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The «Amish» NM_000256.3:c.3330+2T>G splice variant in *MYBPC3* associated with hypertrophic cardiomyopathy is an ancient Swiss mutation

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ABSTRACT

MYBPC3 is the most frequently mutated gene in hypertrophic cardiomyopathy (HCM). Several loss-of-function founder variants have been reported in *MYBPC3* from various geographic regions, altogether suggestive of a modest or absent effect of these variants on reproductive fitness. One of them, a *MYBPC3* splice variant, NM_000256.3:c.3330+2T > G, was first described in homozygous state in newborns presenting with a severe, recessive form of HCM among the Amish population and was later associated with adult-onset dominant HCM in heterozygous carriers. We here report this splice variant in heterozygous state in eight unrelated Swiss families with HCM, making it the most prevalent cardiomyopathy variant in western Switzerland. This variant was identified in patients using targeted (n = 5) or full-genome sequencing (n = 3). Given the prevalence of this variant in the Old Order Amish, Mennonites and Swiss populations, and given that both Amish and Mennonites founders originated from the Bern Canton in Switzerland, the *MYBPC3*, NM_000256.3:c.3330+2T > G variant appears to be of Swiss origin. Neighboring regions that hosted the first Amish settlements (Alsace, South Germany) should be on the lookout for that variant. The existence of *MYBPC3* founder variants in different populations suggests that individuals with early-onset clinical disease may be the tip of the iceberg of a much larger number of asymptomatic carriers. Alternatively, reproductive fitness could even be slightly increased in some variant carriers to compensate for the reduction of fitness in the more severely affected ones, but this remains to be investigated.

1. Introduction

Inherited cardiomyopathies are associated with a significant risk for heart failure, arrhythmias, sudden cardiac death (SCD) and stroke. Among these, HCM is the most common with a revised prevalence of up to 1 in 200 individuals in the general population (Semsarian et al., 2015). HCM is mostly inherited as an autosomal dominant trait but with variable penetrance and expressivity. Moreover, it is genetically heterogenous: variants in at least 11 genes, mostly encoding sarcomeric proteins, have been associated with the disease (Alfares et al., 2015; Ingles et al., 2019; Walsh et al., 2017). *MYBPC3* is the most prevalent HCM-associated gene, accounting for over 40% of all reported HCM variants. Most *MYBPC3* mutations (~75%) are loss-of-function (LoF) variants, all leading to absent or truncated protein and therefore lacking the major binding domains to other sarcomeric proteins. Interestingly, *MYBPC3* mutations are associated with a relatively favorable disease

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outcome and a lower disease penetrance as only 58% of adult carriers below 50 are symptomatic; overall survival also seems to be increased compared to other genes associated with HCM, such as *MYH7* (Christiaans et al., 2010; Niimura et al., 2002). Such an attenuated disease prognosis is compatible with more favorable reproductive fitness and therefore less stringent negative selection and higher carrier frequency rates. In line with the latter, a palette of founder mutations in *MYBPC3* has been described with an allelic frequency among local populations of up to 0.36% (Carrier, 2021).

A few cases with biallelic *MYBPC3* mutations have also been reported with a much more severe, neonatal HCM phenotype (Deprez et al., 2006; Xin et al., 2007; Zahka et al., 2008). We here focus on the *MYBPC3* splice site mutation NM_000256.3:c.3330+2T > G running in eight unrelated Swiss families with dominant HCM, which was originally reported in homozygous state in Amish newborns with severe HCM (Xin et al., 2007). Based on our inpatient clinic population, we find that this is likely the most frequent *MYBPC3* and even HCM-causing mutation in western Switzerland. Haplotype reconstruction indicates that NM_000256.3:c.3330+2T > G is a variant of Swiss origin whose common ancestor dates back to at least 200 years ago and traveled to the US with the first Amish founders. We also highlight that, unlike with other *MYBPC3* variants, the associated HCM phenotype is not particularly mild given that two of our patients underwent heart transplantation before age 50.

2. Subjects and methods

2.1. Patients

Five unrelated probands were referred because of HCM to our multidisciplinary cardiogenetic clinic. All probands underwent a thorough clinical evaluation including electrocardiogram, transthoracic echocardiography, and MRI. A confirmed diagnosis of HCM (left ventricular wall thickness >15 mm; ref. 11,12) by the cardiology division prompted genetic investigations (see Table 1). Three other unrelated patients, with a confirmed diagnosis of HCM, were enrolled based on a familial history of primary cardiomyopathy as part of an independent research study on sudden cardiac death (SCD) susceptibility (Table 1). For all eight individuals, a dedicated, written informed consent – for either clinical diagnostic or research purposes, respectively – was obtained, as approved by local ethical review boards (Ethics Board of the Lausanne University Hospital, Research Ethics Board from Vaud Canton) and in compliance with Swiss laws.

