Supplementary Tables Details on the qPCR assay and PCR, sequencing and RFLP reactions and the variants found in this study.

Supplementary Table 1 Primers, probes, program and mixes of the qPCR assay.

Primers | probes	***MYBPC3* c.91G>C**	***MYBPC3* c.2455C>T**
Forward primer	5'-GAAGCCAAGGTCAGTG-3'	5'-GACCAGAGCTCCTGTC-3'
Reverse primer	5'-CTAGGCCATACTTGTCAC-3'	5'-CGTAGACTCGCATCTCA-3'
Wild-type probe	5'-FAM-TGTCTCGG**C**CTCGAA-BHQ1-3'	5'-FAM-TTCAGCC**G**CATCCACC-BHQ1-3'
Variant probe	5'-TEXASRED-TGTCTCGG**G**CTCGAA-BHQ2-3'	5'-HEX-AGTTCAGCC**A**CATCCACC-BHQ1-3'

qPCR program	**c.91G>C qPCR mix**	**c.2455C>T qPCR mix**
14'40'' - 95 °C	4.9 µl H_2O	4.9 µl H_2O
00'20'' - 95 °C 00'40'' - 59 °C Signal detection } 40x	1.0 µl 10x Key Buffer 1.0 µl Primers (5 µM each) 0.4 µl Wild-type probe (10 µM) 0.4 µl Variant probe (10 µM) 0.2 µl dNTPs (10 mM each) 0.1 µl Polymerase (5 U/µl) 2.0 µl Template	1.0 µl 10x Key Buffer 1.0 µl Primers (5 µM each) 0.5 µl Wild-type probe (5 µM) 0.3 µl Variant probe (5 µM) 0.2 µl dNTPs (10 mM each) 0.1 µl Polymerase (5 U/µl) 2.0 µl Template
	10.0 µl Total volume	10.0 µl Total volume

Supplementary Table 2 PCR primer pairs with the location where each primer binds, the lengths of the amplicons and the respective PCR mixes and programs, as depicted in Supplementary Table 3. Locations are identified like in ENSFCAT00000002530.5 for *MYBPC3* and ENSFCAT00000009094.5 for *MYH7*.

***MYBPC3* cDNA primer pairs**	Location	Amplicon length	PCR mix	PCR program
1f: 5'- TCCTTGGGTGGCCTGTGACT-3'	5' UTR	745	1	1
1R: 5'-TGCGAAAGTAGTCTGGGCATCTGT-3'*	Exon 6			
2f: 5'-TGACCCCATCGGCCTCTTTGTG-3'*	Exon 4	810	1	1
2r: 5'-GCACCCACGGACTCGAAGATGT-3'*	Exon 14			
3f: 5'-GGGCATGAAGCGAGACGAGAAGA-3'*	Exon 11	638	1	1
3r: 5'-ACTTGAACACCGCCTGGTCCTT-3'*	Exon 17			
4f: 5'-ACGGGCAGAGACACCACCTCAT-3'*	Exon 16	697	1	1
4r: 5'-GTGAAGATGCTGCGGTCCTTGGT-3'*	Exon 22			
5f: 5'-AGCTACGCCTGGATGTCCCTATCT-3'*	Exon 20	729	1	1
5r: 5'-ACTCCACGCTGTAGCCATCCAA-3'*	Exon 25			
6f: 5'-TGATTGAGGGCGTGGTGTATGAGA-3'	Exon 24	860	2	1
6r: 5'-AGTGCGACGGTAATGTTCCAAGACA-3'*	Exon 30			
7f: 5'-TGCTGCGGATCGAGAACATGGA-3'*	Exon 28	865	1	1
7r: 5'-AGGCCCGCTCACCTTAATTGC-3'	3' UTR			
***MYH7* cDNA primer pairs**	Location	Amplicon length	PCR mix	PCR program
8f: 5'-TGCTCTGTCTTTCCTTGCTGCTCT-3'*	Exon 1	858	1	2
8r: 5'-TTCCGGTCGCCCCAAAATGGA-3'	Exon 9			
9f: 5'-GGGATCGCAGCAAGAAGGAGCA-3'*	Exon 7	747	1	2
9r: 5'-GCTTGGTCTCCAGGGTGGCATT-3'	Exon 14			
10f: 5'-CATGTACAAGCTGACGGGTGCCAT-3'*	Exon 12	958	1	2
10r: 5'-GGGATGTGTAGAGCGCAAGTTGGT-3'*	Exon 18			

