Myosin light chain mutations in familial hypertrophic cardiomyopathy: phenotypic presentation and frequency in Danish and South African populations

Paal Skytt Andersen, Ole Havndrup, Henning Bundgaard, Johanna Catharina Moolman-Smook, Lars Allan Larsen, Jens Mogensen, Paul Andries Brink, Anders Dupont Børglum, Valerie Ann Corfield, Keld Kjeldsen, Jens Vuust, Michael Christiansen

Methods

DANISH PATIENTS
Sixty-eight families (total number of subjects 395) were consecutively enrolled in a study of FHC at the National University Hospital, Copenhagen. The probands all fulfilled conventional criteria for FHC, and all had a maximum left ventricular wall thickness of 15 mm. After informed consent was obtained (Local Science Ethics Committee, No KF V92213), patient and family histories were taken and physical examinations, 12 lead ECG, and two dimensional and M mode echocardiography were performed. The echocardiographic investigations included Doppler measurements of left ventricular outflow tract, as well as transmural flow patterns and allowed identification of patients with the MV phenotype, which was defined as asymmetrical septal hypertrophy predominantly confined to the midventricular region.

The relatives of these probands, hereafter identified by the family code and pedigree number, were then also clinically investigated to establish segregation of the phenotype. The clinical diagnosis of FHC in relatives was based on (1) ECG criteria of pathological Q waves or signs of left ventricular hypertrophy and/or (2) echocardiography criteria of a maximum left ventricular wall thickness ≥13 mm in the absence of evidence of other specific heart muscle disease.

SOUTH AFRICAN PATIENTS
One hundred and thirty consecutively collected probands of mixed or white ancestry were enrolled as part of an ongoing study at the University at Stellenbosch. Patients were clinically characterised as described elsewhere.
Briefly, the echocardiographic diagnosis of HCM was made in the presence of a maximum ventricular wall thickness ≥13 mm in the absence of evidence of other specific heart muscle disease.

Additionally, blood samples from a group of 150 persons born in Denmark were used as controls in the genetic analysis.

DNA ISOLATION

Genomic DNA was purified from blood samples obtained from all subjects using a QIAamp DNA purification kit (Qiagen, Germany).

MUTATION DETECTION TECHNIQUES

Genomic DNA was PCR amplified using AmpliTaq polymerase (Applied Biosystems, Foster City, CA). All probands were screened for mutations in the MYL2 and MYL3 genes. The primers used for analysis of the MYL2 and MYL3 genes are listed in table 1.

The three probands with mutations in MYL2 (ZY, ZG, and T), as well as the two probands from families with a member with MVH (ZJ and XI), were screened for mutations in all coding exons from the FHC causing genes (MYH7, MYBPC3, TPM1, TNNI3, TNN2, ACTC, MYL2, and MYL3), except in MYL7, where only exons 3–23 were screened. Amplification was performed using intronic primers. Primers and conditions for analysis of these eight FHC genes are essentially as previously described.3–7921

RESULTS

DISEASE-CAUSING MUTATIONS IN THE TWO POPULATIONS

Mutation screening of MYL2 showed three Danish probands (of families ZY, ZG, and T) as well as one previously described,9 but none with mutations in MYL3, while no disease-causing mutations were found in the South African probands. The clinical data of the mutation carriers are given in table 2.

The MVH phenotype was present in three Danish families (ZG, ZJ, and XI), but this was not a constant finding in all affected members of these families. An MYBPC3 mutation and an MYH7 mutation were detected in the probands of families ZJ and XI, respectively.

POLYMORPHISMS IN THE TWO POPULATIONS

The Danish patients were all white, whereas the South African patients were of either white or mixed ethnic descent. Mutation screening of the two populations showed unique, as well as common, polymorphisms. Among South African probands of both white and mixed ancestry, a unique MYL2 intron 1 (g2579C>T) polymorphism was detected, whereas MYL2 polymorphisms identified in exon 3 (g6027T>C) and intron 4 (g8393G>A) were present in both the South African and the Danish probands as well as the Danish control group. The exonic polymorphism did not cause an amino acid change, while the intronic polymorphisms are not expected to affect splicing. In MYL3, three silent variants were identified: exon 1 (c.699C>T), exon 3 (c.261C>T), and exon 4 (c.399C>T). The former was found in both population groups and the control group and the latter two were found only in South African probands of white or mixed ancestry. None of these variants gave rise to amino acid changes.

