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Novel mutations in sarcomeric protein genes in dilated cardiomyopathy

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Abstract

Mutations in sarcomeric protein genes have been reported to cause dilated cardiomyopathy (DCM). In order to detect novel mutations we screened the sarcomeric protein genes β-myosin heavy chain (MYH7), myosin-binding protein C (MYBPC3), troponin T (TNNT2), and α-tropomyosin (TPM1) in 46 young patients with DCM. Mutation screening was done using single-strand conformation polymorphism (SSCP) analysis and DNA sequencing. The mutations in MYH7 were projected onto the protein data bank-structure (pdb) of myosin of striated muscle. In MYH7 two mutations (Ala223Thr and Ser642Leu) were found in two patients. Ser642Leu is part of the actin–myosin interface. Ala223Thr affects a buried residue near the ATP binding site. In MYBPC3 we found one missense mutation (Asn948Thr) in a male patient. None of the mutations were found in 88 healthy controls and in 136 patients with hypertrophic cardiomyopathy (HCM). Thus mutations in HCM causing genes are not rare in DCM and have potential for functional relevance.

Keywords: Dilated cardiomyopathy; Mutation; β Myosin heavy chain; Myosin binding protein C

Dilated and hypertrophic cardiomyopathies are the most frequently occurring types of cardiomyopathies [1]. HCM is characterized by ventricular hypertrophy, DCM by dilatation and systolic contractile dysfunction of the left or both ventricles and results in heart failure [2].

Gene defects account for 20–35% of cases of DCM [3,4]. Four different traits of inheritance are known, the most common trait is autosomal dominant [5] and applies to the genes coding for cardiac actin, desmin, δ-sarcoglycan and lamin A/C. Furthermore, 10 loci are known but the disease-causing genes are not identified. Dystrophin and tafazzin mutations are transmitted by X-linked inheritance [6,7]. The autosomal recessive and mitochondrial traits are rare [5].

Over 150 mutations in 10 genes encoding sarcomeric protein have been identified to cause HCM [8,9]. These are in particular mutations in the genes for the heavy chain of β-myosin, myosin-binding protein C, troponin T, and α-tropomyosin, rarely in the ones for troponin C, troponin I, cardiac actin, the essential and regulatory units of the light chain of myosin, and for titin [6,10].

In 2000, Kamisago et al. [11] discovered that mutations in sarcomeric protein genes are also associated with DCM. Three disease-causing mutations were described: two amino acid substitutions in MYH7 (Ser532Pro and Phe764Leu) and one deletion in TNNT2 (del Lys210). Additional mutations were found: one in TNNT2 (Arg141Trp), two in TPM1 (Glu40Lys and Glu54Lys), two in the titin gene (2-bp ins, 43628AT and Trp930Arg), and two in cardiac actin (Arg312His and Glu361Gly) [12–15].

In this respect we now performed a systematic mutation screening in four specific sarcomeric protein genes: MYH7 (exons 3–23), MYBPC3, TNNT2, and TPM1 using single-strand conformation polymorphism (SSCP) analysis and DNA sequencing.

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Table 1
Rare sequence variants

Gene	Variant	Nucleotide position and substitution
MYH7[a]	IVS 11 +23 A/T	8914 A/T
MYH7	Asp376Asp	9666 C/T

[a] MYH7: GenBank No. M57965.

Materials and methods

Clinical evaluation. This study is based on 46 unrelated patients with a diagnosis of DCM, who gave written informed consent for genotyping and detailed prospective phenotyping. The study was approved by the Ethic Committee of Charité, Humboldt University Berlin. Thirty eight of 46 patients were male, eight female. The patients' age at study was 35 ± 8 years; the age at diagnosis was 29 ± 8 years. Diagnosis was based on echocardiography, coronary angiography, and in selected cases, endomyocardiac biopsy. Diagnostic criteria for DCM were defined as a left ventricular ejection fraction (LVEF) $\leqslant 50\%$, fractional shortening (FS) $\leqslant 27\%$ or both in the presence of a left ventricular end-diastolic dimension (LVEDD) $\geqslant 2.7\,\mathrm{cm/m^2}$ of body surface area (BSA). On the average patients showed a LVEF of $22 \pm 8\%$, FS of $14 \pm 7\%$, and LVEDD of $3.7 \pm 0.7\,\mathrm{cm/m^2}$ of BSA.

