Hypertrophic Cardiomyopathy

Histopathological Features of Sudden Death in Cardiac Troponin T Disease

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Background—Patients with hypertrophic cardiomyopathy (HCM) are at increased risk of premature death; this is particularly apparent for patients with mutations of the troponin T gene. Myocyte disarray and interstitial fibrosis, pathological features of HCM, may be determinants in these deaths. The relation between genotype, pathological phenotype, and mode of death has not been explored.

Methods and Results—Seventy-five hearts with HCM were examined. DNA was available in 50 for screening of the troponin T gene. The macroscopic findings, percentage of disarray, percentage of fibrosis, and percentage of small-vessel disease were correlated with the genotype. A troponin T mutation was identified in 9 of the 50 patients, 8 of whom died suddenly. Patients with a troponin T mutation were younger (mean age, 21.0 years [range, 6 to 37] versus 39.1 years [range, 14 to 72]; P<0.0001), had more sudden death (P=0.02), and had lower heart weights, less fibrosis, and greater disarray than other HCM patients (mean heart weight, 380.3±105.4 g versus 585.0±245.7 g, P=0.002; mean fibrosis, 0.7±0.4% versus 2.6±2.8%, P=0.001; mean disarray, 46.2±7.2% versus 24.1±15.9%, P<0.0001; and mean small-vessel disease, 11.7±14.6 versus 14.1±8.7, P=0.6, respectively). Similarly, patients with troponin T mutations who died suddenly had lower heart weights and greater disarray than patients who died suddenly with unknown genotype (ie, troponin T mutation excluded) (mean heart weight, 429.8±75.4 versus 559.6±204.4 g, P=0.04, and mean disarray, 40.1±9.4% versus 20.2±12.6%, P=0.002, respectively).

Conclusions—Patients with troponin T mutations had severe disarray, with only mild hypertrophy and fibrosis. These patients died suddenly and at an especially early age. We propose that extensive myocyte disarray in the absence of marked hypertrophy is the pathological substrate for sudden death in these patients. (Circulation. 2001;104:1380-1384.)

Key Words: hypertrophy ■ cardiomyopathy ■ pathology

Hypertrophic cardiomyopathy (HCM) is a familial disease characterized by cardiac hypertrophy and an increased frequency of premature death. At least 9 sarcomeric protein gene mutations cause HCM, including mutations of the troponin T1 and the β-myosin heavy chain (βMHC) genes.2,3 There have been many studies relating the genotype to the clinical phenotype of patients with HCM, and although great heterogeneity is seen even within the same gene, it has been possible to draw some conclusions.4–6 It has been shown, for example, that patients with mutations of the βMHC gene show great allelic heterogeneity with regard to both morphology7 and prognosis.8,9 In contrast, the clinical picture for mutations of the troponin T gene is more homogenous, with lesser degrees of hypertrophy and a high proportion of sudden death.4,10 Indeed, despite evidence that marked hypertrophy is a risk for sudden death in HCM,11 patients with mutations of the troponin T gene appear to exhibit a high rate of premature sudden death even in the absence of hypertrophy.12 To understand this apparent paradox, it is important to examine the phenotype not just at the morphological level but also at the histological level. It is likely that myocyte disarray and interstitial fibrosis, which are pathological features of HCM, are determinants in the premature sudden death of these patients, but to date the pathological correlates of troponin T disease have not been studied. We have obtained a large number of hearts with HCM in which blood was available for genotyping. This provided us with an opportunity to screen for mutations of the troponin T gene in a number of HCM hearts and perform an analysis of the relation between genotype, pathological phenotype, and mode of death in HCM.

Methods

Study Population

In the period from 1977 to 1999, 75 hearts with a diagnosis of HCM were obtained after the death of or heart transplantation in patients already under the follow-up of the clinical team or as referred cases to the department of cardiovascular pathology. HCM was diagnosed on the basis of unexplained hypertrophy and the finding of myocyte disarray on postmortem examination (occupying >5% of the myocardium). Family pedigrees were constructed for each of these...
hearts. Clinical investigations were performed and blood obtained for DNA analysis after informed consent and according to local ethics committee approval. As a result, blood was available for 50 HCM hearts (either from alive affected relatives, transplant patients, or patients assessed before death).

Pathological Evaluation
The total heart weight was noted and left ventricular (LV) and right ventricular wall thicknesses measured before sectioning. Nineteen full-thickness blocks were taken from the left and right ventricular free and septal walls at the base, mid-cavity, and apical levels and from the left atrial free wall. All sections were embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin and elastic van Gieson stains. For each histological feature, the section was examined field by field to entirely cover the tissue block without overlap. To quantify disarray, each section was examined and scored as disarray present or absent. The number of fields in which disarray was present was divided by the total number of fields examined and a percentage derived. To quantify fibrosis, sections were examined with the Optomax system (AMS) and quantified as a percentage of fibrosis across each field. To assess small-vessel disease, the external and luminal diameter for all suitable intramyocardial vessels were measured with the use of a digitized pad and the Vids-V program (AMS). Severity of small-vessel disease was considered to be the percentage of vessels within a given section in which external/luminal diameter was ≥3.0.

