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Spectrum of Clinical Phenotypes and Gene Variants in Cardiac Myosin-Binding Protein C Mutation Carriers With Hypertrophic Cardiomyopathy

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OBJECTIVES We studied the clinical and genetic features of hypertrophic cardiomyopathy (HCM) caused by mutations in the myosin-binding protein C gene *(MYBPC3)* in 110 consecutive, unrelated patients and family members of European descent.

BACKGROUND Mutations in the *MYBPC3* gene represent the cause of HCM in ~15% of familial cases. *MYBPC3* mutations were reported to include mainly nonsense versus missense mutations and to be characterized by a delayed onset and benign clinical course of the disease in Japanese and French families. We investigated the features that characterize *MYBPC3* variants in a large, unrelated cohort of consecutive patients.

METHODS The *MYBPC3* gene was screened by single-strand conformational polymorphism analysis and sequencing. The clinical phenotypes were analyzed using rest and 24-h electrocardiography, electrophysiology, two-dimensional and Doppler echocardiography and angiography.

RESULTS We identified 13 mutations in the *MYBPC3* gene: one nonsense, four missense and three splicing mutations and five small deletions and insertions. Of these, 11 were novel, and two were probably founder mutations. Patients with *MYBPC3* mutations presented a broad range of phenotypes. In general, the 16 carriers of protein truncations had a tendency toward earlier disease manifestations (33 ± 13 vs. 48 ± 9 years; $p = 0.06$) and more frequently needed invasive procedures (septal ablation or cardioverter-defibrillator implantation) compared with the 9 carriers of missense mutations or in-frame deletions (12/16 vs. 1/9 patients; $p < 0.01$).

CONCLUSIONS Multiple mutations, which include missense, nonsense and splicing mutations, as well as small deletions and insertions, occur in the *MYBPC3* gene. Protein truncation mutations seem to cause a more severe disease phenotype than missense mutations or in-frame deletions. (J Am Coll Cardiol 2001;38:322–30) © 2001 by the American College of Cardiology

Hypertrophic cardiomyopathy (HCM) is an autosomal-dominant disease characterized by left ventricular hypertrophy, myofibrillar disarray and the risk of premature sudden death (1,2). The disease can be caused by mutations in nine distinct genes that encode for sarcomeric proteins (3–9). More than 120 different mutations have been identified (10). Various reports suggest that some mutations are associated with characteristic phenotypes. Troponin T mutations may cause premature sudden death in patients with only mild or no left ventricular hypertrophy (11). In contrast, the clinical features of patients with mutations in the beta-myosin heavy chain (beta-MHC) gene are more heterogeneous, with some point mutations being associated with a relatively benign outcome (V606M) and others (R403Q) with a high incidence of sudden death (12).

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Since Bonne et al. (7) and Watkins et al. (8) reported the first mutations in *MYBPC3* responsible for HCM, a total of 33 mutations in this gene have been identified (7,8,13–16), which accounts for ~15% of cases of familial HCM. Most of the mutations led to premature stop codons, resulting in proteins lacking myosin- or titin-binding sites, or both (13). Two cross-sectional family studies (14,16) suggest that patients with *MYBPC3* mutations have a favorable clinical profile that is characterized by late-onset HCM and a good prognosis.

Considering the spectrum of phenotypes described in family studies, we investigated how patients with *MYBPC3* mutations were represented in a large cohort of unrelated, consecutive patients presenting with HCM at a tertiary-care referral center and which type of mutation occurred in these patients. We now present the broad spectrum of genetic variability and clinical phenotypes in 15 unrelated patients and their family members with 13 *MYBPC3* mutations, including the first founder mutation in a European cohort.

METHODS

Clinical studies and genetic background. The study is based on 110 consecutive, unrelated patients with a diagnosis of HCM, who gave written, informed consent for

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Abbreviations and Acronyms

del	= deletion
HCM	= hypertrophic cardiomyopathy
ICD	= implantable cardioverter-defibrillator
ins	= insertion
IVS	= intervening sequence (intron)
MHC	= myosin heavy chain
MyBPC	= myosin-binding protein C
MYBPC3	= myosin-binding protein C gene
PCR	= polymerase chain reaction
TASH	= transcoronary ablation of septal hypertrophy

*The single-letter amino acid code is used for description of mutations.