Conditions that may simulate DCM as coronary heart disease, myocarditis, hypertension, and valvular heart disease were excluded.

Genetic studies. DNA was extracted from white blood cells and then amplified using polymerase chain reaction (PCR) as described previously [16]. All primers were designed to include splice binding sites within the intron. Primer sequences and according annealing temperatures are available on request. SSCP analysis at two different temperatures (room temperature and +4 °C) and DNA sequencing using a BigDye RR Terminator Cycle Sequencing Kit on an ABI-Prism 310 Sequencer (Applied Biosystems, Weiterstadt, Germany) were performed as reported previously [16].

None of the mutations altered a restriction site for a commercially available restriction enzyme; therefore, the mutations were confirmed by repeated sequencing of independent PCR products.

The editor for structural alignment of proteins (STRAPs) [17] was used to align myosin sequences from SWISSPROT-sequence database and to locate the mutations in the three-dimensional structure of striated muscle myosin. Visualization of structure and mutations was performed with the protein modeling program INSIGHTII by Accelrys.

Results

By systematic mutation screening of 46 DCM patients using SSCP analysis we identified a total of three mutations, two in MYH7 and one in MYBPC3. All three mutations lead to the substitution of an amino acid. In addition, we found a number of silent variants

Table 2
Polymorphisms

Gene	Variant	Nucleotide position and substitution	Allele frequency (%) ($n = 46$)
MYH7[a]	IVS 2 −25 G/T	5688 G/T	90/10
MYH7	Thr63Thr	5909 C/T	54/46
MYH7	Phe244Phe	7864 C/T	86/14
MYH7	Asp325Asp	8867 C/T	96/4
MYH7	Gly354Gly	9600 C/T	91/9
MYH7	Lys365Lys	9633 G/A	82/18
MYBPC3[b]	Ser236Gly	4229 A/G	
MYBPC3	Thr262Thr	4825 C/T	
MYBPC3	Arg326Gln	6396 G/A	
MYBPC3	IVS 12 −24 C/T	8175 C/T	
MYBPC3	IVS 13 +29 G/A	8360 G/A	
MYBPC3	IVS 19 +47 G/A	10732 G/A	
MYBPC3	IVS 21 +38 A/T	12209 A/T	
MYBPC3	IVS 23 +18 C/G	13314 C/G	
MYBPC3	IVS 29 −21 A/G	18325 A/G	
MYBPC3	Glu1096Glu	18443 A/G	
MYBPC3	IVS 32 +49 C/T	19162 C/T	
MYBPC3	IVS 33 −66 C/T	19731 C/T	
TNNT2[c]	IVS 3 −11$(CTTCT)_1/(CTTCT)_2$	242$(CTTCT)_1/(CTTCT)_2$	60/40
TNNT2	IVS 5 −50 G/A	219 G/A	63/37
TNNT2	Ser69Ser	199 G/A	88/12
TNNT2	Ile106Ile	163 T/C	73/27
TNNT2	IVS 11 −32 C/A	96 C/A	73/27
TNNT2	IVS 14 −33 C/T	128 C/T	87/13
TPM1[d]	Ala151Ala	509 C/A	
TPM1	Tyr162Tyr	542 T/C	

[a] MYH7: GenBank No. M57965.
[b] MYBPC3: GenBank No. Y10129; Allele frequencies were not determined.
[c] TNNT2: GenBank No. AF004409-22.
[d] TPM1: GenBank No. M19713; Allele frequencies were not determined.

and polymorphisms, which were partially already detected in HCM (Tables 1 and 2).

In exon 8 of MYH7 a G to A transition occurs in one male patient at nucleotide 7799 (GenBank No. M57965). This mutation is predicted to result in the substitution of alanine to threonine at residue 223 (Ala223Thr) and leads to the exchange of a nonpolar side chain against an uncharged polar group. Projection of Ala223Thr on the three-dimensional structure of striated muscle myosin using STRAP demonstrates that the mutation is localized in the 50 K upper domain of the 50 kDa central domain near the ATP-binding site (Fig. 1). By the diagnosis of DCM the patient was at the age of 35 years. The patient is classified NYHA II–III and has a sinus rhythm. Additionally, he has been implanted an automatic cardioverter-defibrillator (ICD). Clinical data are shown in Table 3.