Table 2  Clinical and echocardiographic data of mutation carriers in the three FHC families (ZY, ZG, and T) carrying MYL2 mutations

| Family | ID | Age (years) | LVIVS (mm) | LVIVS (mm) | LVPWT (mm) | E/A | LVOT Gradient (H) (mmHg) | LVOT Gradient (V) (mmHg) | Q wave (yes/no) | Voltage (mmHg) | ST/T (mmHg) | LVIVS—left interventricular septal diameter at mid and LVOT level; LVIVPSD—left ventricle posterior wall diameter; LVIVRT—left ventricle isovolumetric relaxation time; peak E/A—transmitral flow velocity; E/A—EA-ratio; gradient LVOT (H, rest and V, valsala)—left ventricle outflow tract gradient; Q wave/Voltage/ST/T (segment)—yes/no refer to the fulfilment of the respective diagnostic ECG criteria; LBBB—left bundle branch block. | *
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Mutation screening of MYL2 identified aberrant SSCP conformers in both exon 5 and exon 7 in the proband from family ZY (ZY:III.2) (fig 1). Subsequent sequencing showed an A>G transition at position g8486 in exon 5, which generated a glutamic acid substitution at the highly evolutionarily conserved lysine at codon 103 (Lys103Glu), while sequencing of exon 7 showed a G>C transversion in the acceptor splice site of intron 6, IVS6-1. Mutation screening of exons 5 and 7 in the rest of family ZY showed that the Lys103Glu substitution was maternally (ZY:II.2) and the IVS6-1 mutation paternally (ZY:II.1) derived. On the maternal side, one other family member carried the Lys103Glu mutation (ZY:II.4). Neither of these mutations was found in any of the other 197 probands or 150 controls. No mutations were detected when screening the other seven FHC genes in this family.

The proband (ZY:III.2), aged 17 years, suffered from exercise induced dyspnoea. He had pronounced septal hypertrophy, diastolic filling abnormalities, but no significantly increased left ventricular outflow tract (LVOT) gradient at rest or during Valsalva manoeuvre. However, during cycle ergonomic exercise there was a significant increase in the LVOT gradient, and he improved symptomatically following percutaneous transcoronary septal myocardial alcohol ablation. The mother (T:I.2) was diagnosed with FHC in later life and died at the age of 72 years. It was not possible to obtain tissue for genotyping. T:II.2 fulfilled the diagnostic criteria for FHC in her mid forties, whereas T:III.1, who was only 10 years old at the time of inclusion, did not. T:II.3, who was not a mutation carrier, had left ventricular hypertrophy, which seemed to relate to arterial hypertension and severe obesity. There was no family history of sudden death.

Mutation screening of exon 2 of the MYL2 gene in the proband (T:II.1) of family T (table 2) identified a G>A transition which causes the previously described FHC associated codon 13 Ala13Thr substitution. Two other family members (T:II.2 and T:III.1) were heterozygous for this mutation, which was not present in either 150 controls or the other 197 probands. No other mutations were identified in this family in the additional seven FHC genes screened.

The proband (T:II.1), aged 42 years, suffered from exercise induced dyspnoea. He had pronounced septal hypertrophy, diastolic filling abnormalities, but no significantly increased left ventricular outflow tract (LVOT) gradient at rest or during Valsalva manoeuvre. However, during cycle ergonomic exercise there was a significant increase in the LVOT gradient, and he improved symptomatically following percutaneous transcoronary septal myocardial alcohol ablation. The mother (T:I.2) was diagnosed with FHC in later life and died at the age of 72 years. It was not possible to obtain tissue for genotyping. T:II.2 fulfilled the diagnostic criteria for FHC in her mid forties, whereas T:III.1, who was only 10 years old at the time of inclusion, did not. T:II.3, who was not a mutation carrier, had left ventricular hypertrophy, which seemed to relate to arterial hypertension and severe obesity. There was no family history of sudden death.