genotyping and detailed prospective phenotyping. The clinical diagnosis of HCM followed internationally recognized criteria. It was based on left ventricular wall thickness ≥15 mm in the absence of confounding disease on two repeated echocardiograms (as defined in the Coronary Artery Risk Development In young Adults [CARDIA] study [17]) or on the presence of borderline hypertrophy (wall thickness of 13 or 14 mm), together with other characteristic features of HCM (i.e., outflow tract gradient). Patients with earlier myectomy were accepted independently on the echocardiographic criteria. In all patients, the clinical investigation included rest and 24-h electrocardiography, prospective evaluation using two-dimensional and Doppler echocardiography, cardiac catheterization and angiography. Patients in whom symptomatic or sustained ventricular tachycardia was suspected were analyzed by electrophysiologic studies. Only life-threatening, symptomatic ventricular tachyarrhythmia was treated with an implantable cardioverter-defibrillator (ICD). In patients with outflow tract obstruction, functional deterioration toward New York Heart Association functional class III, despite long-term medication, was accepted as an indication for subvalvular myectomy or transcoronary ablation of septal hypertrophy (TASH). Family members were invited to undergo genetic analysis and clinical investigation. Nine of 110 patients were from Turkey; all other patients were of German descent. The study was approved by the Ethical Committee of the Charité, Campus Virchow-Clinic, Humboldt University of Berlin, and written, informed consent was obtained from all subjects.

Mutation detection. Isolation of deoxyribonucleic acid (DNA), polymerase chain reaction (PCR) and single-strand conformational polymorphism analysis were performed as reported previously (18). All primer sequences used in this study are available on request (from the authors). Mutations in the *MYBPC3* gene were independently confirmed in DNA samples from probands by restriction-enzyme digestion or, if not available, by repeated sequencing of independent PCR products (Table 1).

Detection of aberrant messenger ribonucleic acid (mRNA) transcripts. To identify the aberrant transcripts that resulted from the splicing mutations IVS7+1G>A, IVS20-2A>G and IVS27+1G>A, total RNA was isolated from lymphocytes. The reverse transcriptase products were amplified by PCR using primers 681F and 913R for IVS7+1G>A, primers 1672F and 2331R for IVS20-2A>G and primers 2669F and 3192R for IVS27+1G>A. The PCR products were cloned using the T/A cloning kit (Clontech), and individual clones were sequenced as described.

Genotyping markers at the *MYBPC3* locus and haplotype construction. Family members of patients 1331 and 169 (IVS7+1G>A) and patients 39 and 315 (Q1233X) were genotyped for IVS7+1G>A and Q1233X mutations, respectively. In addition, an intragenic marker in intron 20 and two *MYBPC3*-flanking markers (D11S1385 and D11S1313) covering a distance of ~0.6 cM were analyzed. Most likely, haplotypes were constructed with reference to gene and marker order on the respective chromosome, in a family context. In the case of a proband without a traced family (DNA 315), evidence of identity by descent was accepted if the alleles present allowed construction of a haplotype (designated as the "assumed haplotype") identical to the disease-associated haplotype.

Statistics. Data on clinical variables are expressed as the mean value ± SD. Student *t* and chi-square tests were used to compare groups.

RESULTS

Mutation data. By systematic mutation screening of 110 patients, 13 heterozygous sequence variants were identified in 15 patients. Of these 13 mutations, only two—insG791 and insAA1042—have been previously reported as being responsible for HCM (16); all others represent novel mutations (Table 1 and Fig. 1), two of which are probably founder mutations.

In exons 9, 17, 18 and 32, four missense mutations occurred—all located at highly conserved positions in humans, mice and chickens. To determine whether these amino acid substitutions are indeed disease-causing mutations and not just rare polymorphisms, we screened 100 chromosomes of 50 healthy blood donors as control subjects. None of the missense mutations were detected. Three mutations (IVS7+1G>A, IVS20-2A>G and IVS27+1G>A) were found in splice consensus sequences and were predicted to lead to aberrant transcripts. For each splicing mutation analysis of patients' lymphocytes, mRNA confirmed the existence of abnormal splice products. IVS7+1G>A led to two aberrant transcripts with skipping of either exon 7 alone or exons 7 and 8 (Fig. 2). Both transcripts led to frame shifts and premature stop codons in exon 9. IVS20-2A>G led to usage of a cryptic splice-donor site in exon 21 and to a premature stop codon after residue 661.IVS27+1G>A led to in-frame skipping of exon 27. In exon 34 at nucleotide 3729, a C>T

