A new mutation of the cardiac troponin T gene causing familial hypertrophic cardiomyopathy without left ventricular hypertrophy

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Abstract

Aim—To screen for a mutation of the cardiac troponin T gene in two families where there had been sudden deaths without an increase in left ventricular mass but with myocardial disarray suggesting hypertrophic cardiomyopathy.

Methods—DNA from affected individuals from both families was used to screen the cardiac troponin T gene on an exon by exon basis. Mutation screening was achieved by polymerase chain reaction and direct sequencing. Where appropriate, a mutation was confirmed by restriction digest.

Results—A novel missense mutation of exon 9 was found in the affected individuals of one of the families. This mutation at amino acid 94 resulted in the substitution of arginine for leucine and was not found in 100 normal control samples. A mutation of the cardiac troponin T gene was excluded in the second family.

Conclusions—A mutation of the gene for the sarcomeric protein cardiac troponin T can cause familial hypertrophic cardiomyopathy with marked myocyte disarray and frequent premature sudden death in the absence of myocardial hypertrophy at clinical or macroscopic level.

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Familial hypertrophic cardiomyopathy is an autosomal dominant disorder characterised clinically by unexplained myocardial hypertrophy.¹ The extent and distribution of this hypertrophy varies between individuals with the condition, but until recently it was acknowledged that the presence of hypertrophy was a requirement for the clinical diagnosis to be made. At the histological level myocyte disarray is a quantitatively specific marker for the condition and is not seen to any significant degree in either normal hearts or hypertrophied hearts from other causes.² We have previously described two families in which widespread myocardial disarray was observed in the absence of an increased myocardial muscle mass.³ These families were both considered to have an atypical form of familial hypertrophic cardiomyopathy, but definitive genetic evidence was lacking. Recent advances in the molecular biology of this condition have identified seven genes that may cause the disease: the β myosin heavy chain gene, the

cardiac troponin T gene, the α tropomyosin gene, the cardiac myosin binding protein C gene, the essential and regulatory light chain genes, and most recently the gene for cardiac troponin I.⁴⁻⁹ All of these genes code for sarcomeric contractile proteins and thus hypertrophic cardiomyopathy is now considered to be a disease of the sarcomere. As mutations of the cardiac troponin T gene are characterised by relatively mild left ventricular hypertrophy (maximum wall thickness of 14 mm in one study) and a high likelihood of sudden death,¹⁰ we elected to screen for mutations of this gene in our two families to investigate further whether so called non-hypertrophied hypertrophic cardiomyopathy was indeed also a disease of the sarcomere.

Methods

Blood was taken for DNA extraction from an affected member of each family. In family A, where a mutation was identified, confirmation of mutation segregation with disease phenotype was established by blood taken from patients 6 and 7 for DNA extraction. In patient 1, no blood was available and thus DNA was extracted from formalin fixed, paraffin embedded tissue using a modified method of the standard phenol-chloroform extraction technique.

A DNA based method for screening of the cardiac troponin T gene was devised. Oligonucleotide primers were designed for the intronic boundaries of each of the 15 transcribed exons of the troponin T gene (intron-exon sequences kindly provided by L Thierfelder, Max-Delbruck-Centrum, Berlin, Germany). Exons 3 and 4, 6 and 7, 10, 11 and 12, and 13 and 14 were co-amplified as single polymerase chain reaction (PCR) products. The PCR products were then screened by automated cycle sequencing using an ABI sequencer (Cambridge Biosciences, Cambridge, UK) and the corresponding forward or reverse primers used.

In family A, a novel mutation of exon 9 at position 94 in the amino acid chain resulted in the generation of a new restriction site for the enzyme Alu I. Patients 1, 4, 6, and 7 were screened by restriction digest, as was an unrelated control panel of 100 DNA samples from a population with no known cardiac disease.

Results

CLINICAL AND PATHOLOGICAL FINDINGS

The clinical and pathological details of both families have been previously described in detail. In brief, family A presented with the

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Figure 1 Pedigree of family A showing clinical status. Filled symbols represent affected individuals; empty symbols, individual not evaluated; N, normal; ?, possibly affected

Figure 2 Pedigree of family B showing clinical status.

sudden cardiac death of patient 1, and the histopathology of three further family members subsequently became available for review.³ All four family members died suddenly and unexpectedly before the age of 45 and all were without any previous cardiac history (fig 1).

