Variable Clinical Manifestation of a Novel Missense Mutation in the Alpha-Tropomyosin (TPM1) Gene in Familial Hypertrophic Cardiomyopathy

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OBJECTIVES
This study was initiated to identify the disease-causing genetic defect in a family with hypertrophic cardiomyopathy (HCM) and high incidence of sudden death.

BACKGROUND
Familial hypertrophic cardiomyopathy (FHC) is an autosomal dominant transmitted disorder that is genetically and clinically heterogeneous. Mutations in 11 genes have been associated with the pathogenesis of the disease.

METHODS
We studied a large FHC family, first by linkage analysis, to identify the gene involved, and subsequently screened the gene, encoding alpha-tropomyosin (TPM1), for mutations by using single-strand conformation polymorphism and sequencing analysis.

RESULTS
Twelve family members presented clinical features of HCM, five of whom died at young age, while others had only mild clinical features. Marker analysis showed linkage for the TPM1 gene on chromosome 15q22 (maximal logarithm of the odds score is 5.16, \(\theta = 0\)); subsequently, a novel missense mutation (Glu62Gln) was identified.

CONCLUSIONS
The novel mutation identified in TPM1 is associated with the clinical features of cardiac hypertrophy in all but one genetically affected member of this large family. The clinical data suggest a malignant phenotype at young age with a variable clinical manifestation and penetrance at older age. The Glu62Gln mutation is the sixth TPM1 mutation identified as the cause of FHC, indicating that mutations in this gene are very rare. This is the first reported amino acid substitution at the \(\phi\)-position within the coiled-coil structure of the tropomyosin protein. (J Am Coll Cardiol 2003;41:981–6) © 2003 by the American College of Cardiology Foundation

Familial hypertrophic cardiomyopathy (FHC) is a clinically and genetically heterogeneous disease with an autosomal dominant mode of inheritance. Main clinical features are increased left ventricular (LV) and/or right ventricular muscle mass often in combination with asymmetric hypertrophy of the septum. On echocardiography, an increased cardiac muscle thickness (13 mm or more) is observed (1). Typical complaints include chest pain (angina pectoris), shortness of breath (dyspnoea), fatigue, palpitations, and syncope. Sudden cardiac death may be the first dramatic symptom of the disease. The motion and contraction force of striated cardiac muscle cells is generated within the sarcomere by interaction between thick and thin filaments. Impaired relaxation and contraction force, a hallmark of FHC is generally caused by mutations in one of the sarcomere encoding genes. Until now, 11 genes have been reported as being involved in the development of FHC (2–4). A total of 10 of the 11 genes encode cardiac proteins that assemble into contractile units (sarcomeres) of the cardiomyocytes. The genes that encode beta-cardiac myosin heavy chain (MYH7), myosin binding protein C (MYBPC3), and troponin T (TNNT2) account for approximately 75% of FHC (based on reported mutations). Recently, the gene PRKAG2 (gamma 2 subunit of AMP-activated protein kinase) encoding a non-sarcomeric protein on chromosome 7q was found to be involved in cardiac hypertrophy (5,6). In these patients, a complex of symptoms including conduction disturbances and Wolf-Parkinson-White syndrome was demonstrated, probably related to glycogen storage (7,8).

In this paper we describe a large five-generation FHC family from the Netherlands. Linkage was identified with the alpha tropomyosin (TPM1) gene, and a novel mutation Glu62Gln (E62Q) was detected. The TPM1 gene is a very rare cause of FHC (approximately 5%), except for the Finnish population where TPM1 mutations account for >11% (9). Until now, only eight missense mutations have

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been described within the TPM1 gene causing either hypertrophic (FHC) or dilated cardiomyopathy (DCM) (10–17). The presence of the new mutation was associated with variable clinical features, although the mutation should be considered malignant, especially at younger age.