Table 1. Mutations in *MYBPC3* That Cause Hypertrophic Cardiomyopathy

Mutation	Exon/ Intron*	Nucleotide Position†	Confirmatory Method	Localization of Mutation/Functional Effects	No. of C-Terminal Residues Lacking	Previous Publication
Missense mutations						
R282W	Exon 9	C>T at nt 876	−*Aci* I	LAGGGRRIS loop/loss of phosphorylation site/change of charge		No
G507R	Exon 17	G>A at nt 1818	Sequencing	Phosphorylation site/change of charge		No
C566R	Exon 18	T>C at nt 1728	+*Tai* I	Phosphorylation site/change of charge		No
V1115I	Exon 32	G>A at nt 3375	−*Taa* I	Fibronectin III module		No
Nonsense mutations						
Q1233X	Exon 34	C>T at nt 3729	−*Cac*8I	Loss of myosin-binding site	41 AS	No
Splicing mutations						
IVS7+1G>A	Intron 7	Donor site +1 G>A	−*Tai* I	Loss of exons 7 and 7 and 8/loss of titin and myosin-binding site	989 AS 1001 AS	No
IVS20−2A>G	Intron 20	Acceptor site −2 A>G	−*Pst* I	Partial loss of exon 21/loss of titin and myosin-binding site	612 AS	No
IVS27+1G>A	Intron 27	Donor site +1 G>A	+*Nld* III	Loss of 56 residues in module C7/loss of A-band incorporation		No
Deletions and insertions						
delC390	Exon 14	Del of C at nt 1200	Sequencing	Loss of titin and myosin-binding site	869 AS	No
insG791	Exon 25	Ins of G at nt 2406	−*Tsp*RI	Loss of titin and myosin-binding site	443 AS	(16)
insAA1042	Exon 30	Ins of AA at nt 3156	Sequencing	Loss of myosin-binding site	228 AS	(16)
delG1047	Exon 30	Del of G at nt 3171	+*Tai* I	Loss of myosin-binding site	199 AS	No
insTTCA1231	Exon 34	Ins of TTCA at nt 3724	−*Mwo* I	Loss of myosin-binding site	32 AS	No

*Exon numeration according to Niimura et al. 1998 (16); †nucleotide position in complementary deoxyribonucleic acid from (19) (accession no. x84075).
del = deletion; ins = insertion; IVS = intervening sequence; nt = nucleotide.

substitution resulted in a premature termination signal (Q1233X) at codon 1233. A total of five small deletions and insertions were identified, all leading to frame shifts and predicted premature protein truncation: delC390, insG791, insAA1042, delG1047 and insTTCA1231 (Table 1).

Clinical features. The 13 different mutations were found in 15 index patients and 14 family members. The clinical features of all mutation carriers are listed in Table 2. The pedigrees are shown in Figure 3. Patients with missense mutations were particularly inhomogeneous. In three index patients (DNA samples 45, 1893 and 1119) and three siblings (DNA 1423, 1999 and 1989), the disease manifested relatively late (51 ± 6 years), with few symptoms, moderate ventricular hypertrophy and no severe arrhythmias. Nevertheless, witnessed sudden death was reported in two elder family members of patient 45. In contrast, patient 70 was diagnosed at age 30 with severe dyspnea, severe hypertrophy, ventricular wall thickness of 30 mm and excessive left atrial enlargement (diameter >70 mm). He had supraventricular arrhythmias, recurrent syncope and repeated monomorphic ventricular tachycardia, despite medical therapy. An ICD and sinoatrial ablation were required. Among the three patients with splice variants, one female patient (DNA sample 7) with an in-frame deletion of exon 27 had only a few symptoms and a modest degree of hypertrophy. Her two children, ages 37 and 32, are both carrying the mutation but exhibit no signs or only very weak signs of a clinical phenotype. In contrast, patient 14 carrying IVS20-2A>G, which led to a frame shift and a premature stop codon, presented at age 43 with moderate hypertrophy, supraventricular and severe ventricular arrhythmias. He exhibited repeated syncopal episodes and sustained symptomatic ventricular tachycardia, and he needed an ICD. The IVS7+1G>A mutation, introducing a frame shift leading to a premature stop codon in exon 9, led to a severe disease manifestation before age 30, severe hypertrophy (TASH or myectomy two times) and symptomatic arrhythmia (one ICD) in three brothers (DNA samples 1331, 1978 and 1992), but to a less severe phenotype in their mother (DNA sample 1975). The IVS7+1G>A mutation led to an almost similar phenotype in patient 169, who presented with the disease at age 24 with severe hypertrophy, angina pectoris and dyspnea and died suddenly at age 54. The five patients carrying small deletions and insertions leading to frame shifts and premature stop codons presented with a clinical phenotype at the of age 34 ± 13 years. All five index