2.2. Exome and whole genome sequencing

Genetic testing strategies varied based on the date and channel through which each patient was recruited. For the five patients seen in the cardiogenetics clinic, blood was obtained for molecular analysis in the context of the diagnostic pathway. Genomic DNA was isolated from peripheral blood. Library preparation was performed using the Illumina TruSight One, TruSight One Expanded or Twist Exome capture kit, and sequencing was performed on an Illumina MiSeq or NextSeq platform, respectively. Raw sequencing data was then processed on-site using routine diagnostic bioinformatic pipelines. For patient 3, a 20-year old girl, segregation studies were performed that showed the presence of the variant in her brother, who also had HCM, and in her mother who was clinically asymptomatic and had a normal cardiac ultrasound. The remaining three patients had been enrolled in a research study and their DNA samples were available at the CHUV institutional biobank. DNA extraction, normalization and quality controls were performed at the CHUV biobank laboratory. Whole-genome sequencing (WGS) was achieved at the Health2030 Genome Center in Geneva using Illumina TruSeq PCR-free library preparation protocols, which include: DNA fragmentation, end-repair, ligation of sequencing adapters and barcodes, size selection of 600-bp fragments, quantitation and normalization of individual libraries. Normalized libraries were loaded to a NovaSeq machine from Illumina for a 150-bp paired-end sequencing run, aiming for a minimal 30X final depth of coverage. Raw sequencing data was then sent to us for further processing. All *MYBPC3* variants were subsequently confirmed using Sanger sequencing at our clinical-grade diagnostic laboratory, prior to disclosure for research participants.

2.3. Bioinformatic pipelines

For patients enrolled from the clinic, raw data processing, variant annotation and variant prioritization were performed as described previously (Royer-Bertrand et al., 2015). For the remaining three patients with full genome investigation, the analytic pipeline was standard and followed GATK best practices (Van der Auwera et al., 2013). For each sample, raw read pairs were converted to unmapped BAMs and then aligned to the human reference genome version hg38 using BWA-MEM v0.7.17 (Li, 2013), independently per sequencing lane and flowcell. Resulting BAM files were merged and read duplicates were marked using PicardTools v2.21.8 MarkDuplicates. Deduplicated BAM files were sorted and indexed using PicardTools v2.21.8 SortSam. Base quality score recalibration was modeled and run using GATK v4.1.3

Table 1
Clinical information for all herein reported individuals carrying the *MYBPC3* NM_000256.3:c.3330+2T > G variant.

	Fam 1	***Fam 2***	***Fam. 3***			***Fam. 7***	***Fam. 8***	***Fam. 4***	***Fam. 5***	***Fam. 6***
	III.2	***III.3***	***IV.1***	***IV.2***	***III.2***	***III.1***	***III.2***	***III.1***	***III.3***	***III.1***
Age	49	65	18	15	50	25	74	65	55	76
Sex	M	M	F	M	F	M	M	M	M	M
Age at diagnosis	41	63	1.5	6	–	–	65	45	50	42
First symptoms	Dyspnea on exertion	Dyspnea upon awakening	Dyspnea on exertion	VSD	None	Rarely palpitations and thoracic pain	Palpitations and thoracic pain	Syncope	Dizziness on exertion	Dyspnea on exertion
Diagnosis	NO-HCM	NO-HCM	NO-HCM	NO-HCM	Normal	NO-HCM	NO-HCM	NO-HCM	NO-HCM	NO-HCM
LV WT (mm)	16	18	28	14	<10	14	21 (septal)	18	19	27
LV EF	34	58	Lower bound	60	Normal	75	62	75	66	65
LVOT > 30 mmHg	No	No	No	No	No	No	No	No	No	No
Syncope	Yes	No	No	No	No	No	No	Yes	No	No
Arrhythmia	Yes	Yes	No	No	No	No	Yes	No	No	Yes
Heart transplant	Yes	–	Yes	–	–	–	–	–	–	–
Genetic test	HCM panel	HCM panel	HCM panel	Sanger	Sanger	HCM panel	HCM panel	WGS	WGS	WGS

LV WT: left ventricular wall thickness; LV EF: left ventricular ejection fraction; LVOT: left ventricular outflow tract gradient; NO-HCM: non-obstructive hypertrophic cardiomyopathy; VSD: ventricular septal defect; WGS: whole genome sequencing.

BaseRecalibrator and GATK v4.1.3 ApplyBQSR. Variant calling was performed individually using GATK v4.1.3 HaplotypeCaller, producing one gVCF file per sample.

Genotyping was performed jointly for the full cohort of >100 samples recruited for this research study using GATK v4.1.3 GenotypeGVCFs (Poplin et al., 2018), producing a unique multi-sample gVCF. Indels and single-nucleotide variants (SNVs) were recalibrated using GATK v4.1.3 VariantRecalibrator and ApplyVQSR. Genotypes were refined with GATK CalculateGenotypePosteriors, using 1 KG VCF files as a gold standard. Low quality variants (read depth <10 and genotype quality <20) were flagged. FASTQ, BAM and VCF files metrics were gathered using PicardTools v2.21.8 CollectQualityYieldMetrics, CollectMultipleMetrics and CollectWgsMetrics, GATK v4.1.3 CollectVariantCallingMetrics, and FastQC (Andrew, 2010). Coverage metrics were obtained using BEDTools v 2.29.2 and Mosdepth (Quinlan, 2014; Pedersen and Quinlan, 2018). Sample relatedness, sex-check and ancestry inference were performed using Peddy (Pedersen and Quinlan, 2017).