Supplementary Table 2 (continued)

***MYH7* cDNA primer pairs**	Location	Amplicon length	PCR mix	PCR program
11f: 5'-GTCCTCCCTCAAGATGCTCAGTAACC-3'*	Exon 16	859	1	2
11r: 5'-GTTGTCTTGTTCCGCCTGCACTT-3'	Exons 22 &23			
12f: 5'-GCTGCTGGGCTCCCTAGACATT-3'*	Exon 20	844	1	2
12r: 5'-GACTTTGGCCTTGGTCAGGGTGTT-3'	Exon 24			
13f: 5'-TGGAGAAGGAGAAGCATGCAACAGA-3'*	Exon 23	936	2	2
13r: 5'-GGCTGGTGAGGTCGTTGACAGAA-3'	Exon 28			
14f: 5'-AACCTGCAGCGTGTGAAGCAGAA-3'*	Exon 27	964	1	2
14r: 5'-GGACTTTCTCCAGCTCATGGATGGTT-3'	Exon 33			
15f: 5'-CCATCCAGAGGACAGAGGAGCTTGA-3'*	Exon 30	805	1	2
15r: 5'-TGGAGACCCTTGACTTGCTTCTGG-3'	Exon 34			
16f: 5'-AACCATCCATGAGCTGGAGAAAGTCC-3'*	Exon 33	615	1	2
16r: 5'-TGGTTGATGAGGCTGGTGTTCTGG-3'	Exons 35 & 36			
17f: 5'-CCGTGCCAACGACGACCTGA-3'*	Exon 35	918	2	2
17r: 5'-TGCTTCATCCAAAGGGGCTGCT-3'*	Exon 40			
***MYH7* gDNA primer pair for PCR-RFLP**	Location	Amplicon length	PCR mix	PCR program
18f: 5'- GGTAACGACCACGGCGGGAGA-3'	Intron 37-38	376	1	3
18r: 5'- CGCTCCTCTGCCTCATCCAGCTC-3'	Exon 39			

*These primers were used as sequencing primers.

Supplementary Table 3 PCR and sequencing mixes and programs.

PCR mix 1	**PCR mix 2**	**Sequencing mix**
5.7 µl H_2O	4.7 µl H_2O	2.0 µl H_2O
1.0 µl 10x Key Buffer	1.0 µl 10x Key Buffer	2.0 µl 5x Sequencing buffer
1.0 µl Primers (5 µM each)	1.0 µl GC-rich	2.0 µl GC-rich
0.2 µl dNTPs (10 mM each)	1.0 µl Primers (5 µM each)	1.5 µl Sequencing primer (2 µM)
0.1 µl Polymerase (5 U/µl)	0.2 µl dNTPs (10 mM each)	0.5 µl RR-mix
2.0 µl Template	0.1 µl Polymerase (5 U/µl)	2.0 µl Template
10.0 µl Total volume	2.0 µl Template	10.0 µl Total volume
	10.0 µl Total volume	

PCR program 1	**PCR program 2**	**PCR program 3**	**Sequencing program**
14'00'' - 95 °C	14'15'' - 95 °C	14'30'' - 95 °C	2'00'' - 95 °C
01'00'' - 95 °C } 40x	00'45'' - 95 °C } 40x	00'30'' - 95 °C } 30x	0'20'' - 95 °C } 30x
01'00'' - 65 °C	00'45'' - 64 °C	00'30'' - 66 °C	4'00'' - 65 °C
02'00'' - 72 °C	01'30'' - 72 °C	01'00'' - 72 °C	Hold - 4 °C
05'00'' - 72 °C	05'00'' - 72 °C	02'00'' - 72 °C	
Hold - 15 °C	Hold - 15 °C	Hold - 15 °C	

Supplementary Table 4 Restriction digest mix and reaction conditions.

Restriction digest mix	**Reaction conditions**
1.0 µl NEBuffer 4	Temperature: 37 °C
2.0 µl *Bse*RI (5 U/µl)	Duration: > 6 hours
7.0 µl Template	
10.0 µl Total volume	

Supplementary Table 5 Variants found in *MYBPC3* and *MYH7* of the described case.