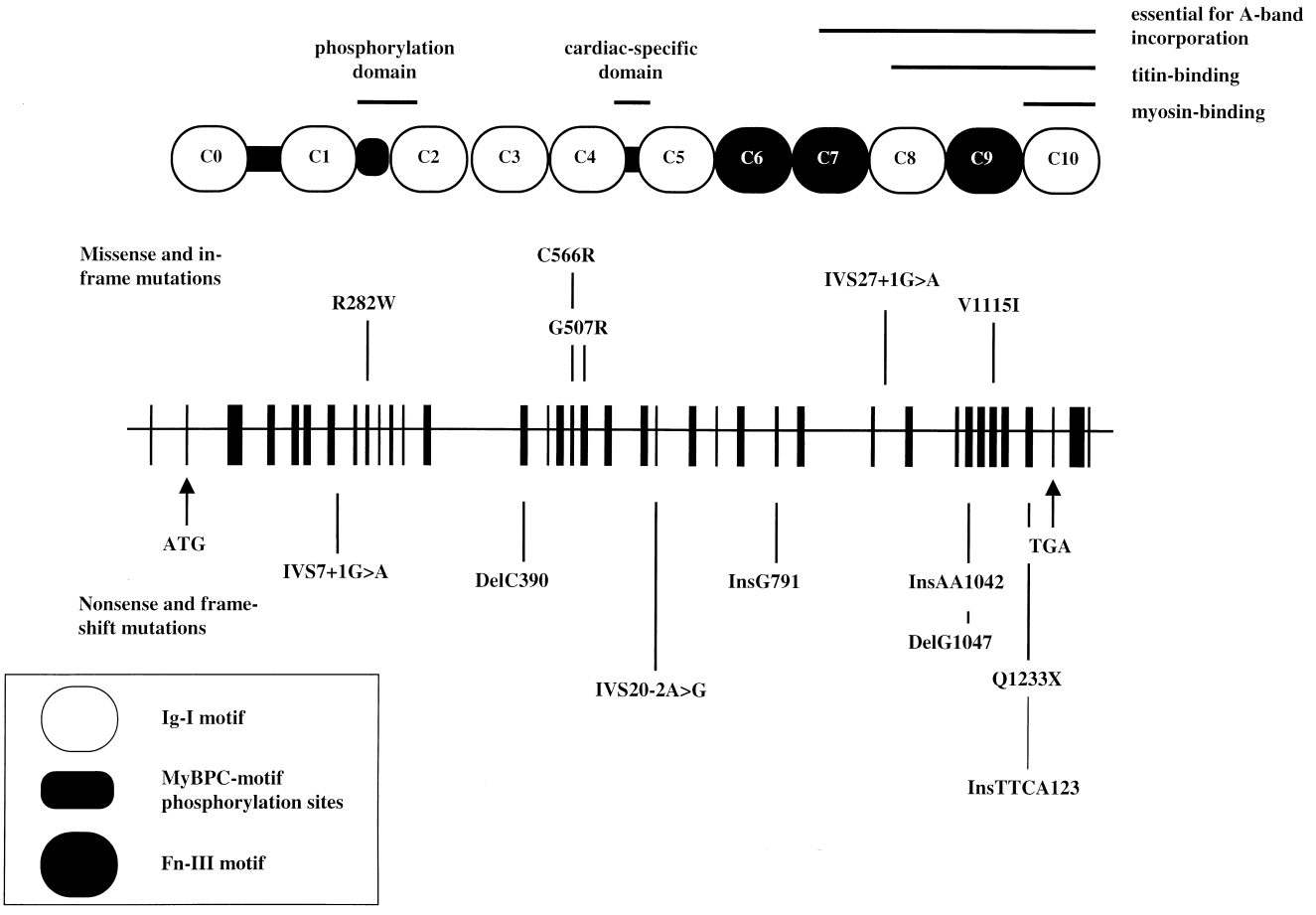

Figure 1. Diagram showing the domain structure of the myosin-binding protein C (MyBPC) polypeptide and the location of hypertrophic cardiomyopathy associated mutations identified in our patients.

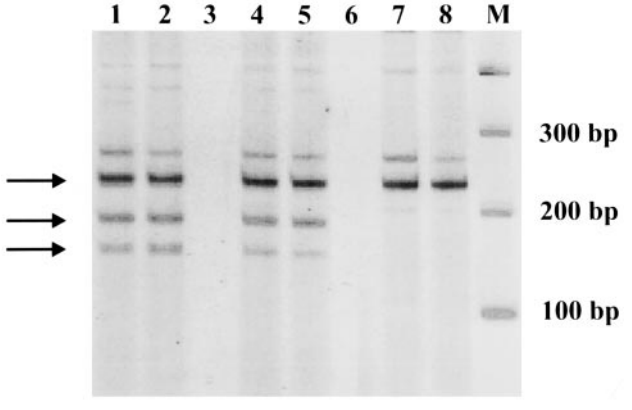

Figure 2. Electrophoresis of polymerase chain reaction (PCR) products after reverse transcriptase (RT)-PCR with primers located in exons 5/6 and 9 of the human *MYBPC3* gene showing aberrant fragments resulting from the substitution of A for G at the splice donor site of exon 7 (IVS7+1G>A). The wild-type splice product is 233–base pair (bp) in length. Aberrant splice products resulting from the IVS7+1G>A mutation are either 184-bp (loss of exon 7) or 154-bp (loss of exons 7 and 8) in length. **Lanes 1 and 2**: RT-PCR products of patient 1331 carrying the IVS7+1G>A mutation; **lane 3**: negative control using water as a template; **lanes 4 and 5**: RT-PCR products of patient 1978 carrying the IVS7+1G>A mutation; **lane 6**: negative control using water as a template; **lanes 7 and 8**: RT-PCR products of control probands carrying only wild-type alleles. M = 100-bp ladder.

patients had severe hypertrophy. In four patients, surgical septal myectomy or TASH was required. Three patients had symptomatic ventricular tachycardia, and two received an ICD. Sudden deaths occurred in the two Turkish families carrying the Q1233X mutation.