The final list of variants was annotated with ANNOVAR and vcfanno (Wang et al., 2010; Pedersen et al., 2016) using external databases. Variants were then ranked and prioritized based on population and cohort allelic frequencies, predicted gene impact, and association to disease. Only variants in genes previously associated with a cardiac phenotype (approximately 600 genes) were screened to minimize the risk of incidental findings.

2.4. Haplotype reconstruction

We first used whatsHap to construct haplotype blocks of nearby heterozygous variants using sequencing reads from the bam/cram files aligned on hg19 for WES data (patients 1, 2, 3a, 7, 8), or on hg38 for WGS data (patient 4, 5, 6) (Patterson et al., 2015). Pre-phased genotypes in VCF files were then passed to shapeit4.0 and compared to population-based phasing calls in order to reconstruct reliable haplotype estimates for chromosome 11 (Delaneau et al., 2019). Genetic recombination maps for hg19 and hg38 were obtained from shapeit4. We used phasing data from the 1000 Genomes Project phase 3 as a reference (3′202 samples total). Final phased data from patients 1, 2, 3a, 7, 8 was then lifted-over to hg38 to be collated to phased data from patients 4–6. WES data from the first three patients were much scarcer and the vast majority of SNP genotypes were missing (see Table 2). Final, reconstructed haplotypes containing the *MYBPC3* variant were then extracted and compared between patients in order to extract the shared region of identity-by-descent (IBD).

2.5. Estimation of mutation age by IBD

We assumed that the shared IBD haplotype is derived from a common ancestor across the six patients. To estimate the age of that common ancestor, we used the size of this shared haplotype as a proxy for the number of meiotic recombination steps that occurred since this ancestor. We used the following formula (Budde et al., 2008):

$$L_{hap} = 200 / K = 200 / (A \times c)$$

where L_{Hap} is the length of the shared IBD region in centimorgans, K is the number of meioses in the descent tree until coalescence, A = age in generations, c = number of copies of the shared haplotype. Solving for A gives an estimate of the age of the common ancestor. We used genetic recombination maps for hg38 to extract the haplotype length into centimorgans.

With the scarcer data yet from eight patients, the smallest IBD region is of 4.9 Mb (first to last common genotypes across all eight patients), and the largest IBD region is of 11.3 Mb (first to last discording genotypes between patients). As there are increased IBD sharing signals on both sides of the centromere, this yields to minor differences in terms of centimorgans and therefore in age estimation:

- $A_{AllPatients\_LowerBound} = 200/(3.63\ cM \times 8) = 6.9$ generations
- $A_{AllPatients\_UpperBound} = 200/(3.86\ cM \times 8) = 6.5$ generation.

With exhaustive genotyping data but from only three patients:

- $A_{ThreePatients} = 200/(3.8 \times 3) = 17.5$.

Assuming 25 years per generation, this traces the last common ancestor back to ~160–170 years ago, therefore in the 19th century.

3. Results

3.1. Same MYBPC3 splice variant identified in eight unrelated Swiss individuals with HCM

Analysis of WGS data from 100 cases with either primary cardiomyopathies (HCM: n = 30, dilated cardiomyopathy – DCM: n = 11, arrhythmogenic right ventricular dysplasia - ARVD: n = 11), arrhythmias (LQTS: n = 13, Brugada syndrome: n = 8), or early-onset SCD (n = 27) identified the same heterozygous variation in *MYBPC3* (NM_000256.3:c.3330+2T > G) in three unrelated male patients (cf. Fig. 1). A parallel screening of our patient database from the cardiogenetic clinic identified five additional, unrelated heterozygous carriers, all presenting with an HCM phenotype (cf. Table 1). The affected sibling of the index case from Family 3 as well as the clinically unaffected mother were later confirmed to also carry the *MYBPC3* variant. Interestingly, mutation carriers from all eight families express a variable disease course, with members of Family 1 and 3 presenting with a more severe phenotype than typically reported for *MYBPC3* variants. Indeed, among them, two members underwent heart transplantation at 46 and 18 years old respectively. The *MYBPC3* variant was clinically reported as *pathogenic* to all patients and families.

This splice site variant was originally reported in homozygous state in newborns with severe, lethal HCM disease among the Amish population (Xin et al., 2007; Zahka et al., 2008). Some probands' relatives presented some HCM-related symptoms such as chest pain, fatigue and palpitation, or even SCD suggesting that heterozygous carriers could be at risk of cardiac disease (Xin et al., 2007). A subsequent study specifically investigating heterozygous carriers of this mutation within the Amish population of Ohio showed that they indeed also harbored HCM features (De et al., 2011). Transcript studies demonstrated that this variant results in the introduction of a premature stop codon via skipping of exon 30, thus leading to a frameshift variant (NP_000247.2:p.Asp1064Glyfs*38), confirming its classification as a Pathogenic variant for dominant HCM according to ACMG guidelines (applied criteria: PVS1, PP1, PM2).