**MYL2 LYS103GLU AND IVS6-1 MUTATIONS IN FAMILY ZY**

Mutation screening of MYL2 identified aberrant SSCP conformers in both exon 5 and exon 7 in the proband from family ZY (ZY:III.2) (fig 1). Subsequent sequencing showed an A>G transition at position g8486 in exon 5, which generated a glutamic acid substitution at the highly evolutionarily conserved lysine at codon 103 (Lys103Glu), while sequencing of exon 7 showed a G>C transversion in the acceptor splice site of intron 6, IVS6-1. Mutation screening of exons 5 and 7 in the rest of family ZY showed that the Lys103Glu substitution was maternally (ZY:II.2) and the IVS6-1 mutation paternally (ZY:II.1) derived. On the maternal side, one other family member carried the Lys103Glu mutation (ZY:II.4). Neither of these mutations was found in any of the other 197 probands or 150 controls. No mutations were detected when screening the other seven FHC genes in this family.

The proband (ZY:III.2) was diagnosed with FHC at the age of 17 years. He had pronounced septal hypertrophy, predominantly confined to the proximal septum, and an inverted transmitral flow pattern indicating diastolic filling abnormalities (table 2). Despite the echocardiographic findings, he had only mild symptoms except for an episode of near syncope, angina, and severe dyspnoea during an attack of paroxysmal atrial fibrillation following intake of amphetamine-like drugs in order to lose weight. His sister (ZY:III.1), aged 42 years, also carrying both the missense and the splice site mutations, had no echocardiographic or ECG signs of hypertrophy, but experienced atypical chest discomfort. Subjects ZY:II.2 and ZY:II.4, carrying the Lys103Glu mutation, were asymptomatic and without ECG or echocardiographic signs of HCM (table 2). There was no family history of sudden death.

**MYL2 ALA13THR MUTATION IN FAMILY T**

Mutation screening of exon 2 of the MYL2 gene in the proband (T:II.1) of family T (table 2) identified a G>A transition which causes the previously described FHC associated codon 13 Ala13Thr substitution. Two other family members (T:II.2 and T:III.1) were heterozygous for this mutation, which was not present in either 150 controls or the other 197 probands. No other mutations were identified in this family in the additional seven FHC genes screened.

The proband (T:II.1), aged 42 years, suffered from exercise induced dyspnoea. He had pronounced septal hypertrophy, diastolic filling abnormalities, but no significantly increased left ventricular outflow tract (LVOT) gradient at rest or during Valsalva manoeuvre. However, during cycle ergonomic exercise there was a significant increase in the LVOT gradient, and he improved symptomatically following percutaneous transcoronary septal myocardial alcohol ablation. The mother (T:I.2) was diagnosed with FHC in later life and died at the age of 72 years. It was not possible to obtain tissue for genotyping. T:II.2 fulfilled the diagnostic criteria for FHC in her mid forties, whereas T:III.1, who was only 10 years old at the time of inclusion, did not. T:II.3, who was not a mutation carrier, had left ventricular hypertrophy, which seemed to relate to arterial hypertension and severe obesity. There was no family history of sudden death.

**Figure 1** Pedigree of family ZY (A) with demonstration of the Lys103Glu MYL2 missense mutation by conventional SSCP/HD analysis (B) and of the IVS6-1 MYL2 splice site mutation by automated SSCP analysis (C), in both cases confirmed by DNA sequencing (D). The arrow denotes the proband.

Denotes heterozygosity for the Lys103Glu mutation, **denotes heterozygosity for the IVS6-1 mutation, and */** denotes compound heterozygosity. WT is the pattern seen in controls and MUT is the pattern seen in patients with a mutation.
A C>A transversion in exon 3 of \textit{MYL2}, resulting in an Asn47Lys substitution, was identified in the proband (ZG:II.1) of family ZG (table 2). It was not possible to obtain blood samples from other family members. The mutation was not identified in the 150 healthy controls or in the other 197 probands. No other mutations were identified in this patient in the additional seven FHC genes screened.