In general, patients and affected family members carrying mutations leading to protein truncation (n = 16) had a tendency toward earlier disease manifestation, compared with patients with missense mutations or in-frame deletions (n = 9) (33 ± 13 vs. 48 ± 9 years, p = 0.06). Myectomy or TASH was performed only in patients with protein truncations (7/16 vs. 0/9 patients; p = 0.02). Five of six ICDs were placed in this patient group (Table 2).

In 11 beta-MHC mutation carriers identified by our group in a separate study, the age of onset of patients with *MYBPC3* mutations seemed comparable (37 ± 10 vs. 41 ± 15 years, p = 0.3). The degree of hypertrophy (20 ± 4 vs. 17 ± 4 mm, p = 0.08) and the necessity for myectomy or TASH (6/15 vs. 2/11 patients) were also similar.

Founder mutation. The mutation IVS7+1G>A was identified in two apparently unrelated patients of German descent. Using three highly polymorphic markers, we constructed the most likely haplotype carrying the disease-causing mutation in

Table 2. Clinical Features of Index Patients and Family Members Carrying MYBPC3 Mutations

DNA	Mutation	Gender	Age at Study (yrs)	Age at Onset (yrs)	Symptoms	TASH/ Myectomy	VT* or ICD	IVS (mm)	IVS/ PW	LVEDD (mm)	FS (%)	SAM	Gradient (mm Hg)	LVEF (%)	LVEDVI (ml^2)	LVEDP (mm Hg)
Missense and in-frame mutations																
45	R282W	M	64	43	AP	No	No	16	1.3	47	46	Yes	25	65	100	12
2002	R282W	M	37	No clinical phenotype	None	No	No	nda	nda	nda	nda	nda	nda	nda	nda	nda
1989	R282W	M	60	57	AF, dyspnea	No	No	†	†	†	†	†	†	†	†	†
1999	R282W	F	65	59	Dyspnea	No	No	Ht,†	Ht,†	Ht,†	Ht,†	Ht,†	Ht,†	Ht,†	Ht,†	Ht,†
70	G507R	M	52	30	Dyspnea, palpitations	No	ICD	30	2.0	44	33	No	43	53	95	17
1893	C566R	M	56	44	AP	No	No	22	2.2	48	33	No	No	62	88	13
1423	C566R	M	56	53	AP	No	No	22	1.8	44	34	No	No	nda	nda	nda
1119	V1115I	F	61	50	Dyspnea	No	No	18	**	46		Yes	69	78	58	8
7	IVS27+1G>A	F	61	41	Palpitations	No	No	13	1.2	50	44	Yes	7	74	76	4
1897	IVS27+1G>A	F	37	No clinical phenotype	None	No	No	nda	nda	nda	nda	nda	nda	nda	nda	nda
1891	IVS27+1G>A	M	32	20	Dizziness, syncopes	No	No	nda	nda	nda	nda	nda	nda	nda	nda	nda
Mean ± SD			56 ± 8	48 ± 9				22 ± 5	1.7 ± 0.4	46 ± 2	37 ± 6		46 ± 18	65 ± 9	85 ± 16	13 ± 3
Small insertions, deletions, out-of-frame and nonsense mutations																
164	delC390	M	51	39	Dyspnea	Myect., 1990	No	22‡	2.0	46	35	No	55	nda	nda	nda
6	insG791	M	69	15	Dyspnea	Myect., 1974	ICD	19‡	1.5	54	20	No	No	70	106	26
314	insAA1042	M	68	54	AP	No	ICD	27	1.8	51	22	No	49	42	76	18
8	delG1047	F	40	25	Palpitations, dizziness	Myect., 1988	Yes	20‡	2.2	44	40	Yes	18	77	nda	nda
1506	delG1047	M	61	44	AP, dyspnea, palpitations	No	Yes	20	1.7	52	42	Yes	7	64	80	3
43	delG1047	M	19	16	None	No	No	13	1.4	47	30	No	No	nda	nda	nda
1206	insTTCA1231	M	42	38	Dyspnea	TASH, 1999	No	20	1.3	48	42	Yes	63	76	97	16
1329	insTTCA1231	M	13	No clinical phenotype	None	No	No	10	1.0	48	31	No	No	nda	nda	nda
169	IVS7+1G>A	M	*	24	AP, dyspnea	No	No	20	1.7	49	40	No	10	43	97	16
1331	IVS7+1G>A	M	40	38	AP, dyspnea	TASH, 1998	No	23	1.9	50	46	Yes	47	70	nda	nda
1975	IVS7+1G>A	F	62	59	AP, dyspnea	No	No	18	1.6	40	43	No	No	nda	nda	nda
1978	IVS7+1G>A	M	36	27	Dyspnea, presyncopes, AP	Myect., 1994	ICD	19‡	1.5	42	45	Yes	85	81	91	17
1992	IVS7+1G>A	M	31	26	AP, dyspnea	TASH, 1999	No	24	1.7	43	30	Yes	60	76	92	41
14	IVS20−2A>G	M	47	43	Dyspnea, palpitations	No	ICD	18	1.3	45	32	No	No	76	64	16
1913	IVS20−2A>G	M	27	18	Dizziness	No	No	13	1.1	46	41	No	No	nda	nda	nda
39	Q1233X	M	50	41	Dyspnea	No	Yes	20	2.0	52	35	Yes	No	68	82	20
2023	Q1233X	F	27	nda	nda	No	No	nda	nda	nda	nda	nda	nda	nda	nda	nda
315	Q1233X	M	29	25	Dyspnea, palpitations	No	ICD	17	1.3	36	44	Yes	70	71	79	10
Mean ± SD			42 ± 16	33 ± 13				19 ± 5	1.6 ± 0.3	47 ± 5	36 ± 8		46 ± 25	68 ± 13	86 ± 12	18 ± 9

