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Prevalence and Clinical Profile of Troponin T Mutations Among Patients With Hypertrophic Cardiomyopathy in Tuscany

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The prevalence and clinical profile of cardiac troponin T gene mutations were evaluated in 150 consecutive patients with hypertrophic cardiomyopathy from the well-defined geographic region of Tuscany. Troponin T mutations had a low prevalence (3.3%; including a newly described Phe110Leu mutation) and were associated with heterogeneous clinical expression and outcome.
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Hypertrophic cardiomyopathy (HC) is a genetic cardiac disease with very heterogeneous clinical presentation and diverse natural history.[1] Familial forms of HC have been associated with mutations of 10 genes encoding sarcomeric proteins, including troponin T.[2–4] The reported prevalence of troponin T mutations among populations with HC varies widely.[5–7] To date, however, studies on well-characterized, geographically homogeneous populations are still lacking, and most published data derive from patients with heterogeneous geographic backgrounds seen at tertiary referral institutions. In the present study, we analyzed the prevalence and clinical profile of troponin T mutations in a regional population of patients with HC from Tuscany in central Italy.

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We studied 150 unrelated index patients with HC, aged >18 years (mean age 42 ± 19). Sixty-three percent were men with an average maximum left ventricular (LV) wall thickness of 23 ± 5 mm; 25% had outflow obstruction. Follow-up was 8 ± 7 years. These patients were seen consecutively at our institution since the beginning of a systematic genetic screening program for troponin T mutations in the year 2000. Azienda Ospedaliera Careggi constitutes the largest and most established center for the management of HC in the Florence metropolitan area and the surrounding Tuscany region. All study patients were born and are permanently living in Tuscany, or in immediately adjacent areas of Umbria, with the exception of 12 patients (8%) who were from other parts of Italy. Detailed clinical features, general outcome, and management strategies used for the overall study population over the years have been previously described.[8] The diagnosis of HC was based on echocardiographic identification of a hypertrophied, nondilated left ventricle, in the absence of another cardiac or systemic disease capable of producing the magnitude of a wall-thickening evident.[1] The cut-off value for LV thickness in the diagnosis of HC was >15 mm.[1]

After informed consent, deoxyribonucleic acid (DNA) was extracted from peripheral blood using a QIAamp DNA Blood kit (QIAGEN GmbH, Hilden, Germany). Coding regions, intron/exon boundaries and 5′ and 3′ regions of troponin T were amplified in 15 reactions using primers.[9] Polymerase chain reaction (PCR) was performed using a 9700 Thermal Cycler (Applied Biosystems, Foster City, Kansas); cycling parameters for the reactions were optimized for each exon. Standard denaturing high-performance liquid chromatography (DHPLC) techniques were used.[10,11] Analysis of heteroduplex and homoduplex

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TABLE 1 Individual Features of Hypertrophic Cardiomyopathy (HC) Associated with Troponin T Mutations

Mutation	Gender	Age at Diagnosis (yrs)	Age at Last Evaluation (yrs)	Relation to Index Case	Echocardiographic Features			ECG Findings	Symptoms/Events* (0–4+)	Family History of HC	Functional Changes Associated With Mutation	Reference
					LV Thickness (mm)	Maron Type	LVOT Obstruction (0–3+)					
Phe110Leu	Female	8	28	‡	32	III	+	Q waves, marked LVH	+++ Recurrent chest pain and syncope, recurrent NSVT	+	Neutral charge substitution; no increase in Ca^{++} sensivity of ATPase	Watkins et al[6]
	Female†	24	48	Mother	21	III	0	Mild LVH	+			
	Male†	73	82	Maternal grandfather	15	II	0	AF, T-wave inversion V_4–V_6	+ Chronic AF			
Arg130Cys	Male	35	55	‡	23	III	0	Marked LVH, Diffuse ST-T abnormalities	0	+	Net charge substitution +1–0	Watkins et al[6]
	Female†	41	58	Sister	13	ND	0	Q waves, mild LVH	0 Paroxysmal AF			
Δ Glu160	Female	33	50	‡	20	III	0	Marked LVH, Diffuse ST-T abnormalities	++ Palpitations, presyncopal spells	+	Net charge loss −1	Harada et al[20]
	Male†	31	55	Brother	22	III	0	RBBB + LAH, mild LVH	+ Dyspnea, paroxysmal AF			
	Male‡	19	31	Nephew	16	I	0	Marked LVH	0			
Arg92Gln	Male	20	23	‡	29	III	0	Q waves, LVH	0	0	Increased Ca^{++} sensivity of myofibril ATPase activity, impaired inhibition of troponin T by troponin I	Thierfelder et al[16]
Arg278Cys	Male	62	66	‡	24	III	+++	LVH, T-wave inversion V_4–V6	++++ Improved after percutaneous septal ablation	0	Net charge substitution +1–0 increased Ca^{++} sensivity of myofibril ATPase activity	Watkin et al[6]

*Events are defined as any acute cardiovascular event requiring hospitalization.