Proband ZG was diagnosed with HCM at the age of 60 years, at which time (fig 2A, B), as well as during follow up (fig 2C, D), he had a high, relatively fixed midventricular flow gradient, as well as diastolic filling abnormalities. The midventricular flow gradient was caused not only by the pronounced septal hypertrophy, but also by a significant increase in the size of the papillary muscle apparatus (fig 2A, C). Interestingly, a marked progression in the septal hypertrophy, from 31 to 45 mm, was seen over two years from the age of 60 to 62 years (fig 2). There was no family history of sudden death.

**Discussion**

The observed frequency of \textit{MYL2} mutations (4.4%) in this study of Danish patients is the second highest reported for this gene in any population to date and justifies \textit{MYL2} mutation screening in the Danish HCM population. The frequency of \textit{MYL2} mutations varies from 7.1% (3/42) in a French study to 0.8% (3/383) in an American study. In contrast, the absence

**MYL2 ASN47LYS MUTATION IN PROBAND ZG**

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**MUTATIONS IN TWO FAMILIES WITH MVH**

In each of these two FHC families, the MVH phenotype was found in one member. In both families, other affected relatives displayed predominant hypertrophy of the proximal part of the septum. No myosin light chain mutations were found in either families. However, screening of the other six FHC causing genes identified a G>A transition at nucleotide g5166 of the \textit{MYBPC3} gene, resulting in an Asp228Asn substitution in proband ZJ, and a C>G transversion at g12739 of the \textit{MYH7} gene, causing an Asp778Glu substitution in proband XI, which was also present in his brother with the MVH phenotype.

**Figure 2** Echocardiographic data from patient ZG:II.1 with Asn47Lys MYL2 mutation showing the findings associated with the rare MVH phenotype. Panel A: parasternal long axis view in 1997 (60 years old) showing pronounced mid septal and papillary muscle apparatus hypertrophy. Panel B: apical two chamber view with left ventricular Doppler flow tracings showing the narrow mid ventricular flow (systole, with gradient) (yellow arrows). Panel C: parasternal long axis view in 1999 (62 years old) showing the increase of septal hypertrophy over two years. Panel D: apical four (two) chamber view showing diastolic flow turbulence through the narrow mid ventricular region (yellow arrows). IVS, interventricular septum. PM, papillary muscle.
of mutations in MYL2 in the South African population significantly reduces its precedence in mutation screening programmes in that population. The absence of MYL3 mutations in both population groups studied confirms previous reports of the small contribution of this gene to the HCM disease load. While frequency studies may be influenced by differences in ethnic background, founder effects, referral bias, and differences in clinical inclusion criteria and mutation detection methods, the patients used in this study were included consecutively and screened in the same manner. However, the inclusion criteria for probands were less stringent in the South African population than in the Danish population (maximum left ventricular wall thickness of at least 13 mm vs 15 mm), and this may partly explain why the MYL2 mutation frequency was lower in the former population.

Family ZY (fig 1) carries two MYL2 mutations. Although the maternally derived Lys103Glu substitution occurs at a well conserved residue, codon 103 has not been included in the so far defined functional domains of the myosin regulatory light chain (RLC) (fig 3). In addition, as neither the mother (ZY:II.2) nor her sib (ZY:II.4), had a clinical phenotype compatible with FHC, it is probable that the Lys103Glu substitution that they carry is an extremely rare normal variant. Its absence in 150 controls supports this proposal; if, on the other hand, this is taken as evidence that Lys103Glu is, in fact, a disease causing mutation, its expression in family ZY would suggest it is associated with a low penetrance. In contrast, the paternally derived IVS6-1 splice site mutation, which is the first of this category described in MYL2, is likely to be the disease causing mutation, as clinical analysis of the proband’s father (ZY:II.1) showed that he had pronounced proximal septal hypertrophy. Furthermore, the G>C transversion affects the highly conserved AG sequence of the splice acceptor site. Neural network splice site prediction analysis predicts that this mutation results in a complete removal of the splice site, which may cause skipping of exon 6 and/or 7, or activation of a cryptic splice site 138 bp downstream from the substituted nucleotide. This would lead to truncation of the protein with loss of the functionally important MYH7 binding site. Deletion of the carboxyl-terminus abolishes the phosphorylation mediated regulation of muscle contraction in smooth muscle. However, the mild phenotype of the proband’s sister (ZY:III.1), who also has the splice site mutation, highlights the variable penetrance frequently associated with FHC causing mutations. The Ala13Thr MYL2 mutation has previously been described as associated with the MVH phenotype of FHC, but this was not the case in family T. The novel Asn47Lys MYL2 mutation is found in the conserved Ca^{2+} binding domain of RLC, and