*Sustained VT ≥ 30 s. †Orthograde measurements were not obtainable. ‡Echocardiography postmyectomy.

AF = atrial fibrillation; AP = angina pectoris; FS = fractional shorting; Ht = hypertrophy; ICD = implantable cardioverter-defibrillator; IVS = intraventricular septum; LVEDP = left ventricular end-diastolic pressure; LVEDD = left ventricular end-diastolic diameter; LVEDVI = left ventricular volume index; LVEF = left ventricular ejection fraction; Myect. = myectomy; nda = no data available; PW = posterior wall; SAM = systolic anterior movement; TASH = transcoronary ablation of septal hypertrophy; VT = ventricular tachycardia.

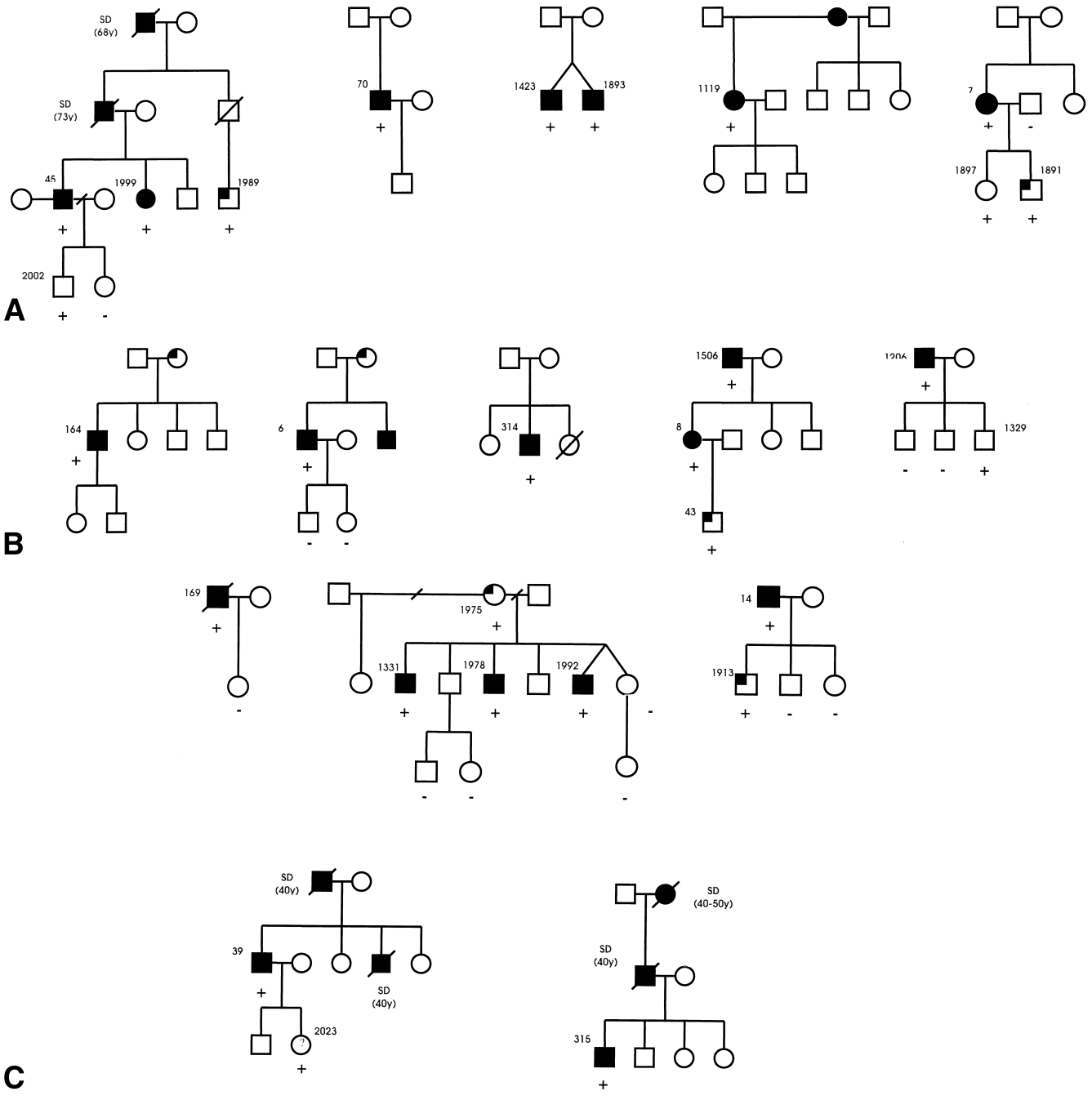

Figure 3. Pedigrees of families with hypertrophic cardiomyopathy. **Symbols** denote gender and disease status: **box** = male; **circle** = female; **darkened** = full phenotype of hypertrophic cardiomyopathy; **quarter** = only discrete signs of the disease; **clear** = unaffected; **slashed** = deceased; **SD** = sudden death; **+** = genetically affected; **-** = genetically unaffected. **(A)** Pedigrees of index-patients (DNA 45, 70, 1893, and 1119) carrying missense mutations (R282W, G507R, C566R, V1115I) and pedigree of index-patient 7 carrying in-frame splicing mutation (IVS27+1G>A). **(B)** Pedigrees of index-patients (DNA 164, 6, 314, 8, 1206) carrying small deletions and insertions (delC390, insG791, insAA1042, delG1047, and insTTCA1231). **(C)** Pedigrees of index-patients (DNA 169, 1331, and 14) carrying splice mutations (IVS7+1G>A, IVS2-2A>G, IVS27+G>A) and pedigrees of index-patients (DNA 315 and 39) carrying nonsense mutations (Q1233X).

