 | 153*-165 | 153*-167 |
| D11SA1 | 11:47741121 | 257* | 257*-251 | 257*-245 | 257*-253 | 257*-251 |
| D11S1784 | 11:48022707 | 143* | 143*-141 | 143*-149 | 143*-141 | 143*-145 |
| D11S4165 | 11:50137951 | 217* | 217*-217 | 217*-217 | 217*-217 | 217*-217 |
| D11S1395 | 11:51382783 | 223* | 223*-223 | 223*-231 | 223*-227 | 223*-227 |

a The c.2541G-haplotype was found by segregation analysis performed in patient 22 and her relatives.

Abbreviations: see TABLE 1

due to early fatigue and chest pain. She mainly complained of nonspecific and episodic chest pain (discomfort) on exertion. Echocardiography showed asymmetric interventricular septal hypertrophy (18 mm), posterior wall of 12 mm, ejection fraction of 70%, normal right ventricle, and maximal (provoked) left ventricular outflow tract gradient of 18 mm Hg without the systolic anterior motion of the mitral valve. Also, severe diastolic dysfunction with no signs of pulmonary hypertension was observed. The 24-hour electrocardiogram highlighted 5 runs of nonsustained ventricular tachycardia (the longest one of 11 beats). Family history was negative for HCM, but the *CSRP3* c.364C>T variant was found to segregate in heterozygosity in a 29-year-old son (Supplementary material, *Figure S1B*), who currently does not suffer from any chronic cardiac or noncardiac diseases. He has no symptoms and is physically active. On echocardiography, he has normal left ventricular (left ventricular end-diastolic diameter, 46 mm) and atrial sizes (left atrial appendage, 17.5 $cm^2$; right atrial appendage, 17 $cm^2$). However, his intraventricular septum was mildly thickened (end-diastolic diameter, 13 mm), with normal systolic function (ejection

**TABLE 3** *CSRP3* variants to date reported in literature or listed in the ClinVar database (for references 51–67, see Supplementary material)

| *CSRP3* variant | Zygosity | Reference(s) | Condition(s) |
|---|---|---|---|
| c.46A>T (p.Thr16Ser) | Not reported | ClinVar | DCM |
| c.50insGCAGATTTCTT (p.Tyr18GlnfsX194) | Het | van Rijsingen et al[60] | HCM |
| c.96G>A (p.Lys32=) | Not reported | ClinVar | DCM |
| c.122_123dupGG (p.Lys42Glyfs) | Het | Bos et al[61] | HCM |
| c.131T>C (p.Leu44Pro) | Het | Geier et al[27]; Geier et al[62]; ClinVar | Familial HCM 12, DCM 1M, not specified, cardiovascular phenotype |
| c.131T>C (p.Leu44Pro) | Het, in association with *MYBPC3* p.Gly1041fs | Bos et al[61] | HCM |
| c.136A>C (p.Ser46Arg) | Het, in association with *TNNI3* p.Arg162Gln | Bos et al[61]; ClinVar | Familial HCM 12, DCM 1M, cardiomyopathy, not specified |
| c.160_164delTCGGAinsAGGGG (p.Ser54_Glu55delinsArgGly) | Het | Geier et al[27]; ClinVar | Familial HCM 12 |
| c.172T>G (p.Cys58Gly) | Het | Geier et al[62]; ClinVar | Familial HCM 12 |
| c.190C>T (p.Arg64Cys) | Het | Bos et al[61] | HCM |
| c.197A>G (p.Tyr66Cys) | Het | Bos et a[61] | HCM |
| c.206A>G (p.Lys69Arg) | Not reported | ClinVar | Familial HCM 12, DCM 1M, not specified, cardiovascular phenotype |
| c.214G>A (p.Gly72Arg) | Het | Hershberger et al[63] | DCM |
| c.233G>T (p.Gly78Val) | Not reported | ClinVar | Cardiovascular phenotype, DCM |
| c.272A>T (p.Gln91Leu) | Het | Bos et al[61] | HCM |
| c.299G>A (p.Arg100His) | Het | Andersen et al[64] | HCM |
| c.336G>A (p.Ala112=) | Not reported | ClinVar | Not specified, HCM, cardiovascular phenotype, DCM, DCM, dominant |
| c.354G>A (p.Glu118=) | Not reported | ClinVar | DCM |
| c.364C>T (p.Arg122Ter) | Hom | This report | HCM |
| c.365G>A (p.Arg122Gln) | Not reported | ClinVar | Familial HCM 12, DCM 1M, cardiomyopathy |
| c.369T>A (p.Cys123Ter) | Hom | Janin et al[11] | HCM |
| c.420G>C (p.Trp140Cys) | Not reported | ClinVar | DCM |
| c.449G>A (p.Cys150Tyr) | Not reported | ClinVar | Familial HCM 12, DCM 1M, not specified, cardiovascular phenotype |
| c.483dup (p.Lys162GlnfsX52) | Hom | Janin et al[11] | Hypertrophic cardiomyopathy |
| c.536C>T (p.Thr179Met) | Not reported | ClinVar | Familial HCM 12, DCM 1M, not specified |

