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Deletion in the Cardiac Troponin I Gene in a Family From Northern Sweden with Hypertrophic Cardiomyopathy

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S. Mörner, P. Richard, E. Kazzam, B. Hainque, K. Schwartz and A. Waldenström. Deletion in the Cardiac Troponin I Gene in a Family From Northern Sweden with Hypertrophic Cardiomyopathy. *Journal of Molecular and Cellular Cardiology* (2000) **32,** 521–525. The cardiac troponin I gene has been described to be associated with hypertrophic cardiomyopathy. Until now, mutations in this gene have been found only in the Japanese population. We now present the first non-Japanese family, from northern Sweden, with a mutation in the cardiac troponin I gene. Clinical diagnose was based on echocardiography, with a maximum left ventricular wall thickness of >13 mm, or major electrocardiographic abnormalities, excluding subjects with other known causes of cardiac hypertrophy. Mutation screening was performed with a single-strand conformation polymorphism analysis and identification of mutation by direct DNA sequencing. We have identified a 33-bp deletion in exon 8 encompassing the stop codon. Nine individuals in three generations were tested, and four were carriers of this deletion. The mother was genetically affected and died of heart failure aged 90. Echocardiography at 71 years of age revealed no hypertrophy, but the electrocardiogram showed signs of left ventricular hypertrophy. Her two sons, also genetically affected, had left ventricular hypertrophy, with maximum wall thickness of 15 and 16 mm, respectively. One daughter and four grandchildren were clinically unaffected, but one of them, a 27-year-old woman with maximum wall thickness of 8 mm and normal electrocardiogram, was found to be genetically affected.

In conclusion, we describe a non-Japanese family in which hypertrophic cardiomyopathy is due to a genetic defect in the cardiac troponin I gene. This mutation is a deletion of 33 bp in the last exon, whereas the previously described mutations in this gene are single nucleotide changes and a single codon deletion. The deletion of the C-terminal part of the cardiac troponin I protein, seems in this particular family to be associated with a mild phenotypic expression of familial hypertrophic cardiomyopathy.

Key Words: Cardiac troponin I; Familial hypertrophic cardiomyopathy; Genetics; Mutation.

Introduction

Hypertrophic cardiomyopathy (HCM) is characterised by left and/or right ventricular hypertrophy, with predominant involvement of the interventricular septum in the absence of other causes of hypertrophy, such as hypertension or valvular heart disease.[1] The prevalence in the general population is about 0.2%.[2] The clinical manifestations of hypertrophic cardiomyopathy are diverse, ranging from benign asymptomatic course to severe heart failure and sudden cardiac death.[1] The disease is an important cause of sudden cardiac death in children and young adults. Typical morphological changes include myocyte hypertrophy and sarcomere disarray surrounding areas of increased loose connective tissue.

In approximately 55% of the cases, hypertrophic

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Table 1 PCR primers, fragment sizes and hybridization PCR temperatures for genomic amplification of the human TNN13 exons.

Exon	Primer sequences (5′→3′)	Fragment size (bp)	PCR temp (°C)
1	forward TCACTGACCCTCCAAACGCC reverse CCTCTCAGCTGCGACCCCTC	149	65→55
2 + 3	forwardTTAGGAGACAGGACACAGCCC reverse CGCCTGTACTCTGCCCCCA	349	70→58
4	forward TGCTGGGGGTGTCTTGAGGT reverse CACCTGCCTGCTCTTTCCCA	163	70→56
5	forward GAGGGTTTAGAAGGGCAGAGG reverse CCGGGACTAGAAACCTCGCA	239	65→55
6	forward GGTCTCCCTGTTTTTGGTTCCC reverse TGGGTCTCCTGGGATGTGCA	251	65→55
7	forward ATGGAGGAGTTGGGTGTGCG reverse ACAGCCCTTCCCCTCAGCAT	287	70→58
8	forward AGACTGGAGAGGAAGAAGAGACCC reverse TCCTGCCTAAGCCCTGGGTAAT	239	65→55

cardiomyopathy is familial, inherited in an autosomal dominant fashion.[3] This is a monogenic disease but there is an important genetic heterogeneity.[4] Mutations in eight sarcomeric protein genes have been identified in families with familial hypertrophic cardiomyopathy. These are the β-myosin heavy chain,[5] the cardiac myosin binding protein C,[6–8] the cardiac troponin T,[9,10], the α-tropomyosin,[9] the cardiac essential and regulatory myosin light chains,[11] the α cardiac actin[12] and the cardiac troponin I.[13]