both families. A common haplotype encompassing the *MYBPC3* locus was segregated with the disease-causing mutation, suggesting descent from a shared ancestor (Fig. 4). The mutation Q1233X was identified in two apparently unrelated patients of Turkish descent. Using the previously described highly polymorphic markers, we constructed the most likely haplotype in the family of patient 39 and an assumed disease-associated haplotype for patient 315. According to these haplotype constructions, the Q1233X mutation was embedded in one haplotype segregated with the disease-causing mutation (data not shown). These data suggest that the mutation Q1233X is identical by descent. Within the analyzed families, the shared haplotype extended ~0.6 cM across the region between D11S1385 and D11S1313.

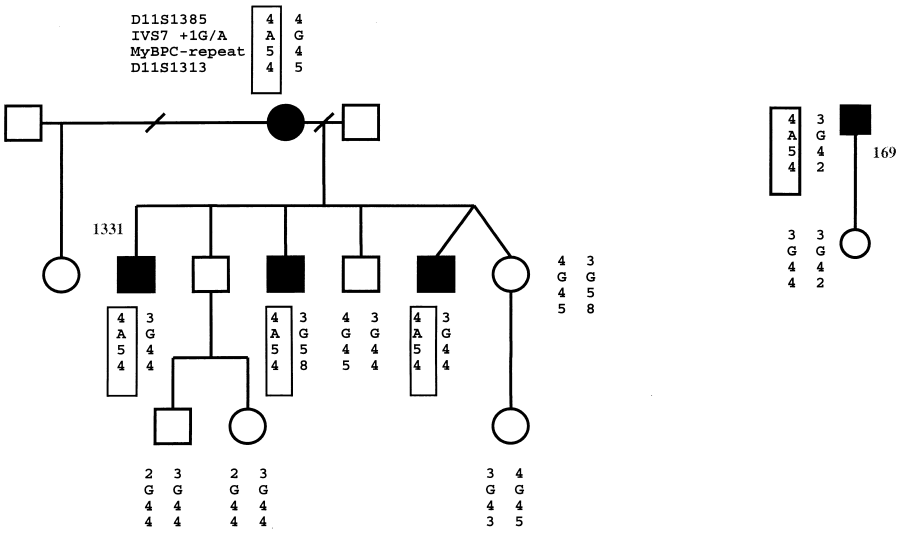

Figure 4. Haplotypes across the myosin-binding protein C gene (*MYBPC3*) locus associated with the IVS7+1G>A mutation are shown. The disease-associated haplotypes of the family members of patients 1331 and 169 are **boxed.** The marker loci used for haplotype construction, covering a distance of ~0.6 cM, are indicated at the left, in telomeric-centromeric order, from top to bottom.

