Clinical and genetic characteristics of α cardiac actin gene mutations in hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is a dominantly inherited disease defined by unexplained myocardial hypertrophy. The prevalence is about 0.2% in the general population. The condition is characterised by a heterogeneous disease expression, and common symptoms include angina, dyspnoea, palpitations, syncope, and exercise limitation. Hypertrophic cardiomyopathy is a frequent cause of sudden cardiac death in young people.1 More than 200 mutations associated with the disease have been identified in sarcomeric contractile protein genes: TNNI3 (troponin T), MYL3 (essential myosin light chain), MYBPC3 (myosin binding protein C), MYL2 (regulatory myosin light chain), MYH7 (β myosin heavy chain), TPM1 (α tropomyosin), ACTC (α cardiac actin), and TNNI3 (troponin I).2–4

In addition, mutations recently have been reported in non-sarcomeric genes.3–7 Mutations in ACTC have also been reported to cause the inherited form of idiopathic dilated cardiomyopathy (DCM). It has been suggested previously that ACTC mutations that affect sarcomere contraction lead to HCM, whereas ACTC mutations that affect force transmission from the sarcomere to the surrounding syncytium lead to DCM.38

We report the clinical and genetic characteristics of ACTC mutations in 206 consecutive patients with HCM.

MATERIALS AND METHODS

Informed consent was obtained from each participant in accordance with local institutional review committee guidelines.

We investigated 206 consecutive Caucasian probands with HCM from Germany (n = 146) or Denmark (n = 60) by mutation analysis of ACTC. We physically and genetically investigated relatives of probands who carried ACTC mutations. The diagnosis of HCM was based on the presence of unexplained myocardial hypertrophy.39 10 In brief, a person was defined as having HCM if the maximal left ventricular wall thickness by echocardiography or cardiac magnetic resonance scan was ≥ 13 mm or the electrocardiogram (ECG) showed major Q wave abnormalities, left ventricular hypertrophy, or marked repolarisation alterations. One patient (pedigree B, participant I-1) was diagnosed with HCM on the basis of results obtained by cardiac catheterisation and cardiac magnetic resonance scans. Participants were suspected of having HCM if their ECG showed incomplete bundle branch block and right axis deviation (pedigree A, participants III-2 and II-3). We classified the phenotype of all participants before DNA analysis.

We isolated genomic DNA from all participants, including controls, from peripheral blood samples, and we amplified ACTC by polymerase chain reaction (PCR) with intronic primers, as previously described.1 We analysed PCR products by single strand conformation polymorphism (SSCP) and heteroduplex analysis of exons 2, 3, and 4 with a precast 12.5% polyacrylamide gel at 4°C and 20°C (Multiphor gel apparatus; Pharmacia Biotech, Uppsala, Sweden). We visualised bands with silver staining and analysed abnormal conformers by direct sequencing.11 Because of difficulties in obtaining reproducible SSCP analysis of exons 1, 5, and 6, we subjected these exons to direct sequencing with standard protocols. We established the haplotypes of families A and B and subjected these exons to direct sequencing with standard protocols. We established the haplotypes of families A and B and subjected these exons to direct sequencing with standard protocols. We established the haplotypes of families A and B and subjected these exons to direct sequencing with standard protocols. We established the haplotypes of families A and B and subjected these exons to direct sequencing with standard protocols. We established the haplotypes of families A and B and subjected these exons to direct sequencing with standard protocols.

RESULTS

Genetic investigations

The proband of family A (individual II-1) had an 1153A→R nucleotide substitution in exon 3, which resulted in a Tyr166CyS amino acid exchange. Subsequent mutation analysis of this proband’s relatives identified three additional carriers of the mutated allele (fig 1 and table 1).

The proband of family B (individual I-1) had a 283A→C nucleotide substitution in exon 5, which resulted in a Met305Leu amino acid substitution (fig 1 and table 1). The