†Familial case; not originally included among the 150 study patients, were identified after systematic family screening of index cases with known troponin T mutations.

‡Index case.

AF = atrial fibrillation; ATPase = adenosine triphosphatase; Ca^{++} = calcium ion; ECG = electrocardiographic; LAH = left anterior hemiblock; LVH = LV hypertrophy; LVOT = LV outflow tract; ND = not determined owing to borderline phenotypic expression; NSVT = nonsustained ventricular tachycardia; RBBB = right bundle branch block.

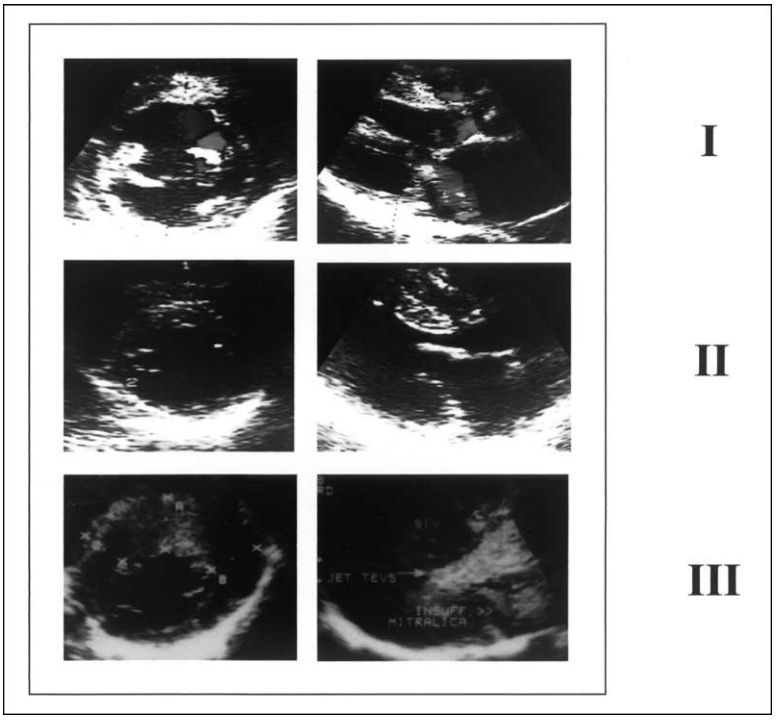

FIGURE 1. Heterogeneous morphologic expression of the newly described Phe110Leu troponin T mutation. Two-dimensional echocardiograms: parasternal short-axis *(left panels)* and long-axis *(right panels)* views are shown. *(I)* Maternal grandfather, aged 82 years, mild hypertrophy (maximum 15 mm) in the context of an initial hypokinetic-dilated evolution associated with moderate mitral regurgitation and chronic atrial fibrillation. *(II)* Mother, aged 48 years, moderate septal hypertrophy (maximum 21 mm), nonobstructive. *(III)* Index case, woman, aged 28 years, severe septal hypertrophy (maximum 32 mm) with mild basal outflow obstruction (peak gradient 25 mm Hg) and mitral regurgitation. See Table 1 for patients' clinical details.

was performed on a WAVE DNA Fragment Analysis System (Transgenomic, San Jose, California). The conditions of DHPLC were developed on the basis of exon-specific melting profiles predicted by WAVEMAKER software (Transgenomic, San José, California). Samples showing heteroduplex by DHPLC were sequenced on an ABI sequencer (ABI Prism 377) using Big Dye Terminator chemistry (Applied Biosystems). PCR products were purified according to QIAquick PCR Purification Kit (Quiagen). Finally, data obtained from the Sequence Analysis Software (Applied Biosystems) were aligned with the wild-type troponin T gene sequence (GenBank Database; *http://www.ebi.ac.uk/queries/bq.html*). A sequence mismatch was considered as a disease-causing mutation only if absent in 150 healthy controls and associated with amino acidic change. Each mutation was confirmed by a new, independent PCR, and whenever possible, by restriction enzyme digestion.[12] In selected patients, genes of the β-cardiac myosin heavy chain, cardiac myosin binding protein C, cardiac troponin I, and myosin regulatory light chain 2 were screened at the cardiogenetics unit of the Hôpital de la Salpêtrière by the single strand conformation analysis sequencing approach to rule out a double mutation.[9]

Systematic clinical and genetic screening was performed among relatives of patients with an identified troponin T mutation to confirm its causative role and evaluate the associated phenotype. Genetic counseling was offered to all patients and relatives.