The second mutation in MYH7 is located in exon 17. A transition from C to T at nucleotide 12164 leads to an exchange of polar serine to unpolar leucine at residue 642 (Ser642Leu). We observed that this variation is located in the actin–myosin interaction region and may influence the local structure at this position (Fig. 1). By diagnosis the patient was 18 years of age. The individual is classified NYHA III and an automatic cardioverter-defibrillator has been implanted.

In MYBPC3 we found an A to C transversion in exon 27 at nucleotide 16,575 (GenBank No. Y10129). This transversion is predicted to substitute asparagine to threonine at residue 948 (Asn948Thr). The patient's age at diagnosis was 36 years. Medically treated he was classified NYHA III and also received an ICD.

To eliminate the possibility that these amino acid substitutions were polymorphisms within the general population we tested 88 unrelated control individuals (176 chromosomes). No anomalous SSCP conformers were identified in these individuals. In addition, the evaluation of 136 patients with HCM failed to detect these three base variations.

Two of the three amino acid substitutions, Ser642Leu and Asn948Thr, are highly conserved across many species.

No mutation in either TPM1 or TNNT2 was found, only a number of polymorphisms were detected, which are shown in Table 2.

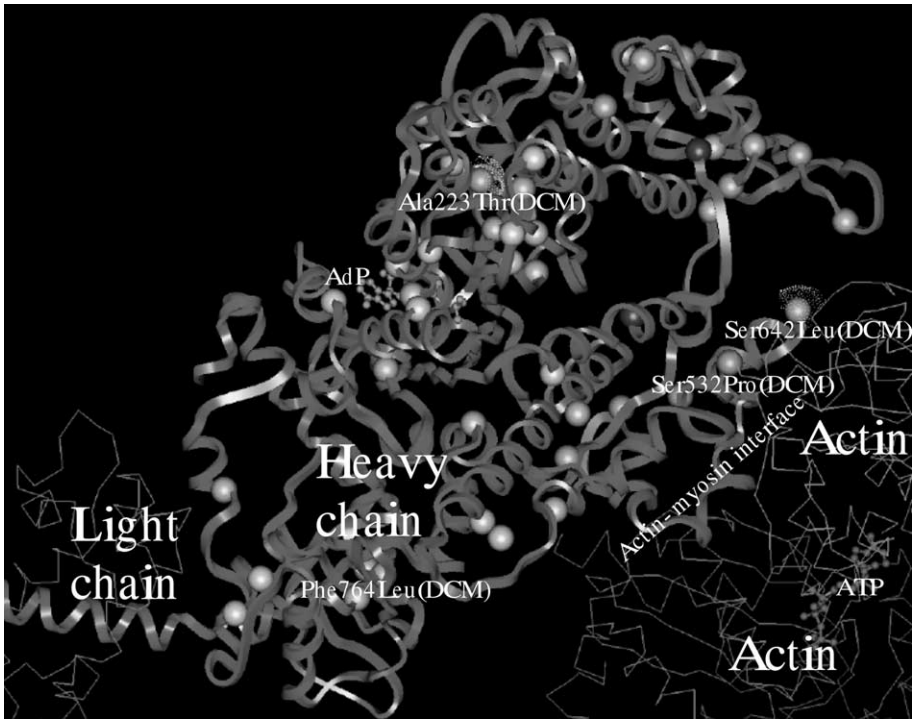

Fig. 1. STRAP analysis of mutations in MYH7. Location of mutations in MYH7 retrieved from two databases http://genetics.med.harvard.edu/~seidman/cg3 [18] and http://www.angis.org.au/pbin/Databases/Heart/fhcquery.cgi [9]. Mutations that are associated with HCM are depicted as gray balls and those with DCM as yellow balls. Mutations found by our laboratory (Ala223Thr and Ser642Leu) are highlighted with a yellow dotted surface. In the figure two mutations with DCM are located within the interface of actin and myosin whereas almost all mutations located elsewhere present with a hypertrophic phenotype. The only mutations outside this interface with a dilated phenotype are Ala223Thr and Phe764Leu. At present no structure file of the cardiac protein is available. Therefore, the mutations were mapped onto myosin of striated muscle (pdb2MYS). The relative positions of actin and myosin had been modeled by Mendelson [19] (pdb1ALM) from crystallographic structures and electron microscopy images of the complex. The location of ADP-ATP was obtained from pdb1BR2.