- Mutations in the genes, MYL2 and MYL3, coding for the sarcomeric myosin regulatory and essential light chains, respectively, cause familial hypertrophic cardiomyopathy (FHC).
- We performed mutation screening of consecutively collected Danish (68) and South African (130) FHC probands to establish the frequency of MYL2 and MYL3 mutations. Subsequently, we undertook genotype-phenotype correlations to determine if these mutations are associated with the previously reported midventricular hypertrophy (MVH) phenotype. The coding regions of MYL2 and MYL3 were screened using single strand conformation polymorphism/heteroduplex analysis and mutations were identified by DNA sequencing.
- In the Danish cohort, a novel splice site mutation (IVS6-1) and a novel missense mutation (N47K) were found in MYL2, in addition to one previously described mutation, but no MYL3 mutations were detected. No disease causing mutations were found in the South African cohort. Patients with MVH were identified in three Danish families, one carrying the MYL2 N47K mutation and the other two carrying mutations in the myosin light chain binding region of the β-myosin heavy chain gene or in the cardiac myosin binding protein C gene. Hence, MVH is neither unique to, nor invariably associated with, myosin light chain mutations.
- The observed frequency of MYL2 FHC causing mutations (4.4%) in the Danish cohort warrants mutation screening of this gene in affected subjects in this population. The absence of light chain mutations in the South African probands corroborates reports of geographical variation in the frequency of FHC causing mutations. The absence of reported sudden death indicates that MYL2 mutations may be associated with a good prognosis.
we suggest that a changed affinity towards Ca\(^{2+}\) may be the pathophysiological mechanism of the mutation.

Phenotypically, most patients with HCM are of the classical type with predominately proximal septal hypertrophy. Much more rare are the apical hypertrophy and the MVH phenotypes.\(^6\) The latter phenotype has in some cases been associated with mutations in the myosin light chains.\(^7\) However, others have also identified mutations in the light chains associated with the classical phenotype.\(^8\) We have found mutations in \textit{MYL2} associated with both classical (IVS6-1 and Ala13Thr) and the MVH form (Asp47Lys) (fig 2). The only common finding among the missense mutations presented here are the family history of a good prognosis, that is, good survival (none of our families had members who had died unexpectedly), and late age of onset (children were free of symptoms of cardiac disease). However, it must be taken into account that the numbers of family members studied were small and more extensive investigations are necessary to confirm the good prognosis.

We also identified two additional families in which one affected family member had the MVH phenotype that was not caused by a mutation in either of the myosin light chains. One of these patients carried a mutation (Asp778Glu) in \textit{MYH7} in the region that has been characterised as the light chain binding region,\(^9\) while the other carried a mutation (Asp230Asn) in \textit{MYBPC3} in the amino (N)-proximal C1 domain. Interestingly, it has been speculated that the N-terminal region of myosin binding protein C may contribute to the regulation of muscle contractility, possibly in concert with the regulatory light chain.\(^9\) In this region of \textit{MYBPC3}, one other missense mutation has previously been identified (Glu258Lys); however, this mutation has not been reported to be associated with the MVH phenotype. It is possible that mutations in this region may determine a hypercontractile state, which could induce hypertrophy directly.\(^26\)

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