Key points

- The study aimed to investigate the prevalence and clinical characteristics of α cardiac actin mutations (ACTC) in 206 consecutive families with hypertrophic cardiomyopathy (HCM). Mutation analysis was performed by SSCP analysis and direct sequencing. In addition, the likely impact of ACTC mutations on sarcomeric function was investigated by analysis of the crystal structure of the actin protein.
- The prevalence of ACTC mutations was 1.5%. Two novel mutations were identified. Available phenotypic information in carriers of ACTC mutations suggested a heterogeneous clinical appearance that ranged from unaffected cases to patients with severe disease presentation.
- Examination of the crystal structure of actin showed that amino acids mutated in HCM were exposed to the surface domain of actin that interacts with myosin.
- ACTC mutations are rare in HCM, and no specific genotype–phenotype correlation is apparent. The localisation of ACTC mutations in conserved and functional important regions of the gene substantiate the current hypothesis that ACTC mutations in HCM affect sarcomeric contraction, whereas ACTC mutations in dilated cardiomyopathy affect force transmission.
presence of the mutation in proband B was confirmed by a Msl1 restriction enzyme assay, which cleaved the wildtype allele, as expected, and left the mutated allele intact. To diminish the possibility that the ACTC mutations identified (Tyr166Cys and Met305Leu) were common polymorphisms, 150 control chromosomes were investigated by SSCP analysis (exon 3) or direct sequencing (exon 5). No sequence variations were identified, which confirmed the result of previous SSCP analyses of controls of exon 3 (300 chromosomes) and exon 5 (870 chromosomes). These observations are in accordance with the fact that cardiac actin is a highly conserved protein throughout evolution including the Tyr166 and Met305 amino acids that are conserved in the 37 vertebrate and invertebrate actins that are part of the protein alignment database. Compared with the original sequence published more than a decade ago, five silent homozygous base changes were seen in all 206 individuals investigated (exon 2, 274T>R; intron 3, 1089G>R; exon 4, 1488T>C and 1532C>T; and exon 6, 284T>C), which is likely to reflect the contemporary, more accurate method of sequencing. Others have performed mutation analysis of ACTC in the context of DCM and identified a total of eight silent single nucleotide polymorphisms in populations of Asian and South African origin, respectively.

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**Table 1** Clinical features of families A and B

<table>
<thead>
<tr>
<th>Pedigree</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Symptoms</th>
<th>Electrocardiogram findings</th>
<th>Echocardiogram findings</th>
<th>Phenotypic assignment</th>
<th>ACTC genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-1</td>
<td>51</td>
<td>Man</td>
<td>Angina</td>
<td>Atrial fibrillation</td>
<td>HCM</td>
<td>HCM</td>
<td>Tyr166Cys</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dyspnoea</td>
<td>(New York Heart Association grade III)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III-1</td>
<td>27</td>
<td>Man</td>
<td>No</td>
<td>Incomplete right bundle branch block</td>
<td>HCM</td>
<td>HCM</td>
<td>Tyr166Cys</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Septum 15 mm</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Systolic anterior movement of mitral valve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Left ventricle outflow tract gradient at rest 35 mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III-2</td>
<td>24</td>
<td>Man</td>
<td>No</td>
<td>Incomplete right bundle branch block and right axis deviation</td>
<td>HCM</td>
<td>Suspected HCM</td>
<td>Tyr166Cys</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Septum 18 mm</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td>Systolic anterior movement of mitral valve</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Left ventricle outflow tract gradient at rest 35 mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-3</td>
<td>47</td>
<td>Man</td>
<td>No</td>
<td>Incomplete right bundle branch black and right axis deviation</td>
<td>Normal</td>
<td>Suspected HCM</td>
<td>Tyr166Cys</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>Septum 15 mm</td>
<td></td>
<td></td>
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<td>Systolic anterior movement of mitral valve</td>
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<td></td>
<td></td>
<td>Left ventricle outflow tract gradient at rest 35 mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td>Atrial fibrillation</td>
<td>Not obtainable*</td>
<td>HCM</td>
<td>Met305Leu</td>
</tr>
<tr>
<td>I-1</td>
<td>61</td>
<td>Man</td>
<td>Angina</td>
<td>(New York Heart Association grade III)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Cardiac catheterisation showed apical systolic obstruction and normal left ventricular function and coronary arteries.
Clinical investigations

The proband of family A was diagnosed with HCM at the age of 51 years. He had severe symptoms of angina and dyspnoea (New York Heart Association (NYHA) class III) (fig 1, table 1). An echocardiogram showed septal hypertrophy of 18 mm, mild mitral regurgitation, systolic anterior movement of the mitral valve, and a left ventricle outflow tract gradient of 35 mm Hg at rest, which increased to 60 mm Hg after the Valsalva manoeuvre. Transaortal myectomy abolished the outflow tract obstruction and gave considerable symptomatic improvement. Part of the left ventricular septum was removed by transaortal myectomy and stained with haematoxylin and eosin. Histology of the removed myocardium showed myocyte hypertrophy and myofibrillar disarray consistent with a diagnosis of HCM15 (fig 1). The proband’s mother, individual I-2, had dyspnoea on exertion, which had started in her late twenties. She later developed atrial fibrillation and mitral regurgitation and died of congestive heart failure at the age of 50 years. The remaining relatives had no symptoms of disease, but the echocardiogram of participant III-1 showed septal hypertrophy of 15 mm. In addition, the electrocardiograms of participants III-2 and II-3 had minor abnormalities in terms of incomplete right bundle branch block and right axis deviation, while their echocardiograms were within the upper normal range of the maximal left ventricular wall thickness (both 12 mm).