METHODS

Clinical studies. A large five-generation Dutch family with FHC was analyzed in our institute. Family members were clinically evaluated by physical examination, two-dimensional echocardiographic examination, and 12-lead electrocardiography (ECG) analysis. Histopathological data of deceased family members were obtained when available. The clinical diagnosis of hypertrophic cardiomyopathy (HCM) was made if the interventricular septal thickness was >13 mm, in the absence of other cardiac or systemic causes of LV hypertrophy. In some family members, phenotypic assignment of cardiomyopathy was based on histopathological features of HCM at postmortem examination. Informed consent was obtained from each family member for genetic analysis.

Genetic studies. MARKER ANALYSIS. Blood samples were collected from 22 individuals (clinically affected members and family members with no clinical symptoms of cardiac hypertrophy). Genomic DNA was extracted according to standard protocols (18). Marker sets, used to test four candidate genes, were either located in the 5′ and 3′ flanking regions of the genes or were intragenic. D1S2716 and D1S2622 at 2 to 2.5 centiMorgan (cM) proximal of the troponin T gene (TNNT2; chromosome 1q32), marker D11S1344 and D11S1357 at 0.8 cM of the myosin binding protein C gene (MYBPC3; chromosome 11p11.2), two intragenic markers MYOI and MYOII for the beta-cardiac myosin heavy chain (MYH7; chromosome 11q32), marker D15S159 and D15S993 at 0.5 cM for the alpha-tropomyosin (TPM1; chromosome 15q22) (19–22), comprising the entire coding sequence of the TPM1 gene in cardiac muscle, were amplified by using intragenic primers as previously described (10). Single-strand conformation polymorphism analysis was performed at 5°C and 15°C, and amplicons with aberrant conformers were purified and analyzed by sequencing as described previously (24). For diagnostic analysis, the mutation detection was performed by MnII endonuclease (Roche, Almere, the Netherlands) of the PCR product of exon 2b and analyzed by gel electrophoresis on a 3% Nusieve Agarose gel (FMC, Sanvertech, Boechout, Belgium).

RESULTS

Clinical characteristics. The pedigree of the FHC family is presented in Figure 1, and main clinical characteristics are presented in Table 1. The family came to our attention because of the early sudden death of a mother (III.11) and four of her children (IV:16, IV:17, IV:18, and IV:19). The cause of her death was unclear, and no autopsy was performed. Postmortem evaluation of the two children (IV:16, IV:17) revealed severe asymmetric septal hypertrophy. Histology indicated myofibrillar disarray with interstitial fibrosis in the apical part of the septum and abnormal intramural coronary vessels. The third son (IV:8) was examined after the death of the oldest two sibs. He suddenly died at age 15. The youngest son (IV:19) was examined at age 17, and, at that time, he complained about fatigue. A few months later, he died suddenly. The father of this family remarried to a younger sister (III.13) of his first wife. Two children (IV:21 and IV:22) again showed HCM at age 33 and 35 years, respectively. Besides cardiac abnormalities, both were mentally retarded suffered spastic paralysis. The origin of their mental deficiency remains unclear. Their mother remained without complaints at age 67 years. She was frequently reinvestigated and did not show any signs of HCM on ECG or two-dimensional echocardiography. Only on magnetic resonance imaging investigation a septal hypertrophy (max, 17 mm) was found. In the other branch of this family, the diagnosis HCM was made for individual IV:9 at routine screening for military services. At age 7, his daughter (V:3) was investigated for signs of HCM. Electrocardiographic examination and echocardiography demonstrated signs of LV hypertrophy. At present she remains without complaints.