A review of the necropsy findings revealed a normal macroscopic appearance of all four hearts, with all heart weights falling within the normal range. Histology revealed widespread fibrosis with myocyte disarray. The surviving daughter of patient 1 was found at family screening to have an abnormal ECG, normal echo, and abnormal blood pressure response to upright exercise. Her clinical status has remained stable since the first evaluation. The offspring of patient 4 have subsequently been investigated: patient 7 is five years old and has a normal ECG and echo; patient 6 is now seven years old and has an abnormal ECG and a normal echo.

Family B (fig 2) was investigated following the death at catheterisation of patient 1, who had evidence of paroxysmal atrial fibrillation

Table 1 Clinical details of family B

Patient	Age (years)	ECG	LVWT on echo (mm)	LA on echo (mm)	Diastolic abnormalities
1	43	AF	13	40	+
2	56	AF	11	59	+
3	49	SR/Dom RV1/repolarisation abnormalities	13	42	–
4	24	AF	13	71	+
5	27	SR/Dom RV1/repolarisation abnormalities	11	45	+

AF, atrial fibrillation; Dom RV1, dominant R wave in lead V1; LA, left atrium; LVWT, left ventricular wall thickness; SR, sinus rhythm; +, present; –, absent.

Figure 3 Restriction digest of family A. Lane 1, patient 4; lane 2, patient 7; lane 3, patient 6; lane 4, normal control. Undigested product of 418 base pairs (bp) is seen in all four lanes (upper band). Mutation creates a restriction digest site producing two products of 318 bp and 110 bp. Affected patients (lanes 1 and 3) are heterozygotes and thus show both the undigested fragment of 418 bp and two digested fragments (only the larger 318 bp fragment seen).

and maximum wall thickness on echo at the upper limit of normal (13 mm). Necropsy revealed a macroscopically normal heart with a normal heart weight; histology, however, showed severe myocyte disarray with areas of fibrosis. Screening of the eight surviving family members revealed four asymptomatic individuals, all with repolarisation abnormalities of the ECG, normal wall thickness on echo, but abnormal ventricular filling patterns and left atrial enlargement. Subsequent evaluation of these family members has revealed a high incidence of progression to chronic atrial fibrillation and left atrial dilatation, despite persistently normal left ventricular wall thicknesses and preserved systolic function (table 1). This family is considered to have a restrictive form of hypertrophic cardiomyopathy.

GENETIC SCREENING

In family A, a novel mutation in exon 9 of the cardiac troponin T gene is seen to segregate with the diseased phenotype (fig 3). This was established with both direct DNA sequencing and restriction digest assay in all but one of the family members assessed (it was not possible to establish clearly a mutated haplotype in patient 1 on restriction digest as the PCR product using DNA extracted from formalin fixed tissue was not of sufficient quantity; the mutation was, however, established with direct sequencing). This missense mutation at position 94 in the amino acid sequence is at an evolutionary conserved site and results in a non-conservative substitution of the amino acid arginine to leucine, thus also giving rise to a change in the amino acid charge. This mutation was not found after screening the DNA of 100 normal control subjects.

No mutation of the cardiac troponin T gene was found in family B. This family has also been screened by linkage analysis and shown to have no linkage with either the β myosin heavy chain or cardiac troponin T loci.

Discussion

We have attempted to find the genetic basis for familial myocardial disarray in the absence of hypertrophy. We elected to investigate the

troponin T gene as our candidate gene in view of the known phenotype in patients with this gene mutation who are characterised by minimal left ventricular hypertrophy, marked myocyte disarray, and premature sudden cardiac death. Family A fitted most closely to this pattern and indeed has been shown to have a mutation of this gene. Family B was also characterised by disarray in the absence of hypertrophy; however, there is no history of sudden death and the echo data reveal enlarged left atria and marked abnormalities of left ventricular filling. It is therefore suggested that this family has a restrictive form of hypertrophic cardiomyopathy, with so far a benign natural history, and as such differs significantly from the recognised troponin T phenotype.