The proband of family B was diagnosed at the age of 61 years. He had symptoms of angina on exertion and dyspnoea (NYHA II) (fig 1, table 1). Images of the heart by echocardiography were unobtainable, but cardiac catheterisation showed apical systolic obliteration of the left ventricle consistent with a diagnosis of apical HCM. This finding was confirmed by a cardiac magnetic resonance scan, which showed a maximal left ventricular wall thickness of 14 mm. He had no family history of cardiac disease, and subsequent physical examination and mutation analysis of his children did not identify additional disease carriers.

Analysis of actin crystal structure

Actins are major constituents of the thin filaments in the muscle sarcomere. They generate force within the sarcomere in companion with myosin and transmit force from the sarcomere to the surrounding syncytium by the thin filament. Previous studies of ACTC mutations in families with either HCM or the hereditary form of DCM have led to the hypothesis that mutations in force transmitting domains lead to HCM, while mutations in force generating domains lead to DCM.2 The mutations identified in families A and B, as well as the Ala295 previously reported to be mutated in HCM, shown in red. All HCM mutations are localised at the actin surface that interacts with myosin. Two amino acids previously reported to cause DCM (Arg312 and Glu361) are shown in purple.6 Amino acid differences between α cardiac actin and γ enteric actin expressed in a transgenic ACTC knock out mouse highlighted in green.16

To confirm this hypothesis, we used a number of approaches.

Conventional page gel SSCP analysis was used for mutation analysis of three exons of ACTC. This method is presumed to be less sensitive than direct sequencing and more recent methods developed for mutation screening.22 The frequency of ACTC mutations in our study, however, was similar to that in other studies, and it is unlikely therefore, that the SSCP method we used missed a significant number of mutations. Although the number of affected participants was limited in this study, the two amino acid substitutions identified (Tyr166Cys and Met305Leu) were believed to be disease causing mutations, because all clinically affected individuals were mutation carriers, no sequence variations were identified in ethnically matched control individuals, both mutations changed highly conserved amino acids, and both mutations were likely to change protein–protein interactions on the basis of analysis of the crystal structure of actin.

The phenotypes associated with the Tyr166Cys and Met305Leu amino acid substitutions seemed to be heterogeneous. Both probands of families A and B had reduced exercise capacity and symptoms of disease in contrast with the remaining asymptomatic mutation carriers of family A. In addition, the proband of family A had pronounced hypertrophy of the septum that needed myectomy compared with the mild or absent hypertrophy of the remaining participants who carried the mutated allele.

The clinical findings associated with ACTC mutations described in previous studies further strengthen the impression of variable disease expression (table 2). The first study to...
report on ACTC mutations identified an Ala295Ser amino acid substitution in a large Danish pedigree. Only a few of the affected people had symptoms of disease, including two with end stage dilated HCM and one with tachycardia caused by a Wolff-Parkinson-White syndrome, which preceded a later development of ventricular hypertrophy. The remaining carriers of the mutation, except one, fulfilled the diagnostic criteria for HCM, but none had symptoms of disease. The Ala295Ser mutation seemed to be associated with a high penetrance, diverse phenotypes, a relatively low morbidity, and no sudden deaths. These findings were different from those reported recently by Olson et al.19 They identified three novel ACTC mutations, of which two occurred de novo and one appeared in a family with seven disease carriers (table 2). Both de novo mutations were associated with early onset and severe disease expression, as is the case in patients who also have de novo mutations in other cardiac sarcomeric contractile protein genes.20 Most of the family members who carried the Glu99Lys mutation were reported to have symptoms of disease. Two had experienced ventricular arrhythmia, of which one had been resuscitated from cardiac arrest (no age reported). The predominant finding on echocardiograms in this family was that most of the affected members had apical hypertrophy of the myocardium.

The heterogeneous disease expression associated with ACTC mutations in this study accords well with the results of recent studies that investigated the clinical expression of mutations in other genes associated with HCM.21 Environmental factors and individual genetic backgrounds are likely to modify the phenotype of mutation carriers.22

**Table 2 Clinical profile associated with previous reported ACTC mutations**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Carriers of mutations</th>
<th>Phenotype positive</th>
<th>Symptomatic</th>
<th>Abnormal electrocardiogram</th>
<th>Ventricular arrhythmia</th>
<th>Abnormal echocardiogram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala295Ser</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pro164Ala</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ala331Pro</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Glu99Lys</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

* Episodes of non-sustained ventricular tachycardia accompanied by syncope. † Ventricular fibrillation and cardiac arrest. ‡ with ventricular fibrillation and cardiac arrest and one with ventricular tachycardia. ** Seven with wall hypertrophy and two with end stage dilation. †† Septal and apical hypertrophy. ††† Five with predominant apical hypertrophy and one with septal hypertrophy.

**Conclusion**

Mutations of the ACTC gene in patients with HCM are rare, and no gene specific phenotype is apparent. Localisation of ACTC mutations in conserved and functionally important regions of the gene substantiate the hypothesis that ACTC mutations in patients with HCM affect sarcomere contraction, whereas ACTC mutations in patients with DCM affect force transmission from the sarcomere to the surrounding syncytiun.

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