Linkage of the TPM1 gene. Because of maternal transmission in the right part of the pedigree (Fig. 1), HCM-related and specific mitochondrial DNA mutations (A3243G, A3260G, C3303T, A4300G, A4269G, A43417G, G8344A, and T8993C/G) were excluded, as well as mitochondrial DNA deletions (data not shown).
Marker analysis of 20 family members and two spouses of deceased members excluded the involvement of the MYH7, the MYBPC3, and the TNNT2 genes. Segregation of the alpha-tropomyosin (TPM1) markers were in line with a possible involvement of the TPM1 gene. Linkage analysis software revealed a maximum LOD score $Z(\theta) = 5.16$ for markers D15S159 and D15S993. Subsequently, nine amplified exons of the TPM1 gene were screened by SSCP analysis. An aberrant conformer was identified in exon 2b indicating a sequence variation. Sequence analysis showed a GAG to CAG transition at position 184 (complementary DNA reference sequence of TPM1, Genebank accession number M19713). The nucleotide substitution changes the amino acid glutamic acid of codon 62 into glutamine (Glu62Gln). In this family, the missense mutation segregated with the disease. As the G to C substitution abolishes a MnlI restriction site, we applied MnlI restriction analysis to identify carriers of the genetic defect in all family members at risk. The pathogenicity of the mutation was based on exclusion of the mutation within a control group of 100 unrelated subjects (200 chromosomes) and by the localization of the substituted amino acid in an evolutionary highly conserved domain of the alpha-tropomyosin protein.

**DISCUSSION**

Familial HCM is a clinically and genetically heterogeneous disorder. Incomplete penetrance and variable age-related clinical expression is often observed within and between families, even if an identical mutation is involved. At the moment, mutations in 11 genes have been identified that are involved in FHC, making linkage analysis the first step in identifying the genetic defect, as has been demonstrated in this family. In FHC families, however, a major problem is the high incidence of sudden death of affected family members and, because of that, no tissue or DNA is available for linkage studies. In this study, marker analysis showed statistically significant linkage with the TPM1 gene on chromosome 15q22, and the causative mutation was identified in all clinically affected family members. The high conservation of the protein domain involved, and the absence in the control population, indicated that this mutation was very likely the cause of FHC in this family. However, in this pedigree, the penetrance of the Glu62Gln mutation is incomplete, as one of the carriers with the mutation presents only with very mild hypertrophy at older age that might be missed by routine echocardiographic screening. These findings suggest that other genetic or environmental factors, yet unidentified, must be involved in modifying the effect of the mutation between family members. The clinical presentation of the novel mutation points towards a malignant (high incidence of sudden death) form of FHC with varying hypertrophy from mild to severe, in particular, asymmetrical septal hypertrophy. An interesting finding is the coronary abnormalities encountered during autopsy in patient IV:16. Despite marked initial thickening, there were no signs of old or fresh myocardial infarction. In addition, the localization of the fibrosis was not related to the site of coronary abnormalities. These findings make coronary disease a less likely cause of sudden death in this patient. In contrast, the extensive disarray combined with fibrosis and maybe local subendocardial ischemia could provide an arrhythmic substrate. Unfortunately, no serial measurements or Holter monitoring were performed in affected family members. No cases presented with HCM progressing into DCM.

Up to the present, only few TPM1 mutations have been reported, causing either hypertrophic (FHC) or DCM. Two mutations in exon 5, near the Ca$_{2+}$-dependent troponin-T
Table 1. Clinical Features of the HCM Family