Pedigree Evaluation
To identify affected relatives for genotyping, all available family members underwent standard history, physical examination, 12-lead ECG, and 2D echocardiographic investigation. A diagnosis of HCM was made on the demonstration of a maximal LV wall thickness of ≥15 mm in the absence of a known cause or in accordance with the proposed criteria for a diagnosis of HCM in the context of familial disease.13

Genetic Screening
Genomic DNA was extracted for polymerase chain reaction amplification from blood and screened for mutations of the troponin T gene. Screening was performed by automated direct DNA sequencing (ABI automated sequencer, Cambridge Biosciences) on all 15 transcribed exons. Where possible, mutations were confirmed with restriction enzyme digestion.

Statistical Analysis
All calculations were performed with the SPSS/WIN programs (SPSS). Data are presented as mean±SD. The pathological characteristics of patients with troponin T mutations were compared with patients with unknown genotype by use of the Student’s t test for independent samples (2-tailed) and χ² analysis. A value of P<0.05 was taken as evidence of significance.

Results
A troponin T mutation was identified in 9 hearts (see Table). All mutations found were considered to be disease causing in view of the following: cosegregation of the mutation with the disease in affected relatives; the same sequence variations were not found in at least 180 chromosomes from normal control subjects; all missense mutations caused an amino acid change and were at a highly conserved site in the protein.
Clinical and Genetic Profile of Genotyped Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age, y</th>
<th>Mode of Death</th>
<th>Gene</th>
<th>Mutation</th>
</tr>
</thead>
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<td>M</td>
<td>22</td>
<td>SCD</td>
<td>TT</td>
<td>Arg92Trp</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>26</td>
<td>SCD</td>
<td>TT</td>
<td>Arg92Leu</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>17</td>
<td>SCD</td>
<td>TT</td>
<td>Arg94Leu</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>21</td>
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<td>TT</td>
<td>Arg94Leu</td>
</tr>
<tr>
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<td>F</td>
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<td>TT</td>
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<td>TT</td>
<td>Pro77Leu</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>29</td>
<td>SCD</td>
<td>TT</td>
<td>Ser69Phe</td>
</tr>
</tbody>
</table>

SCD indicates sudden cardiac death; Tx, transplant; and TT, troponin T.

Of the 9 patients found to have mutations of the cardiac troponin T gene, 2 were from the same family (patients 3 and 4). The mutations for all 9 patients are shown in the Table. Eight patients with a troponin T had sudden cardiac death, compared with 19 patients in whom a genotype was not identified (ie, troponin T mutations excluded). For 6 of the 9 patients who died suddenly, death was the first clinical manifestation. One patient underwent heart transplantation for symptoms and arrhythmias that were refractory to medical treatment (systolic function was well preserved).

Troponin T Mutations Versus Patients With HCM With Unknown Genotype

Patients with a troponin T mutation were of a younger age (mean age, 21.0 years [range, 6 to 37] versus 39.1 years [range, 14 to 72], \( P<0.0001 \)) and had more sudden deaths (8 of 9 patients versus 19 of 41 patients, \( P=0.02 \)) than HCM patients with unknown genotype (ie, troponin T mutations excluded).

Troponin T hearts had significantly lower heart weights, less fibrosis, and greater disarray than patients in whom the gene was not identified (mean heart weight, 380.3 \( \pm 105.4 \) g versus 585.0 \( \pm 245.7 \) g, \( P=0.002 \); mean fibrosis, 0.7 \( \pm 0.4\)\% versus 2.6 \( \pm 2.8\)\%, \( P=0.001 \); mean disarray, 46.2 \( \pm 7.2\)\% versus 24.1 \( \pm 15.9\)\%, \( P<0.0001 \); and mean small vessel disease, 11.7 \( \pm 14.6 \) versus 14.1 \( \pm 8.7 \), \( P=0.6 \), respectively). Similar results were found after excluding patients with dilated HCM (data not shown).

Patients With Sudden Death With Troponin T Mutation Versus Those With Sudden Death With Unknown Genotype

Patients who died suddenly with a troponin T mutation were younger, and had significantly lower heart weights and greater disarray than patients with sudden cardiac death without a troponin T mutation (ie, unknown genotype) (mean age was 22.9 years [11.9] versus 35.8 years [14.4], \( P=0.08 \), mean heart weight, 429.8 [75.4] g versus 559.6 [204.4] g, \( P=0.04 \), and mean disarray, 40.1 [9.4] versus 20.2 [12.6]\%, \( P=0.002 \), respectively). The degree of fibrosis and small-vessel disease did not differ significantly between these patient groups (mean fibrosis, 1.05 [1.4]\% versus 1.45 [1.3]\%, \( P=0.5 \), and mean small-vessel disease, 11.3 [13.4]\% versus 12.9 [8.8]\%, \( P=0.6 \), respectively).

Figure 2. Proposed relation between genotype, histology, phenotype, and sudden death. BPR indicates blood pressure response to exercise; LV, left ventricle; and SCD, sudden cardiac death.