3.2. Most frequent MYBPC3, HCM-associated variant in western Switzerland

Approximately 170 individuals with HCM have visited the Cardiogenetics clinic at our hospital. Among those who underwent genetic testing, only a few pathogenic variants were observed more than once in our cohort (e.g. *MYH7*: NM_000257.4:c.2652_2654del (p.Lys884del) and *MYBPC3*: NM_000256.3:c.2905C > T (p.Gln969Ter) observed four and three times respectively in presumably unrelated families). The *MYBPC3,* NM_000256.3:c.3330+2T > G splice variant is thus remarkable because it was observed in five apparently unrelated families from our clinic, as well as in three unrelated individuals from our parallel research study. This is the most common disease-causing variant in our HCM patient population (*MYBPC3* splice variant allelic frequency in our inpatient clinic cohort: 5/340 = 1.47E-02, expected carrier frequency: 2.92E-02), leading to an extrapolated carrier frequency of about 1.46E-04 and corresponding allelic frequency of 7.3E-05 within the Swiss population when considering an HCM prevalence of 1 in 200 (Semsarian et al., 2015). This is likely an underestimate given the incomplete

Table 2
Haplotypes associated with the *MYBPC3* NM_000256.3:c.3330+2T > G variant across patients.

CHR	POSITION	RS#	REF/ALT	MAF (EUR)	Patient #1 Fam. 1. III.2		Patient #2 Fam. 2. III.3		Patient #3a Fam. 3 IV.1		Patient #7 Fam. 7 III.1		Patient #8 Fam. 8 III.2		Patient #4 Fam. 4 III.1		Patient #5 Fam. 6 III.1		Patient #6 Fam. 5 III.3		
chr11	44107536	rs11037864	A/T	32.4%	0	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	
chr11	44264044	rs149719812	CTT/-	10.2%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0	1	0	0	0	0	
chr11	44264307	rs4755796	C/T	23.5%	0	L.C.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0	1	0	0	0	0	
chr11	44264337	rs4755797	A/G	31.3%	0	L.C.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0	1	0	1	1	1	*
chr11	44267521	rs12419361	G/A	10.6%	0	1	0	0	0	1	N.A.	N.A.	L.C.	1	0	0	0	0	0	0	
chr11	44267762	rs10769024	G/C	40.6%	0	0	1	1	1	0	1	1	1	0	1	0	1	0	1	0	**
chr11	44275278	rs11037927	C/T	48.3%	1	0	1	0	1	1	1	0	1	0	1	0	1	1	1	1	
chr11	44297967	rs77191467	A/G	3.1%	N.A.	N.A.	1	L.C.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	44482563	rs146250581	A/G	0.7%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	44495956	rs57046569	G/A	6.6%	N.A.	N.A.	1	L.C.	N.A.	N.A.	L.C.	0	N.A.	N.A.	1	1	1	0	1	0	
chr11	44497812	rs145767164	G/A	0.3%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	L.C.	0	1	0	1	0	1	0	
chr11	44618466	rs2303861	G/A	28.2%	N.A.	N.A.	0	L.C.	0	1	0	1	0	1	0	0	0	0	0	1	
chr11	44738935	rs74980176	A/G	2.8%	N.A.	0	1	L.C.	L.C.	0	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	44796080	rs1563054	T/C	24.2%	N.A.	N.A.	1	L.C.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	44983492	rs35813597	G/A	0.5%	N.A.	N.A.	N.A.	N.A.	1	L.C.	N.A.	N.A.	L.C.	0	1	0	1	0	1	0	
chr11	45011815	rs184724151	A/T	0.3%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	45119606	rs113066447	C/T	0.5%	N.A.	N.A.	L.C.	0	N.A.	N.A.	N.A.	N.A.	L.C.	0	1	0	1	0	1	0	
chr11	45265773	rs113337087	G/A	0.8%	N.A.	N.A.	1	L.C.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	45839854	rs72900694	T/C	22.4%	1	L.C.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	45913702	rs2271845	C/T	30.1%	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	0	
chr11	46013837	rs924885251	C/T	0.0%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	46283644	rs55798544	C/A	24.4%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
chr11	46410414	rs180716764	G/A	0.9%	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	1	0	1	0	1	0	
chr11	46465207	rs117447018	A/T	0.4%	1	L.C.	N.A.	N.A.	1	0	1	0	1	0	1	0	1	0	1	0	
chr11	46508349	rs143880001	G/A	0.5%	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	1	0	1	0	1	0	
chr11	46895074	rs540384558	-/TACCT	3.2%	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	1	
chr11	47161522	rs11551406	C/T	0.7%	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	1	0	1	0	1	0	
chr11	47161876	rs117749721	C/G	0.9%	N.A.	N.A.	L.C.	0	1	0	1	0	1	0	1	0	1	0	1	0	
chr11	47162139	rs78112569	G/T	1.9%	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	1	0	1	0	1	0	
chr11	47162140	rs75741689	G/A	2.1%	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	1	0	1	0	1	0	
chr11	**47333192**	**MUTATION**	**A/C**	0.0%	1	0	1	0	1	0					1	0	1	0	1	0	
chr11	47350027	.	G/A	3.3%	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	
chr11	47410415	rs755555	C/T	32.4%	1	1	1	1	1	0	1	0	1	0	1	0	1	0	1	1	
chr11	47501917	rs371313278	-/T	0.5%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	47559884	rs117677903	C/T	2.5%	1	0	1	L.C.	N.A.	N.A.	N.A.	N.A.	L.C.	0	1	0	1	0	1	0	