DISCUSSION

We identified 15 *MYBPC3* mutation carriers after systematic mutation screening of 110 consecutive patients with HCM in our institute. Eleven of these mutations were novel, only two mutations—insG791 and insAA1042—have already been reported as being responsible for HCM (16). Because each of the novel sequence variants was found only in clinically affected patients and was predicted to alter the encoded protein, all were considered mutations that cause HCM. The mutation spectrum included four missense mutations, three splicing mutations, one nonsense mutation and five small deletions and insertions. For two mutations (IVS7+1G>A and Q1233X), the existence of a common founder was shown. The spectrum of clinical phenotypes was broad. Severe hypertrophy necessitating myectomy or TASH and life-threatening arrhythmias were more frequently found in patients with protein truncations. Severe arrhythmias were the most prominent feature, and sudden death was reported in four of the 15 families.

Four amino acid exchanges that were not present in 100 chromosomes of healthy control subjects were found, all of which affect amino acids that are conserved at least among the human, mouse and chicken cardiac *MYBPC3* protein and between human fast- and slow-type skeletal myosin-binding proteins. All of them concern functionally relevant regions: R282W lies within the LAGGGRRIS loop, and G507R and C566R are clustered in a highly conserved segment that spans the phosphorylation domain of the molecule (19) and are located in proximity of a total of four missense mutations, which have already been reported as HCM-causing variants (16). The V1115I substitution is located in a stretch of highly conserved amino acids in the fibronectin type 3-like motif of module C9, which is responsible for titin binding (20). The position of these mutations in functionally important regions of the protein, the loss or incorporation of charged amino acids leading to conformational changes of the protein and the nonappearance of the mutations in 100 chromosomes of healthy control subjects are strong hints for a disease-causing action of these mutations.

Three splicing variants occur in splice consensus sequences of the *MYBPC3* gene. The IVS7+1G>A mutation led to two alternative transcripts. Both aberrant transcripts were due to skipping of either exon 7 alone or exons 7 and 8, which in both cases led to a frame shift and the incorporation of either 26 or 16 aberrant residues before a premature stop codon occurred. The IVS20-2A>G mutation altered the consensus AG of the splice-acceptor site of exon 21 and led to use of a cryptic splice-acceptor site and subsequently to the deletion of 11 base pairs, which introduces a premature stop codon at residue 661. The splice variant IVS27+1G>A led to in-frame skipping of exon 27 and loss of 56 highly conserved amino acid residues that are important for the incorporation of MyBPC into the A band (21).

Eight of our 13 mutations (two splice variants, one nonsense mutation and five small deletions and insertions) led to premature protein truncation and subsequently to the lack of carboxyl-terminal amino acids, which are required for the incorporation of cardiac MyBPC into the A band, titin interaction and myosin binding (20,22–24). It is not yet clear whether these defects will result in stable, truncated proteins, which than act as "poison polypeptides" through a dominant negative effect (25), or whether these defects act as null alleles and cause HCM by haplo-insufficiency. Recent studies have published conflicting results: Gilbert et al. (21) and Yang et al. (26) demonstrated the incorporation of stable, truncated proteins encoding truncation mutants of *MYBPC3* in transfected skeletal muscle myoblasts (ΔC9–

10) and in a transgenic mouse model, respectively. These truncated proteins did not correctly incorporate into the sarcomere and were only weakly associated with the rest of the sarcomeric proteins. In contrast, Rottbauer et al. (27) and Moolman et al. (28) were unable to detect the truncated protein in human myocardial biopsies.

Nine of the 13 HCM-causing mutations detected in our study group are novel and thus far represent private mutations, as do most of the HCM-associated mutations. This is in accordance with the fact that mutations associated with significant mortality confer a reproductive disadvantage. Consequently, within populations, recurring identical disease-causing mutations are more likely to have arisen independently, rather than reflecting a common ancestor (29). Two of our mutations (IVS7+1G>A and Q1233X) were identified in two apparently unrelated patients. Haplotype analysis across the *MYBPC3* locus in the relevant families revealed that all of the individuals who carried the same mutation also shared either a disease-associated haplotype or a haplotype that was assumed to be disease-associated. The haplotypes were conserved over a region of ~0.6 cM. To the best of our knowledge, this report is the first to show the existence of a founder effect as a cause of HCM in a German and Turkish population. Until now, the report by Moolman-Smook et al. (30) was the first to describe strong founder effects as a cause of HCM in a South-African population.

According to our findings, *MYBPC3* mutations may cause a broad spectrum of phenotypes. The most severe manifestations of the disease, including life-threatening arrhythmia and the necessity for myectomy or TASH, accumulated in the group with protein truncations. In contrast, the patients and family members with missense mutations and in-frame deletions generally exhibited less pronounced signs and symptoms of the disease. In comparison to 11 beta-MHC mutation carriers, the age of onset, degree of hypertrophy and necessity for ICD, myectomy or TASH were comparable.

Study limitations. We clearly realize that phenotype-genotype relations require large families with identical mutations, which cannot be established in our patient cohort. Therefore, we did not aim to study disease penetrance and limited ourselves to describing the characteristic clinical features of the index patients and available family members.

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