Abbreviations: DCM, dilated cardiomyopathy; others, see **TABLE 1**

fraction, 67%), no valvular abnormalities, and no sign of systolic anterior motion and left ventricular outflow tract obstruction.

The *CSRP3* c.364C>T (p.Arg122Ter) mutation is not a common variant since it is absent in GnomAD-Genomes and GnomAD-Exomes--European databases. In total, only 3 *CSRP3* c.364C>T alleles are listed in GnomAD-Exomes (ie, 2 in African and 1 in South Asian populations), but never in a homozygous state. It has not been described in the literature before, but it is listed as likely pathogenic in the ClinVar database because of the following evidence: 1) it is a rare variant; 2) it is predicted to cause the loss of protein function either by protein truncation or nonsense-mediated mRNA decay; 3) *CSRP3*-null mice develop cardiomyopathy and heart failure due to a disrupted cardiomyocyte architecture[26]; and 4) myocardial biopsies of a HCM patient with a heterozygous *CSRP3* missense variant showed myocyte disarray and a reduced level of MLP, suggesting that cardiomyopathy may stem from *CSRP3* haploinsufficiency.[27] To date, 24 carriers of a *CSRP3* alteration were reported in the literature or included in the ClinVar database: 18 had HCM and 6 were affected by dilated cardiomyopathy. Among the HCM patients, 9 carried a heterozygous *CSRP3* variant (including 7 amino acid substitutions and 2 truncating variants), 2 cases were heterozygous but also carried a second variant in another gene, while 2 patients harbored a truncating alteration in homozygosity (**TABLE 3**).[11] Therefore, patient 58 described herein is the third *CSRP3* human knockout case reported so far. Our finding strengthens the assumption that at least several *CSRP3* variants

**TABLE 4** Secondary molecular findings (for references 51–67, see Supplementary material)

| Patient | Gene | Location | Ref | Alt | RefSeq | Nucleotide | AA | Coding impact | Zygosity | dbSNP | GnomAD-Exomes allele frequency (%) | | ClinVar | ACMG/AMP 2015 | | ClinVar conditions | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | European Non-Finnish | Total | | Classification | Activated rules | | |
| Pt 64 | *PMM2* | 16:8941632 | G | A | NM_000303.2 | c.691G>A | Val231Met | Missense | Het | rs80338707 | 0.011 | 0.007 | P | VUS-3B | PP3, PP5, PM1 | Carbohydrate-deficient glycoprotein syndrome type Ia | Reported in several individuals affected with PMM2-CDG (Barone et al[65]) |
| Pt 161 | *SCO2* | 22:50962423 | C | T | NM_005138.2 | c.418G>A | Glu140Lys | Missense | Het | rs74315511 | 0.017 | 0.008 | P | VUS-3B | PP3, PP5, PM1 | Cardioencephalomyopathy, fatal infantile, due to cytochrome c oxidase deficiency 1a<br>Myopia 6 | Previously reported as c.1541G>A in homozygosity or compound heterozygosity with a second *SCO2* variant in individuals with *SCO2*-related clinical features including HCM (Papadopoulou et al[32]; Jaksch et al[31])<br>Reported as a heterozygous mutation in 1 individual with autosomal dominant high-grade myopia (Tran-Viet et al[33]) |
| Pt 211 | *GUSB* | 7:65444841 | C | T | NM_000181.3 | c.454G>A | Asp152Asn | Missense | Het | rs149606212 | 0.189 | 0.113 | VUS | LP | PP3, PM1, PM2 | MPS type VIIa | Identified in homozygosity in a child with MPS type VIIa (Vervoort et al[66]). Transfection studies showed that the D152N substitution resulted in decreased enzyme activity. Vervoort et al[66] referred to D152N as a "pseudodeficiency allele" that leads to greatly reduced levels of beta-glucuronidase activity without apparent deleterious consequences. |
| Pt 218 | *GYG1* | 3:148714249 | G | C | NM_004130.3 | c.304G>C | Asp102His | Missense | Het | rs143137713 | 0.189 | 0.102 | P/LP | VUS-3B | PP3, PP5, PM1 | Glycogen storage disease XVa | Reported in homozygosity or compound heterozygosity in individuals with glycogenin-1 deficiency (Malfatti et al[67]; Hedberg-Oldfors et al[29]) |