Discussion

The relation between the genotype and the specific histological changes seen in HCM has not been established. Much work has focused on the development of animal transgenes. However, it is difficult to know how far the results from such studies can be extrapolated to the human disease. A mouse transgene analogous to the \( \beta \)-MHC mutation Arg403Gln has been developed.\(^\text{14} \) Despite the lethal nature of the human mutant, the \( \alpha \)-MHC\(^{203/-} \) mice had normal life expectancies when involved in normal daily activities,\(^\text{15} \) suggesting differences between animal and human disease expression. Furthermore, microscopic assessment of disarray is difficult in such small animals, and it is uncertain whether the pathological phenotype (which does not have gross cardiac hypertrophy\(^\text{14} \)) is truly analogous to the human disease. This study is the first to examine the relation between the genotype and the pathological phenotype in a group of patients with HCM.

We have shown that patients with a troponin T mutation have greater disarray, less fibrosis, and lower heart weights than other patients with HCM. These differences were apparent when dilated HCM cases were excluded, suggesting that the greater disarray and reduced fibrosis in patients with troponin T mutations was as a direct result of the gene mutation and not caused by differences in comparing hearts with typical HCM with those with dilated HCM.

Furthermore, patients with a troponin T mutation who died suddenly also had greater disarray and lower heart weights than patients who died suddenly but who did not have a troponin T mutation (Figure 1). Thus for patients with troponin T mutations, who have been widely reported as at increased risk of premature death,\(^\text{4,10,12} \) the pathological substrate for sudden death appears to be widespread myocyte disarray.

At the clinical level, cardiac hypertrophy has been considered the primary phenotypic feature of HCM,\(^\text{16} \) whereas at the histological level, myocyte disarray is a quantitatively specific marker of the disease.\(^\text{17} \) It has therefore been assumed that cardiac hypertrophy develops in relation to myocyte disarray. However, in a pathological study of HCM hearts, Maron et al\(^\text{18} \) found no correlation between the LV wall thickness and the degree of myocyte...
disarray. Our results reveal that HCM hearts with different mutations of the troponin T gene manifest widespread disarray in the absence of marked hypertrophy. These findings suggest that at least for patients with troponin T disease, the risk of premature death is determined by the level of myocyte disarray and is independent of the degree of cardiac hypertrophy. The results also have important implications with regard to the pathogenesis of both disarray and hypertrophy, suggesting that hypertrophy is not a necessary consequence of myocyte disarray. Instead, cardiac hypertrophy may develop in response to only certain sarcomeric mutations, representing a compensatory mechanism that may not be appropriate in the presence of most troponin T mutations.

A link between troponin T mutations and ischemia has been postulated from in vitro work showing that for at least some troponin T mutations, the energy requirement for cross-bridge cycling is increased. Thus, even in the absence of hypertrophy, energy demands may be increased to a level that cannot be met, resulting in ischemia. Preliminary clinical data suggest an association between both disarray and ischemia and disarray and several of the accepted risk factors for sudden death (including an abnormal vascular response to exercise). Because our patients with troponin T mutations exhibited widespread disarray, we suggest that ischemia and associated abnormalities of the vascular response to exercise may be particularly important in patients with troponin T mutations. Because both ischemia and an abnormal vascular response to exercise are recognized risk factors for sudden death, a pathway from mutant sarcomere protein to myocyte disarray to sudden death is suggested (see Figure 2).

In contrast, hearts in which a mutation of the troponin T gene was excluded had greater fibrosis, which has been shown to relate to episodes of nonsustained ventricular tachycardia and probably is a substrate for arrhythmogenesis. Because both fibrosis and episodes of nonsustained ventricular tachycardia increase with increasing age, whereas disarray is likely to be present at least early childhood (as demonstrated in patient 5), these patients would most likely suffer sudden arrhythmic death at a later age than their troponin T counterparts. Indeed, our results show that patients who died suddenly but in whom the underlying abnormality was not a troponin T mutation, died so at a later age. It is therefore suggested that for HCM patients in whom the phenotype is characterized by greater fibrosis than that found in relation to a troponin T mutation, death may occur later in life, either as a result of an arrhythmic episode or from progression to the dilated phase of the disease (see Figure 2).

**Study Limitations**

Although only 9 patients with troponin T mutations were studied, we believe that the results derived from hearts with a troponin T mutation are indeed reflective of the histological phenotype as a whole, because a high degree of concordance has been seen among all but one of the studies examining the clinical phenotype (it should be noted that the study in which a more heterogeneous phenotype was observed was hampered by a limited assessment of the pedigree).

In conclusion, our study has shown that patients with troponin T mutations had severe disarray with only mild hypertrophy and fibrosis. These patients died suddenly and at an especially early age. Obviously, the numbers involved are small because of the problems of obtaining whole hearts from genotyped patients. However, we believe that this study offers a preliminary insight into the relation between the cardiac pathology and the pathogenesis of sudden death in patients with HCM with mutations of the troponin T gene.

**Acknowledgments**

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**References**

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