chr11	47581177	rs2030166	C/T	35.7%	1	1	1	1	1	1	1	0	1	0	1	0	1	0	1	1	
chr11	48030993	rs78284435	T/C	1.8%	N.A.	N.A.	N.A.	N.A.	1	L.C.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	48128203	rs905475	C/T	24.4%	1	1	1	0	1	1	1	0	1	1	1	0	1	1	1	1	
chr11	48147112	rs3741410	T/G	13.4%	1	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0	
chr11	48344583	rs191713647	T/C	0.7%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	L.C.	0	N.A.	N.A.	1	0	1	0	1	0	
chr11	48364345	rs180852357	G/C	0.6%	N.A.	N.A.	L.C.	0	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	48365454	rs200471892	A/G	2.2%	N.A.	N.A.	L.C.	0	1	0	1	0	1	0	1	0	1	0	1	0	
chr11	48491245	rs118158813	C/T	0.8%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	48590372	rs72904694	T/G	2.7%	N.A.	N.A.	N.A.	N.A.	1	0	1	1	1	0	1	0	1	0	1	0	
chr11	48814949	rs535084347	A/T	0.4%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	48819919	rs181653558	G/A	2.5%	1	L.C.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	L.C.	0	1	0	1	0	1	0	
chr11	48822413	rs554711147	T/C	0.1%	L.C.	0	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	49034299	rs61743193	C/G	2.7%	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	1	0	1	0	1	0	
chr11	49149116	rs11608163	T/G	4.6%	1	L.C.	N.A.	N.A.	N.A.	N.A.	1	L.C.	N.A.	N.A.	1	0	1	0	1	0	
chr11	49175100	rs202715	T/C	13.4%	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	
chr11	49200053	rs11603728	G/A	2.7%	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	
chr11	49207061	rs202675	C/G	36.5%	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	***
chr11	49413857	rs72910356	T/G	2.5%	1	L.C.	N.A.	N.A.	N.A.	N.A.	N.A.	0	N.A.	N.A.	1	0	1	0	1	0	
chr11	49418150	rs2866496	C/A	1.8%	1	L.C.	1	L.C.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	49682977	rs192853061	C/A	0.6%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	49821529	rs139012182	T/G	4.2%	1	L.C.	1	L.C.	N.A.	N.A.	1	0	N.A.	N.A.	1	0	1	0	1	0	
chr11	49855298	rs139416665	G/C	3.5%	1	L.C.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	L.C.	0	1	0	1	0	1	0	
chr11	49874820	rs190597597	G/A	0.8%	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	1	0	1	0	1	0	
chr11	49963872	rs192136010	G/A	0.5%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	L.C.	N.A.	N.A.	1	0	1	0	1	0	
chr11	49981655	rs4598671	A/C	40.8%	N.A.	N.A.	N.A.	N.A.	0	0	0	1	0	0	0	1	0	0	0	0	
chr11	50105317	rs11601458	G/C	0.9%	L.C.	0	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	L.C.	0	1	0	1	0	1	0	
chr11	50139756	rs146995652	G/A	3.3%	N.A.	N.A.	1	L.C.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	50157441	rs144483800	A/C	3.6%	N.A.	N.A.	N.A.	N.A.	1	L.C.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	50447939	rs187618390	T/C	0.7%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	50530110	rs192586068	C/A	0.7%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	50649425	rs144476692	C/T	3.1%	N.A.	N.A.	1	L.C.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
CENTROMERE																					
chr11	54565747	rs149236977	C/T	1.2%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	54571303	rs143860428	C/T	3.1%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	54603136	rs11246609	T/C	70.6%	N.A.	N.A.	N.A.	N.A.	1	1	1	0	1	1	1	0	1	1	1	1	
chr11	55572903	rs559449	T/C	49.7%	N.A.	N.A.	N.A.	N.A.	1	1	1	0	1	1	1	0	1	1	1	1	
chr11	55595794	rs511492	C/T	24.7%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	1	1	0	****
chr11	55603905	rs75423534	G/A	13.2%	N.A.	N.A.	N.A.	N.A.	0	0	1	1	N.A.	N.A.	0	0	0	0	0	0	
chr11	55986275	rs116885398	C/A	9.1%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	0	1	0	1	0	
chr11	55986857	rs185570181	G/T	9.3%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	1	0	0	0	0	
chr11	55986887	rs116532166	T/C	9.5%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1	1	0	0	0	0	
chr11	55991501	rs7396287	G/T	88.1%	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0	0	1	1	1	1	
chr11	56043453	rs61890054	C/T	9.3%	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	

Only a selection of variants is reported in Table 3 for visualization purposes. Selected markers include the ones with: a/ MAF < 10% in non-Finnish Europeans, and/or b/ genotypes available across at least four patients. *first discordant genotype across patients 4-6 (dense, WGS data). **: first discordant genotype across patients 1, 2, 3a, 4, 5, 6, 7, 8; ***: last common genotype for all 8 patients; ****: last common genotype for patients 4-6 (dense, WGS data). N.A.: genotype unavailable because the position is not covered, L.C.: genotype unknown because region is of low coverage (<5X).