Table 3
Clinical features

Mutation	Gene	Sex	Age at study (years)	Age at diagnosis (years)	NYHA class	Rhythm	ICD	LVEDD (mm)	LVESD (mm)	LVEF (%)	FS (%)	PAPm (mm Hg)
Ala223Thr	MYH7	Male	39	35	II–III	SR	+	77	68	15	12	30
Ser642Leu	MYH7	Male	24	18	III	SR	+	68	59	13	13	38
Asn948Thr	MYBPC3	Male	40	36	III	SR	+	76	69	20	9	17

NYHA, New York Heart Association; SR, sinus rhythm; ICD, implantable cardioverter-defibrillator; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; LVEF, left ventricular ejection fraction, as determined by echocardiography; FS, fractional shortening, as determined by echocardiography; PAPm, pulmonary artery pressure, mean.

Discussion

The novel findings of our study consist in the detection of three novel rare mutations, leading to amino acid exchanges in the HCM related genes MYH7 and MYBPC3 in patients with DCM.

Inspection of the protein structure of β-MHC predicts effects on thermostability and protein folding for the mutation Ala223Thr and severe conformational changes for Ser642Leu located close to the actin–myosin interaction site. Both mutations did not occur in healthy controls or HCM patients. Since both occurred in patients in whom family members were not available for the analysis substantiation of disease-causing potential by two-point linkage analysis was not possible.

Mutations in sarcomeric protein genes are known to cause HCM and some of them have recently been described to cause also DCM. We selected MYH7, MYBPC3, and TNNT2 because these were the genes that most frequently cause HCM [8]. TPM1 was selected because it causes a transition from HCM to DCM [20].

Novel mutations were searched by a carefully optimized and validated SSCP technique. We may assume that we missed less than 5% of mutations in the genes under investigation. This mutation detection rate is in the upper range of sensitivity rates published for SSCP [21–23].

To increase the likelihood to detect genetic defects as causes for DCM, we limited the investigations to patients that were young at presentation, although we do well realize that this does not exclude other causes of disease.

Left ventricular wall thickness was normal or subnormal (<10 mm) in all patients. Transition from HCM to DCM was excluded in all patients.

Some arguments are in favor of the disease-causing potential of the novel mutations.

Ser642Leu did not occur in 136 HCM patients and 88 controls and therefore does not present a frequent polymorphism. The high degree of conservation also favors functional relevance. The findings of the STRAP analysis predict severe functional changes in the actin–myosin interaction. Ser642Leu is situated in a loop which directly interacts with actin and may alter binding affinity and binding kinetics. It could enforce the interaction by increasing hydrophobic interaction or conversely decrease the affinity by loss of one H-bond. Steric hindrance could also occur because leucine is larger than serine. Our hypothesis is that mutations in the interface of actin and myosin lead to DCM by altering the affinity to actin. Recent investigations suggest that the molecular cause of DCM could be deficits in force transmission as well as force production [24]. The second cause would be consistent with our mutation in the actin–myosin-binding site. Furthermore, a DCM-causing mutation is located nearby (Ser532Pro) [11]. Since HCM-causing mutations are frequently clustered around specific locations [25], this may also be true for DCM-causing variants and increases the likelihood of functional relevance.

Ala223Thr did not occur in 136 HCM patients and 88 controls either. Threonine is slightly bigger than alanine and it may be difficult to accommodate it. It is not solvent exposed by at least one atomic layer from all sides. The exchange could decrease thermostability or affect the protein folding and thus the protein motility [26]. In addition, the mutation Ala223Thr is situated next to the HCM-causing variant Gln222Lys [25]. However, the multiple sequence alignment of various myosin heavy chains reveals some proteins that have already a threonine there.

The Asn948Thr mutation in MYBPC3 could not be evaluated by STRAP analysis because crystal structure is not available.

Unfortunately, family members for the calculation of two-point LOD scores could not be obtained for further investigation. Therefore, definite conclusions on a disease-causing nature of the variants are not possible. If families with the respective mutations are not detected by us or other investigators, transgenic animal model may represent the next relevant step to obtain further evidence of relevance.

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