<table>
<thead>
<tr>
<th>Pedigree</th>
<th>Gender</th>
<th>Age</th>
<th>E62Q</th>
<th>Clinical History</th>
<th>2D Echo</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>II.2</td>
<td>F</td>
<td>(+)*</td>
<td>SCD at 69 yrs</td>
<td>NA</td>
<td>NA</td>
<td>Obligate carrier</td>
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<tr>
<td>II.4</td>
<td>F</td>
<td>(+)</td>
<td></td>
<td>NA</td>
<td>NA</td>
<td>Obligate carrier</td>
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<td></td>
<td>Obligate carrier;</td>
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<td>gynecological cancer at</td>
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<td></td>
<td></td>
<td>age 71 yrs</td>
</tr>
<tr>
<td>III.4</td>
<td>F</td>
<td>(+)</td>
<td>SCD at 47 yrs</td>
<td>NA</td>
<td>NA</td>
<td>Obligate carrier</td>
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<tr>
<td>III.5</td>
<td>M</td>
<td>(+)</td>
<td>SCD at 45 yrs</td>
<td>NA</td>
<td>NA</td>
<td>Obligate carrier</td>
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<tr>
<td>III.11</td>
<td>F</td>
<td>?</td>
<td>SCD at 35 yrs</td>
<td>NA</td>
<td>NA</td>
<td>Obligate carrier</td>
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<tr>
<td>III.13</td>
<td>F</td>
<td>67</td>
<td>+‡</td>
<td>No complaints</td>
<td>Normal</td>
<td>Normal</td>
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<tr>
<td>III.3</td>
<td>M</td>
<td>46</td>
<td>+</td>
<td>No complaints</td>
<td>66%</td>
<td>16 14 23 41 LVH, abnormal ST-segments</td>
</tr>
<tr>
<td>IV.4</td>
<td>F</td>
<td>34</td>
<td>+</td>
<td>Atypical complaints</td>
<td>Normal</td>
<td>LVH, abnormal ST-segments</td>
</tr>
<tr>
<td>IV.8</td>
<td>M</td>
<td>44</td>
<td>+</td>
<td>PAF</td>
<td>64%</td>
<td>18 8 30 46 SAM, MI</td>
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<tr>
<td>IV.9</td>
<td>M</td>
<td>34</td>
<td>+</td>
<td>PAF, dyspnea</td>
<td>70%</td>
<td>14 10 33 55 SAM, MI</td>
</tr>
<tr>
<td>IV.11</td>
<td>M</td>
<td>18</td>
<td>(+)</td>
<td>SCD at 18 yrs, arrhythmia</td>
<td>NA</td>
<td>31 10 24 38 SAM LVH, abnormal ST-segments Died during catheterization</td>
</tr>
<tr>
<td>IV.16</td>
<td>M</td>
<td>19</td>
<td>(+)</td>
<td>SCD at 19 yrs</td>
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<td>LVH</td>
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<td>IV.17</td>
<td>F</td>
<td>17</td>
<td>(+)</td>
<td>SCD at 17 yrs</td>
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<td>LVH</td>
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<td>PM: septal hypertrophy,</td>
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<td>myofibrillar disarray +</td>
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<td>fibrosis</td>
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<td>PM: extensive septal</td>
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<td>hypertrophy + LVH,</td>
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<td>RVH, myofibrillar</td>
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<td>disarray + fibrosis</td>
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<td>PM: muscle degeneration,</td>
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<td>interstitial fibrosis</td>
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<td></td>
<td>Mentally retarded spasticity</td>
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<td>Hypertrophy at age 5 yrs</td>
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</tbody>
</table>

*The (+) symbol indicates possible carrier of the mutation Glu62Gln; ‡the + symbol indicates carrier of the mutation Glu62Gln; §MRI: normal value <12 mm.

Age = age (yr) at cardiac evaluation; ASH = asymmetrical septum hypertrophy; EDD = end-diastolic diameter; ESD = end-systolic diameter; HCM = hypertrophic cardiomyopathy; LVH = left ventricular hypertrophy; LVEF = left ventricular ejection fraction; MI = mitral incompetence; MRI = magnetic resonance imaging; NA = not available; PAF = paroxysmal atrial fibrillation; PM = post mortem; PW = posterior wall; RVH = right ventricular hypertrophy; SAM = systolic anterior movement of the mitral valve; SCD = sudden cardiac death; SH = septum hypertrophy.
binding domain of the alpha-tropomyosin protein, have
been associated with a transition from severe hypertrophy to
DCM (Glu180Val) and with mild LV hypertrophy but
poor prognosis (Glu180Gly) (10,15). Within the same
protein domain, a mutational hot spot at position
Asp175Asn has been identified in five unrelated families
(four Caucasian, one Japanese) with FHC (9,10,12,13). The
Asp175Asn mutation was studied in three families with full
penetance and seemed to be associated with a favorable
prognosis (25). Unique features of mild cardiac hypertrophy
with a high mortality rate were described for the mutation
Val95Ala in exon 3 (16). The mutations Glu40Lys and
Glu54Lys in exon 2b occurred in an area that may alter the
binding of alpha-tropomyosin to actin and have been
associated with clinical features of DCM (17).