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Of the 150 index patients with HC, 5 had heterozygous troponin T mutations: 1 deletion of a whole codon resulting in the loss of 1 amino acid from the protein sequence, and 4 nucleotide substitutions resulting in missense mutations (Table 1) No patient had homozygous mutations. Thus, the prevalence of troponin T mutations in our population with HC was 3.3%. Missense mutations included the newly described Phe110Leu, located in a highly conserved region coding for the tropomyosin binding site.[13] Because multiple independent occurrences of another mutation have been reported in the same site (Phe110Ile), our findings support the hypothesis of a mutational hot spot at codon 110.[14,15]

The clinical course and morphologic expression of HC caused by troponin T mutations were very heterogeneous (Table 1). Only in the family with the deletion (ΔGlu160) was there a clear history of sudden cardiac death (a brother and a niece of the index patient, aged $\leq$20 years, died suddenly and unexpectedly) associated with mild to moderate LV hypertrophy. All remaining patients had a negative familial and personal history of cardiac arrest during follow-up. In most patients, the clinical course was favorable, with 3 patients totally asymptomatic and free of cardiovascular events during follow-up, and only 1 judged to require an implantable defibrillator for primary prevention of sudden cardiac death (Table 1).

A wide range of maximum LV thickness values occurred among patients with troponin T mutations (average 22 $\pm$ 6 vs 23 $\pm$ 5 mm in the overall study cohort; $p = 0.4$). Morphologic heterogeneity was particularly striking in the family with the newly described Phe110Leu mutation (Figure 1 and Table 1). In this family, other predominant HC-causing genes (β-cardiac myosin heavy chain, cardiac myosin-binding protein C, cardiac troponin I, and myosin regulatory light chain 2) were also screened to search for double mutations accounting for the clinical and morphologic heterogeneity. However, no additional mutation was found.

Finally, five different DNA polymorphisms were found in our HC cohort (Table 2). Base substitutions not affecting the amino acid composition of the protein were found within exons 8 and 9, and variations in sequence length were found in intron 3.[9] A previously undescribed polymorphism was found in exon 14, where it caused the change in amino acid compo-

TABLE 2 Frequency of Troponin T Polymorphisms

Polymorphism	DNA Change	Amino Acid Change	Frequency Among Patients With HC	Reference
Exon 8	TCG→TCA	Ser69Ser	10%	9
Exon 9	ATT→ATC	Ile106Ile	50%	9
Exon 14	AAG→AGG	Lys253Arg	2%	—
Intron 3	Deletion - ctctt		49%	9
Intron 11	. . .ttttct[c]tgggcctctctctcatggtt. . . [a]		35%	9

sition Lys253Arg (Table 2); this could not be considered a mutation because it occurred in 2% of 150 healthy controls.

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Troponin T mutations have been repeatedly associated with a higher risk of sudden death despite milder myocardial hypertrophy.[16,17] These reports have contributed to the widespread perception of troponin T-related HC as a lethal condition, based on a limited number of families (i.e., <15) seen at tertiary referral centers. However, not all patients with troponin T mutations appear to be at increased risk.[5,14,18,19] To date, only 6 of the 16 different mutations identified have been characterized by a high incidence of sudden death.[17] Thus, the relevance of a systematic screening for troponin T mutations among patients with HC is still unresolved.[7] For this reason, in the last 2 years, we set up a comprehensive screening program in a regional, well-characterized patient population with HC followed at our institution for almost 3 decades.

Five different troponin T mutations were identified among 150 consecutive and unrelated patients with HC (an overall prevalence of 3.3%). Although any comparison with different populations with HC should be interpreted with caution owing to the different impact of patient referral bias in each study, this value is sensibly lower than most reported prevalence rates (range up to 15%).[6] Our findings show that the prevalence of troponin T mutations in the community may be very low, and that higher rates reported by tertiary referral centers may at least in part be due to patient selection.[7] Only 1 previous study[5] reported a 3% troponin T mutation rate in a small German cohort with prevalently obstructive HC, although the investigators admit that the epidemiologic value of such a highly selected group is unclear. We acknowledge that because of the reported association with minimal hypertrophy,[6,17] troponin T-related HC may have been more often overlooked, thus contributing to the low prevalence of troponin T mutations in our population. However, this bias is likely to have equally affected previous studies providing higher prevalence rates.