**a** Autosomal recessive

Abbreviations: MPS, mucopolysaccharidosis; others, see **TABLE 1**

lead to HCM with an autosomal recessive inheritance[11] rather than with an autosomal dominant transmission as recorded in the Online Mendelian Inheritance in Man (OMIM) database (OMIM: *600824). The mouse model knock-in for *CSRP3* pathogenic variants also corroborates the hypothesis of autosomal recessive inheritance of the cardiac disease and recalls the findings displayed by patient 58 and his son.[28] Indeed, the heterozygous mice for *CSRP3* pathogenic variants do not display an overt cardiac phenotype (except for an increase in anterior wall thickness), while the homozygous mutated mouse shows a clear cardiomyopathy phenotype.[28]

The "non–core HCM genes" with mutations in the present HCM cohort were *GYG1*, *GUSB, PMM2* and *SCO2.* Cases 64, 161, 211, and 218 were indeed identified as heterozygous carriers of autosomal recessive alterations associated with *PMM2*, *SCO2*, *GUSB*, and *GYG1* deficiency, respectively (**TABLE 4**). The *GYG1*, *GUSB, PMM2*, and *SCO2* variants were prioritized by the TGex software since they were related to the cardiomyopathy phenotype: *GYG1* c.304G>C, found in patient 218, has previously been described in homozygosity in cases affected by severe cardiomyopathy without skeletal muscle weakness[29]; the *GUSB* and *PMM2* genes are responsible for genetic disorders associated with HCM[30]; the *SCO2* c.418G>A variant has been previously reported by the name of "c.1541G>A" in homozygosity or in compound heterozygosity in individuals with *SCO2*-related clinical features including HCM.[31,32] Therefore, these results can be considered "secondary findings" for which we cannot exclude a possible hypomorphic impact over the patients' phenotype. Also, the *SCO2* p.Glu140Lys variant was previously associated with autosomal dominant high-grade myopia.[33] In the present patient, presenting low myopia of –2.00 diopters in both eyes, the *SCO2* c.418G>A variant exhibited reduced penetrance.

In conclusion, this report expands the mutational spectrum and the inheritance pattern of HCM. The ultra-rare *MYBPC3* c.2541C>G (p.Tyr847Ter) alteration, found in 9 cases (of which 4 were index patients), while absent in databases from large-scale sequencing projects, acts as a variant hotspot in the present Polish cohort and correlates with a younger age at HCM diagnosis. These findings, if confirmed in a wider population of the same ethnic origin, will increase the number of truncating founder *MYBPC3* alterations.

The identification of the novel homozygous null *CSRP3* variant leading to HCM suggests that the autosomal recessive inheritance pattern could be more frequent in HCM than reported so far. In heterozygosity, the null *CSRP3* allele seems to correlate only with a mild thickening of the intraventricular septum.

## SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

## ARTICLE INFORMATION

**NOTE** For references 51–67, see Supplementary material at www.mp.pl/paim.

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