The TPM1 gene encodes a rigid rod-shaped protein.
This protein is abundantly expressed in the striated muscle
cells and various other tissues and forms a double helix
coiled-coil structure by head-to-tail polymerization.
Several isoforms result from the TPM1 gene by alternative splicing,
and the adult cardiac isoform is encoded by only nine of the
14 exons. The protein is located within the thin filament
of the sarcomere where it is associated with actin by twisting
around the long axis of the actin filament as a coiled-coil
dimer. It can bind seven consecutive actin monomers and,
so, contributes to the stability of the thin filament (26).
Another thin filament component troponin T also has a
binding site for tropomyosin. This binding site is thought to
be responsible for positioning the troponin complex, which
is composed of three polypeptides, troponin T, I, and C,
into the thin filament. The troponin-tropomyosin complex
is Ca
2+ -sensitive. By raising the level of free calcium, the
position of the tropomyosin-troponin complex is shifted,
and the actin filaments can interact with the myosin heads
of the thick filaments, which are active in the contracting
muscle.

Our findings suggest that differences in clinical presen-
tation may depend on the location of the amino acid
substitution in the TPM1 helix. The novel mutation
Glu62Gln is the most 5’ proximal missense mutation that
has been reported in the TPM1 gene thus far, and which is
associated with a malignant form of FHC.

The negative-charged polar amino acid is replaced by an
uncharged polar residue at a position on the outer surface of
the coiled-coil (26). In the coiled-coil filament of the
tropomyosin molecule, the positions of the amino acids are
arranged in an order of highly conserved heptad repeats
along the entire length of the TPM1 protein (26,27). All
mutations that have been reported previously and associated
with FHC have occurred at the g-1, d-1, or e-position of
the heptad repeat (Fig. 2). The altered amino acids, which have
been correlated with DCM, only occurred at the e-position.
Two of the DCM-associated mutations change the acidic
amino acid residue into a basic residue. Olson et al. (17)
suggested that these mutations create a locally increased
positive charge in the negatively charged surface of tropo-
myosin, changing the electrostatic interactions between actin and tropomyosin filaments. This novel Glu62Gln
mutation is the first reported amino acid substitution at the
f-position within the coiled-coil that changes the polarity of
the amino acid and which is associated with hypertrophy.
Further, it should be noticed that the amino acid variants at
the positions 62, 63, and 70 are located within a protein
domain with a function yet unidentified.

Reports of novel mutations within the TPM1 gene may
help to unravel and define functionally important domains
that are crucial for specific interaction with other (sarcomer-
ic) proteins. Preliminary data of mutated amino acids in
specific regions might indicate in which way the cardiomyo-
pathy might develop, hypertrophy or dilation of the cardiac
ventricles. By identifying the important domains for either
disorder, better diagnostic and prognostic tools become
available. Furthermore, the knowledge will help to elucidate
the complex architecture of the sarcomere.

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REFERENCES
2. Carrier L, Jongbloed R, Smeets H, Doevendans PA. Hypertrophic
cardiomyopathy. In: Doevendans PA, Wilde AA, editors. Cardiovas-
cular Genetics for Clinicians. Dordrecht: Kluwer Academic Publish-
3. Marian AJ. Pathogenesis of diverse clinical and pathological pheno-
17. Olson TM, Kishimoto NY, Whitby FG, Michels VV. Mutations that alter the surface charge of alpha-tropomyosin are associated with dilated cardiomyopathy. J Mol Cell Cardiol 2001;33:723–32.