Troponin I is a constituent protein of the troponin complex located on the thin filament of striated muscle that provides a calcium-sensitive switch for contraction. The cardiac isoform of troponin I (cTnI) is expressed only in the cardiac muscle.[14] The cardiac troponin I gene (*TNNI-3*) is located on chromosome 19p13.2-q13.2. It contains eight exons and encodes a polypeptide of 210 amino acids.

Genotype-phenotype correlations are important in determining the clinical impact from different mutations. Until now, mutations in the cardiac troponin I have been described only in the Japanese population.[13] Six different mutations have been published, five missense mutations and one codon deletion, which thus does not disrupt the reading frame. We present the first non-Japanese family with a mutation in the troponin I gene. This mutation is also a new type of genetic defect in this gene as it is a 33-bp deletion in exon 8, encompassing the last eight amino acids and the stop codon. The aim of our work is to describe the phenotype associated with this unusual mutation.

Material and Methods

Clinical evaluation

Nine family members in three generations were investigated with echocardiography and electrocardiogram (ECG). Eight subjects were investigated at the same time as a venous blood sample was taken for the genotyping. For one individual, the 90-year-old mother, we only had access to previous ECG and echocardiography examinations, performed at the age of 71 years. Informed consent was obtained from each individual and the protocol was approved by the ethics committee of Umeå University. Diagnosis of affected family members was based on criteria previously described.[15] Briefly, a left ventricular wall thickness of at least 13 mm or major ECG-abnormalities (left ventricular hypertrophy (LVH), abnormal Q-waves or marked T-wave inversion) was required for a positive diagnose. The presence of other possible causes of hypertrophy such as valvular heart disease, hypertension and obesity were taken into account when designating clinical status.

Genetic analysis

DNA was extracted from peripheral blood leucocytes. The β-myosin heavy chain, the cardiac myosin binding protein C and the cardiac regulatory myosin light chain genes were screened for mutations as previously described.[4] For the screening of TNNI-3, intronic sets of oligonucleotide primers