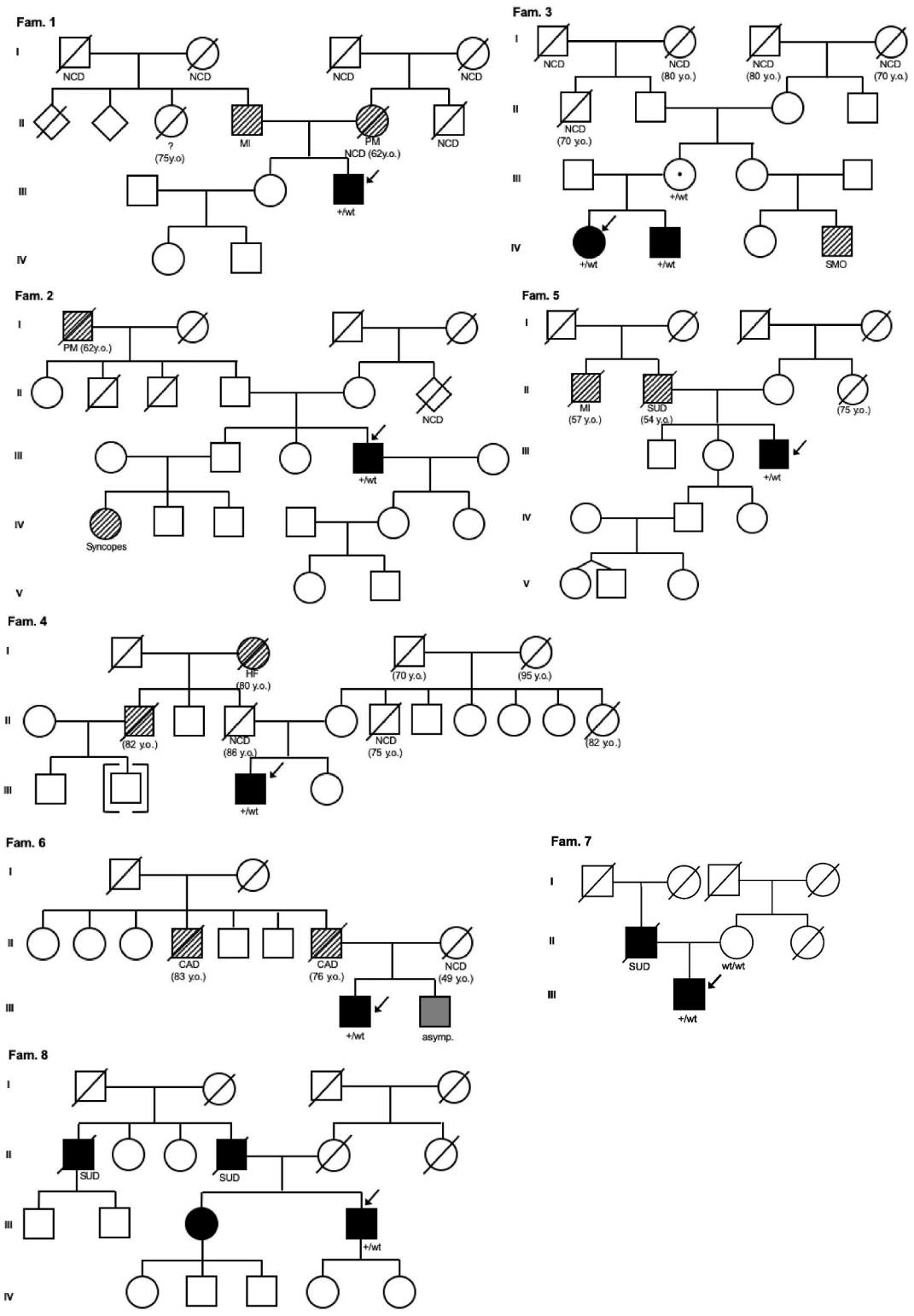

Fig. 1. Pedigrees of all eight families.

penetrance of the mutation, and given that not all HCM patients have undergone genetic testing; however, it matches closely the allelic frequency reported in Europeans from gnomAD (MAF: 7E-05 in non-Finnish Europeans). The variant allelic frequency is below the MAF threshold for ACMG BA1 criteria for *MYBPC3* variants using conservative disease attributes, which was estimated at 1.7E-4 (HCM prevalence of 1:200, variant penetrance of 60%, *MYBPC3* gene contribution in HCM of 40%, maximum allelic heterogeneity contribution of 10%, computed using https://www.cardiodb.org/allelefrequencyapp/).