In family A we identified a novel mutation in exon 9 of the cardiac troponin T gene that segregated with the disease phenotype and was not found in 100 normal controls. The mutation is a missense mutation at a highly conserved site in the troponin T protein. The switch from arginine to leucine at this position results in a non-conservative amino acid substitution, associated with a change in hydrophilicity and in the electrical charge of the amino acid. It is likely therefore that this change in amino acid would lead to a significant change in the protein conformation at this position, which is known to be an important site for interaction between cardiac troponin T and α tropomyosin.¹¹

So far all genotype phenotype studies on patients with hypertrophic cardiomyopathy and a mutation of troponin T have been characterised by minimal left ventricular hypertrophy and a high rate of sudden cardiac death.¹⁰ In this paper we confirm this pattern and present a model for familial hypertrophic cardiomyopathy secondary to a troponin T gene mutation which has myocyte disarray without hypertrophy as the anatomical basis for sudden death. It would appear that myocardial hypertrophy is not required for either the appearance of disarray or the occurrence of sudden death in patients with hypertrophic cardiomyopathy. This therefore raises a question about the pathological basis for the disarray. Recent studies have suggested that for most β myosin mutations the mutated protein is able to assemble into the sarcomere, but that sarcomeric function is impaired and thus myocyte hypertrophy develops as a secondary and compensatory process.¹²⁻¹³ Myocyte disarray is thus seen to be a result of myofibrillar disarray and myocyte hypertrophy. This may hold true for β myosin mutations where in vitro and in vivo studies have suggested a reduction in the sliding velocity of the sarcomere unit.¹⁴⁻¹⁵ Studies to date on mutated troponin T have provided conflicting results, with evidence of both an impaired force of contraction in isolated myocyte studies¹⁶ and an increase in the sliding speed of thin filaments in an in vitro motility assay.¹⁷ This last study might suggest that at least some mutations of the cardiac troponin T gene can actually enhance the contractile performance of the sarcomere and thus lead directly to a hypercontractile state

without the need for secondary hypertrophy. These differences in the function of the mutated sarcomere may help to explain the absence of hypertrophy in our present study and suggest that, despite a mutation of a sarcomere contractile protein, myocyte disarray can develop in the absence of significant myocyte hypertrophy. It is not possible to determine whether the incidence of sudden death in this family was a direct result of the marked disarray. Significant fibrosis was also present on histological examination and these areas of fibrosis may act as an arrhythmogenic focus. Alternatively the only surviving adult who was assessed during exercise showed a markedly abnormal vascular response and has experienced syncope. The mechanism of death may be related to this abnormal blood pressure response and a fall in cardiac output during exertion, promoting myocardial hypoperfusion (especially in areas with small vessel disease) and a possible arrhythmia. Once initiated ventricular tachycardia would be perpetuated, and in a heart with severe disarray may rapidly deteriorate to ventricular fibrillation.

We have thus established that patients with a mutation of a sarcomere protein may develop hypertrophic cardiomyopathy in the absence of myocardial hypertrophy detectable at the clinical or histological level. These patients appear to be at high risk of sudden death and yet fail to show the characteristic increase in wall thickness that has been a hallmark of the clinical diagnosis. The clinician is then faced with the difficult process of attempting to screen for hypertrophic cardiomyopathy in patients who may present with a family history of sudden death and an abnormal ECG, but normal echocardiography. It now appears that we can attempt to clarify this evaluation with a genetic diagnosis.

CONCLUSIONS

We provide evidence that familial hypertrophic cardiomyopathy with marked myocardial disarray in the absence of an increase in cardiac muscle mass (detectable at the clinical or macroscopic level) is indeed a disease of the sarcomere, and is caused by a mutation in the cardiac troponin T gene. Patients with this mutation appear at high risk of sudden death. This has important implications for clinical screening of patients for hypertrophic cardiomyopathy and confirms the evolving importance of a genetic diagnosis in these families.

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