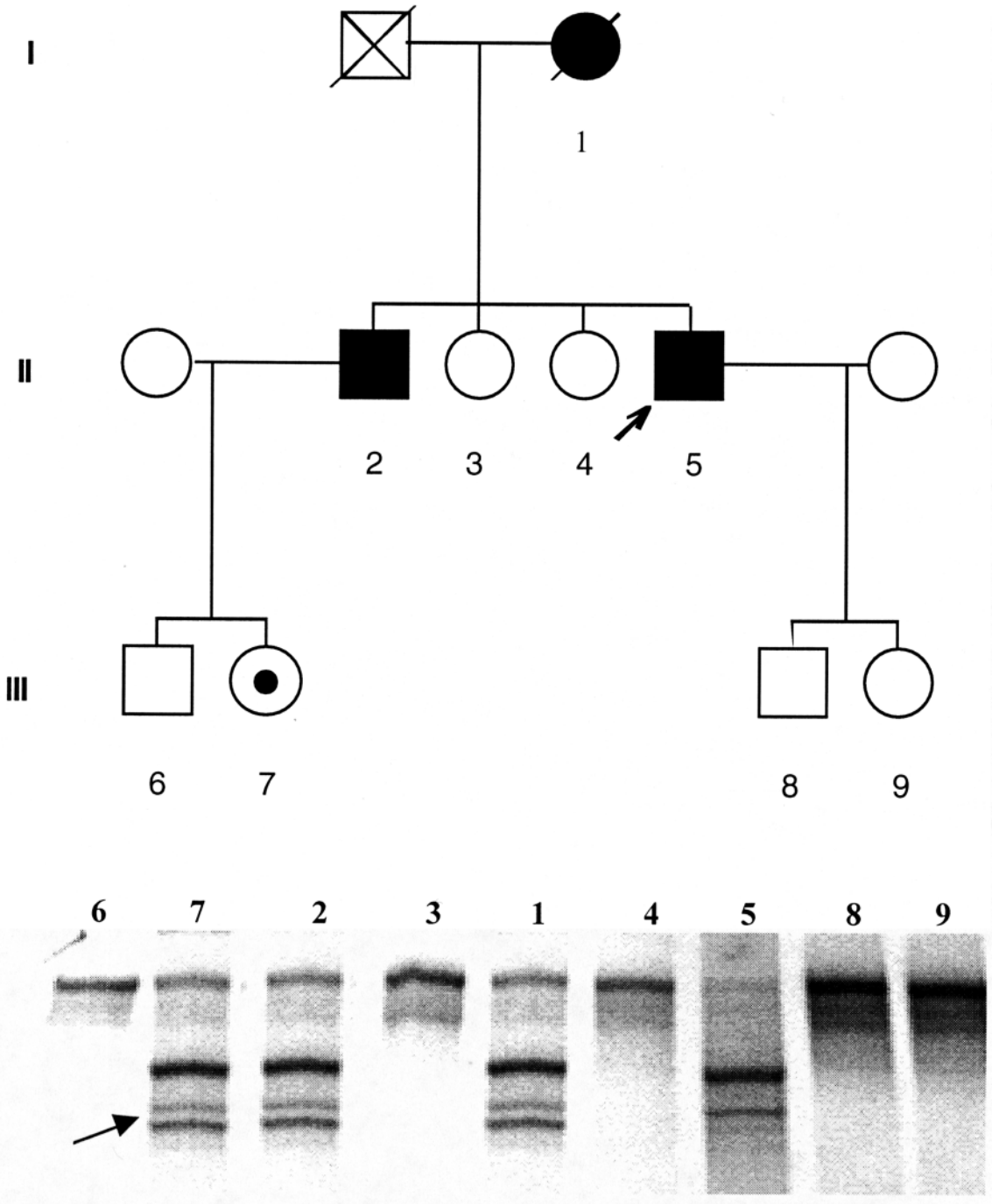

Figure 1 Family pedigree and SSCP profiles. Squares, males; Circles, females; filled symbols, clinically affected subjects; circle with dot, genetically affected but clinically unaffected; the proband is indicated by an arrow; abnormal SSCP profiles indicated by arrow.

were designed according to the published genomic sequence of the cardiac troponin I gene (available at the genome database, http://gdbwww.gdb.org/, DNA Sequence ID; ×90780, ×90781 and ×90782). Each of the eight exons was amplified using a "touch-down" polymerase chain reaction (PCR) protocole and then subjected to a single-strand conformation polymorphism analysis (SSCP). Primer sequences (5′→3′) and PCR annealing temperatures are given in Table 1. After amplification, PCR products were heat denatured in a saccharose buffer, and resolved on a 10%

Table 2 Clinical evaluation of the family.

Subject	Age* (years)	Genotype	Phenotype				
			Symptoms	Electrocardiogram	IVSD (mm)	LVPW (mm)	Clinical status
I-1	71	Pos	Dys	LVH, AF	9	9	A
II-2	61	Pos	0	T-wave inv.	15	8	A
II-3	54	Neg	0	Normal	10	10	H
II-4	55	Neg	CP, Dys	Normal	13	10	H
II-5	64	Pos	0	ST-T-changes, #, AF	16	14	A
III-6	33	Neg	0	Incr. QRS voltage	10	8	H
III-7	27	Pos	0	Normal	8	7	H
III-8	32	Neg	0	Normal	9	9	H
III-9	34	Neg	0	Normal	9	10	H

* Age at echocardiography and ECG investigation. LVH, left ventricular hypertrophy as defined by Romhilt-Estes score; IVS, interventricular septum; LVPW, left ventricular posterior wall. Clinical status: A, affected; H, healthy. CP, chest pain; Dys, dyspnoea; AF, atrial fibrillation; #, with digoxin treatment.

polyacrylamide gel (acrylamide/bisacrylamide ratio of 37.5:1) at 8 mA per gel in Tris-Borate-EDTA (TBE) buffer 0.8X, run at 7 and 25°C. After migration, DNA was visualised by silver staining (Pharmacia) of the gels.[16] The nature of the mutation was then determined by direct sequencing of the PCR product of both forward and reverse strands on an automated laser fluorescent DNA sequencer (Applied Biosystems, ABI 377).