The long-term outcome of our patients with troponin T mutations was variable, although usually not adverse. Specifically, 1/3 of the patients had a favorable clinical course because they remained asymptomatic and had no acute cardiovascular event related to HC. Only the family carrying the deletion had a clear history of sudden cardiac death; of note, affected patients in this family had only mild or moderate hypertrophy.[6,17,20] Among all other patients, only 1 was at high risk according to standard definitions.[1] Thus, the notion that troponin T-related disease may be per se a high-risk condition is misleading and may lead to overaggressive management in patients with HC.

Finally, our data do not support the view of a milder phenotypic expression of troponin T-related HC. On average, study patients with troponin T mutations had a wide range of maximum LV wall thickness values and did not exhibit a lesser degree of hypertrophy or any other distinctive feature compared with the overall population with HC. Therefore, any effort to improve the yield of genetic screening in our cohort by preselecting patients based on morphologic parameters would have been unreliable.

In conclusion, in a regional population with HC from central Italy, troponin T mutations were rare and associated with heterogeneous morphologic expression. Troponin T-related HC did not appear to represent a high-risk condition per se, when compared with the overall patient population. A new mutation (Phe110Leu) was identified.

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Effects of Increased Concentrations of Glucose on Platelet Reactivity in Healthy Subjects and in Patients With and Without Diabetes Mellitus

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Hyperglycemia has been linked to adverse outcomes after myocardial infarction. We characterized the effect of selected concentrations of glucose or mannitol on platelet function in whole blood samples from healthy volunteers and from patients with and without diabetes mellitus. Activation of platelet glycoprotein IIb/IIIa and P-selectin expression was increased similarly after addition of isosmotic concentrations of glucose and mannitol, suggesting that increased osmolarity associated with hyperglycemia increases platelet reactivity. ©2003 by Excerpta Medica, Inc. **(Am J Cardiol 2003;92:1362–1365)**

The extent of hyperglycemia influences both short- and long-term outcomes after acute myocardial infarction.[1–3] For patients with diabetes mellitus, poor glycemic control is associated with adverse outcomes after coronary bypass surgery.[4] We have found that increased platelet reactivity is associated with an increased incidence of ischemic complications after coronary intervention.[5] Accordingly, this study was performed to determine whether high concentrations of glucose, per se, alter platelet reactivity.

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All subjects participating in this study gave written informed consent to participate in protocols approved by the institutional review board of the University of Vermont. Blood samples were obtained from 14 healthy volunteers and from hospitalized patients with (n = 14) or without (n = 7) type 2 diabetes mellitus. Healthy volunteers had not taken aspirin or any other antiplatelet agent for at least 10 days before blood was obtained. All patients were treated with aspirin (325 mg/day orally) and intravenous unfractionated heparin or low molecular-weight heparin at the time the blood was obtained. No patient had been treated with an antiplatelet agent other than aspirin. Patients were eligible if they had no history of diabetes and a hemoglobin (Hb)A1c level of <6.5%, or a history of type 2 diabetes mellitus and a HbA1c level of >7.5%. HbA1c was determined with the use of a point-of-care instrument (Axis-Shield, Oslo, Norway).

Blood was obtained with the use of a 2-syringe technique. For healthy volunteers, the first 3 ml of blood was discarded. For patients with and without diabetes mellitus, the first 3 ml of blood was used to determine blood glucose with the use of an instant-read glucometer (Accu-Chek, Roche Diagnostics, Indianapolis, Indiana) and HbA1c. The next 10 ml of blood was drawn into a separate syringe and anticoagulated with D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone, Calbiochem (Calbiochem, San Diego, California) (76 μmol/L final concentration) to inhibit coagulation and hence platelet activation during incubation in vitro. Aliquots were incubated for 1 hour in the absence or presence of added glucose or mannitol (25 mmol/L) to identify effects of each on platelet reactivity. Glucose and mannitol were prepared as equimolar stock solutions in Tyrode's buffer (137 mmol/L sodium chloride, 2.7 mmol/L sodium bicarbonate, 0.36 mmol/L monobasic sodium phosphate, 2 mmol/L calcium chloride 2, and 4 mmol/L magnesium chloride 2, pH 7.35).

Platelet activation was subsequently assessed as previously described.[6] Flow cytometric analysis was performed with the use of a Coulter EPICS Elite flow cytometer (Hialeah, Florida). The population of platelets was identified on the basis of particle size and association with a peridinin chlorophyll protein-conjugated antibody to glycoprotein (GP) IIIa (CD61; Becton Dickinson, San Jose, California). Fluorescein isothiocyanate-conjugated fibrinogen was added to assess the activation

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