3.3. Hypothesis of a founder mutation of Swiss origin

The majority of the homozygous carriers of the NM_000256.3: c.3330+2T > G mutation originate from the Geauga settlement of Ohio (McKusick et al., 1964; Greksa, 2002). Yet, multiple cases from other Amish families across the US were reported as presumable carriers,

suggesting the mutation came from a common ancestor. The NM_000256.3:c.3330+2T > G mutation was also found in one family from the Mennonite community (Xin et al., 2007). Both high prevalence and large geographic distribution are suggestive of more distant common ancestors. Interestingly, Old Order Amish and Old Order Mennonite populations share a similar geographic origin, as both emigrated from Switzerland to the US. Indeed, from 1683 to 1880, the ancestors of the present-day Old Order Mennonites and Old Order Amish emigrated in eastern Pennsylvania from Switzerland and South Germany (Puffenberger, 2003). The first Mennonite settlement in Pennsylvania dates back to the 1680s, while the first Amish settlement dates back to 1714 (Guss, 2007). Before their migration, they were mostly located in Bern Canton (Switzerland), Palatinate (southwestern Germany) and Alsace (eastern France).

Notably, all the mutation-carrying individuals and families from this study had at least one branch in their pedigrees originating from Central or Western Switzerland (cf. Fig. 2). From public databases, this variant is listed in ClinVar (VCV000008621.10), reported from diagnostic laboratories from the United States but also Germany and Switzerland. In gnomAD, the variant was exclusively observed within the non-Finnish European population. Haplotype reconstruction of this variant in our patients identified an IBD region of 3.6–3.9 centimorgans across patients (cf. Table 2), dating the last common ancestor to less than 200 years ago.

Altogether, these data suggest that the NM_000256.3:c.3330+2T > G variant is of Swiss/South-German origin, and spread through a common ancestor that we can date back to the 19th century.

4. Discussion

In this study, we report the same variant, NM_000256.3:c.3330+2T > G, in *MYBPC3* identified either by exome or whole-genome sequencing, in eight unrelated probands with HCM. Clinical examination revealed typical non-obstructive HCM phenotypes. Interestingly, two affected siblings including the patient with the earliest-onset and more severe phenotype inherited the variant from their mother, who is asymptomatic and has a normal cardiac ultrasound at age 50 years, suggesting a reduced penetrance.

This variant, with an estimated allelic frequency of 7.3E-05 in the Swiss population, appears to be the most common variant associated with dominant HCM in Switzerland. Its initial report in the Amish and Mennonite populations along with its predominance in Europeans from both diagnostic (ClinVar) and population databases (gnomADv3) all