***MYBPC3* variants (XM_019812396.1)**			***MYH7* variants (XM_006932746.4)**		
c.220G>A	p.(Ala74Thr)	heterozygote	c.975T>C	p.(Asp325=)	heterozygote
c.414T>C	p.(Ser138=)	homozygote	c.1128C>T	p.(Asp376=)	heterozygote
c.772G>A	p.(Val258Ile)	heterozygote	c.1572T>C	p.(Ile524=)	heterozygote
c.1032T>C	p.(Arg344=)	heterozygote	c.1719G>A	p.(Pro573=)	heterozygote
c.1311C>T	p.(Gly437=)	heterozygote	c.3546G>A	p.(Thr1182=)	homozygote
c.1326C>T	p.(Ser442=)	heterozygote	c.4053A>G	p.(Thr1351=)	heterozygote
c.1956C>T	p.(Arg652=)	heterozygote	c.4308T>C	p.(Asn1436=)	heterozygote
c.2765C>T	p.(Pro922Leu)	heterozygote	c.4314C>T	p.(Ala1438=)	heterozygote
c.2847A>G	p.(Ala949=)	homozygote	c.4815G>A	p.(Thr1605=)	homozygote
c.3109G>A	p.(Ala1037Thr)	heterozygote	c.5647G>A	p.(Glu1883Lys)	heterozygote
c.3267A>G	p.(Gln1089=)	heterozygote			

Supplementary Table 6 Variants in *MYBPC3* and *MYH7* of the 8 other HCM-affected cats where the coding regions of these genes were sequenced. These cats were 5 Domestic Shorthairs, 2 British Shorthairs and one crossbreed Domestic Shorthair x Persian. The allele frequency in this group of 8 cats is given for each variant. None of these variants was considered to cause HCM. All variants were archived in the EVA database together with the variants in the described case (https://www.ebi.ac.uk/eva/?Study-Browser&browserType=sgv; project ID: PRJEB30318; analysis ID: ERZ795310).

***MYBPC3* variants (XM_019812396.1)**			***MYH7* variants (XM_006932746.4)**		
c.175G>A	p.(Ala59Thr)	0.0625	c.85C>A	p.(Arg29=)	0.0625
c.220G>A	p.(Ala74Thr)	0.125	c.975T>C	p.(Asp325=)	0.4375
c.311C>T	p.(Pro104Leu)	0.0625	c.1128C>T	p.(Asp376=)	0.25
c.414T>C	p.(Ser138=)	1.0	c.1572T>C	p.(Ile524=)	0.375
c.772G>A	p.(Val258Ile)	0.25	c.1719G>A	p.(Pro573=)	0.1875
c.1032T>C	p.(Arg344=)	1.0	c.1746C>T	p.(Tyr582=)	0.1875
c.1806C>T	p.(Asp602=)	0.25	c.1872T>C	p.(Tyr624=)	0.0625
c.1956C>T	p.(Arg652=)	1.0	c.2289G>A	p.(Val763=)	0.125
c.2095G>A	p.(Ala699Thr)	0.0625	c.2886C>T	p.(Ala962=)	0.0625
c.2607C>T	p.(Pro859=)	0.3125	c.2943A>G	p.(Glu981=)	0.125
c.2765C>T	p.(Pro922Leu)	0.9375	c.3132G>T	p.(Val1044=)	0.0625
c.2847A>G	p.(Ala949=)	0.875	c.3171C>T	p.(Gly1057=)	0.0625
c.2976C>A	p.(Leu992=)	0.0625	c.3459C>T	p.(Ala1153=)	0.1875
c.3109G>A	p.(Ala1037Thr)	0.9375	c.3546G>A	p.(Thr1182=)	0.1875
c.3126C>T	p.(Tyr1042=)	0.125	c.3813C>T	p.(Asn1271=)	0.125
c.3267A>G	p.(Gln1089=)	0.0625	c.4053A>G	p.(Thr1351=)	0.3125
c.3388A>G	p.(Ile1130Val)	0.0625	c.4200C>G	p.(Ala1400=)	0.125
c.3525C>T	p.(Asp1175=)	0.1875	c.4308T>C	p.(Asn1436=)	0.25
c.3799C>T	p.(Leu1267=)	0.0625	c.4314C>T	p.(Ala1438=)	0.125
			c.4509A>G	p.(Lys1503=)	0.8125
			c.4815G>A	p.(Thr1605=)	0.9375
			c.4959C>T	p.(Thr1653=)	0.3125
			c.5106G>A	p.(Ala1702=)	0.1875
			c.5550C>G	p.(Leu1850=)	0.25