Results and Discussion

The pedigree of the family is shown in Figure 1. Nine individuals were examined, and their clinical characteristics are shown in Table 2. Three patients fulfilled echocardiographic or ECG criteria for HCM. The mother (individual I-1) was diagnosed with heart failure at 78 years of age. Echocardiography at the age of 71 showed no hypertrophy, but her ECG was consistent with LVH. She died of heart failure at 90 years of age. Her two sons (II-2 and II-5), 61 and 64 years old, respectively, both showed LVH with a septum of 15 and 16 mm. They have not experienced symptoms of syncope or heart failure. One daughter (II-3) had no LVH, but one daughter (II-4) presented with chest pain and dyspnoea. She had borderline LVH, which could well be explained by severe obesity, with a body mass index of 34. Among the third-generation individuals III-6 and III-7 (children of II-2) and III-8 and III-9 (children of II-3), no clinical signs of HCM were detected. Individual III-6 did have increased QRS-voltage in the ECG, but no other abnormalities, and thus did not fulfil the criteria for HCM.

In the β-myosin heavy chain, the cardiac myosin binding protein C and the cardiac regulatory myosin light chain genes we did not find any mutations. All exons of the TNNI-3 were investigated, the seven first exons showed normal SSCP-profiles, but in the genetically affected individuals, the amplification of exon 8 led to two amplified fragments, a 239-bp fragment and a smaller one. Analysis of SSCP pattern showed the presence of an abnormal profile which was not found in 200 normal chromosomes. Sequence analysis of exon 8 identified a 33-bp deletion. The deletion starts in position 125–164, encompassing the stop codon and another six nucleotides further downstream (sequence access number N° × 90782). Thereby, at the protein level, this mutation leads to the deletion of the last eight amino acids, the termination codon, and generates 19 abnormal amino acids before the next UGA stop codon. In the present family, four individuals (I-1, II-2, II-5 and III-7) were genetically affected.

The troponin complex is an essential modulator of calcium-stimulated actomyosin interaction of ATPase activity in striated muscle, and troponin I is an inhibitory component of the complex. An actin-binding domain is essential for this inhibition activity and the C-terminal region of troponin I is also required for binding to troponin C. The molecular mechanisms of cardiac hypertrophy caused by cTNI mutations remains unclear. cTNI is expressed only in the cardiac muscle, thus cTNI mutations only affect the heart. All previously described mutations in cTNI are located in exons 7 and 8, corresponding to the C-terminal part of the protein which shows a high degree of homology between isoforms (fast-skeletal and slow-skeletal isoforms) and different species,[13] whereas the N-terminal part

of the protein is more divergent. So, mutations in the C-terminal part of cTNI might affect the inhibitory function and lead to an increased contractility of myocytes. This could lead to cardiac hypertrophy as a compensatory phenomenon.

Conclusion

We report here a family from northern Sweden presenting a familial and classical form of hypertrophic cardiomyopathy in which a deletion encompassing the stop codon of *TNNI3* gene was found to be responsible of the disease. Four patients were genetically affected, of whom three presented echocardiographic or ECG criteria for HCM and a woman (III-7), 27 years old, was asymptomatic. The phenotypes associated with previously described mutations[13] in the same region of the protein were heterogeneous, with patients presenting a classical form of HCM as well as the apical type and HCM associated with the Wolff–Parkinson–White syndrome. The family described here shows a classical form of HCM with a mild phenotype and without severe hypertrophy, outflow obstruction or premature death. This suggests that this deletion has either less influence on the function of the protein, or others factors such as environmental or genetic factors (modifier genes) may influence the severity of the phenotype.

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