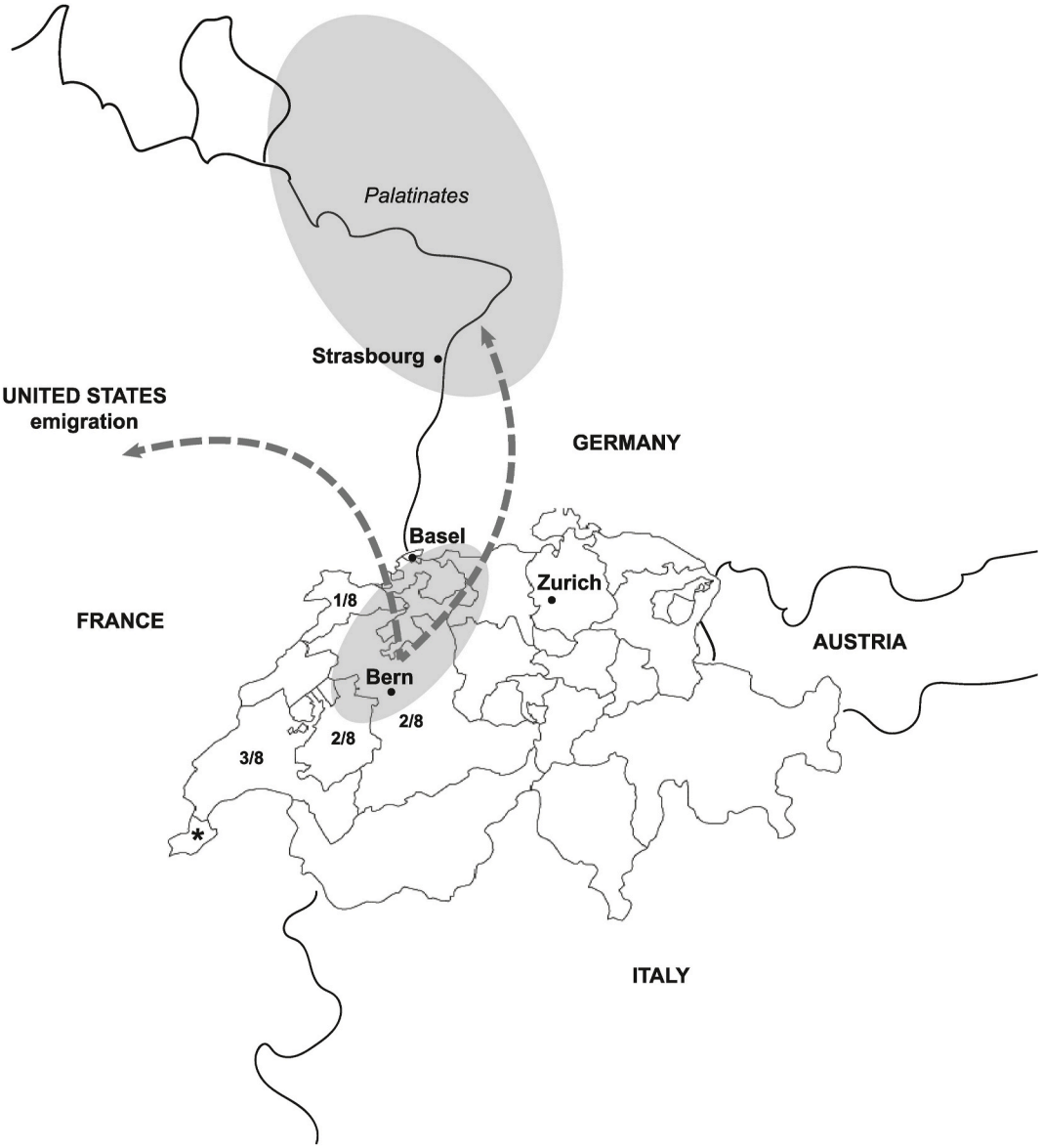

Fig. 2. Distribution of the *MYBPC3* NM_000256.3:c.3330+2T > G carriers in Switzerland and migration of the variant in Europe.

suggest that it is a founder variant whose common ancestor originated in Switzerland/Southern-Germany back in the 1800s. Given the putatively reduced penetrance of this variant, its frequency in the general population may be underestimated, meaning that many carriers are left undiagnosed. Some associated recessive cases may arise. Neighboring regions that hosted the first Amish settlements (Alsace, South Germany) should be on the lookout for that variant.

Interestingly, numerous other founder mutations in *MYBPC3* have been reported in various geographical regions across the globe: USA, Iceland, Poland, Spain, France, Finland, Japan (Kubo et al., 2005; Jaaskelainen et al., 2004; van Velzen et al., 2017; Sabater-Molina et al., 2017; Calore et al., 2015; Alders et al., 2003; Adalsteinsdottir et al., 2014, 2020). The *MYBPC3* gene itself does not seem to be a mutational hotspot (there are not many recurrent variants) but once mutations arise in a given population, they appear to be fixed in spite of their pathogenic potential. Such a significant prevalence of founder mutations in different populations raises interesting hypotheses (Semsarian et al., 2015): the phenotypic penetrance of these variants must be reduced and/or age-dependent, indicating that many carriers are probably not diagnosed (Alfares et al., 2015); the independent occurrence of *MYBPC3* LoF variants in various geographic regions and their persistence across generations indicate that overall genetic fitness is at most mildly reduced. If this is true, severe phenotypes as observed in two of our patients must be rare, perhaps being the tip of the iceberg of a much larger number of individuals who remain asymptomatic (Ingles et al., 2019). Finally, reproductive fitness could even be slightly increased in some young adults to compensate for the reduction of fitness in the more severely affected individuals. Does a *MYBPC3* HCM variant confer a transient period of cardiac « overperformance » before becoming clinically relevant? This hypothesis is purely speculative but deserves to be kept in mind.

Ethical approval

Written informed consent was obtained for all reported patients. The study was approved by the local and institutional research ethics boards (study# 2018-00328) and was performed in compliance with Swiss laws, the 1964 Helsinki declaration and its later amendments.

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Data availability

Complete genotyping data for all eight patients for the 12-Mb candidate IBD region are provided in Supplementary Table 1 (of which Table 2 is a subset, for visualization purposes). Individual sequencing data have not been made publicly available because of privacy protection.

CAD: coronary artery disease; HF: heart failure; MI: myocardial infarct; NCD: non-cardiac death; PM: pacemaker; SUD: sudden unexplained death; asymp. asymptomatic; mut: *MYBPC3:* NM_000256.3: c.3330+2T > G, wt: *MYBPC3* wild-type NM_000256.3:c.3330+2T.

The figures indicate the distribution of the eight *MYBPC3* NM_000256.3:c.3330+2T > G carrier families in Swiss cantons (* denotes one additional patient from the Geneva canton listed in ClinVar). All patients originate from Central/Western Switzerland, which is from where the Old-Order Amish and Old-Order Mennonites populations (greyed areas) emigrated to Palatinates, Strasbourg region but also the United States (Pennsylvania and Ohio in particular, where >40 mutation carriers have been identified).

CRediT authorship contribution statement

Claire Redin: Conceptualization, Formal analysis, Investigation, Supervision, Writing – original draft, Writing – review & editing. **Despina Christina Pavlidou:** Investigation, Writing – review & editing. **Zahurul Bhuiyan:** Investigation, Writing – review & editing. **Alessandra Pia Porretta:** Investigation, Writing – review & editing. **Pierre Monney:** Investigation, Writing – review & editing. **Nicola Bedoni:** Investigation, Writing – review & editing. **Fabienne Maurer:** Investigation, Writing – review & editing. **Nicole Sekarski:** Investigation, Writing – review & editing. **Isis Atallah:** Investigation, Writing – review & editing. **Davoine Émeline:** Investigation, Writing – review & editing. **Xavier Jeanrenaud:** Investigation, Writing – review & editing. **Etienne Pruvot:** Investigation, Supervision, Writing – review & editing. **Jacques Fellay:** Funding acquisition, Conceptualization, Supervision, Writing – review & editing. **Andrea Superti-Furga:** Funding acquisition, Conceptualization, Formal analysis, Investigation, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2022.104627.

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