CASE REPORT

A previously undescribed de novo insertion-deletion mutation in the β myosin heavy chain gene in a kindred with familial hypertrophic cardiomyopathy

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Abstract

A previously undescribed de novo insertion-deletion mutation in the β cardiac myosin heavy chain gene was found in a kindred with familial hypertrophic cardiomyopathy. In the mutated allele there is an inserted-deleted guanine at nucleotides 8823 and 8850 of the β myosin heavy chain gene, resulting in a dramatic change of the aminoacid sequence (AA 395–404). Such a mutation, detected in the proband and in his son but not in the proband's parents, is likely to produce major impairment of myosin function.

(*Heart* 1996;76:451–452)

Keywords: hypertrophic cardiomyopathy; myosin; mutation.

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Accepted for publication
4 June 1996

Familial hypertrophic cardiomyopathy is an autosomal dominant inherited cardiac disorder characterised by unexplained thickening of the interventricular septum and the free wall of the left ventricle. It is the most common cause of sudden cardiac death in otherwise healthy young individuals. The genetic heterogeneity of familial hypertrophic cardiomyopathy has been confirmed by several groups. Four genes (on chromosomes 1, 11, 14, and 15) encoding for sarcomeric proteins (cardiac troponin T, cardiac myosin binding protein-C, β myosin heavy chain (β-MHC), and α tropomyosin, respectively), have been associated to the disease.[1–4] We have recently shown that β myosin purified from soleus muscle biopsies of individuals with two distinct missense mutations in the β-MHC gene (Arg403Gln and Leu908Val) translocates phalloidin-labelled actin filaments at a slower velocity than that of normal controls in an in vitro motility assay,[5] suggesting that cardiac hypertrophy may be a compensatory mechanism.

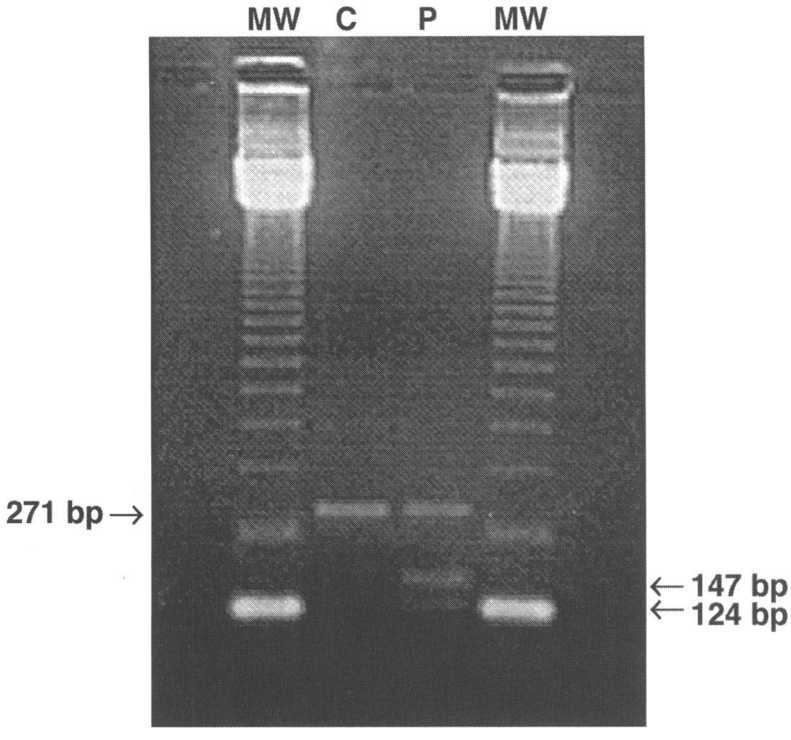
CASE REPORT

A 44 year old man with clinical features of hypertrophic cardiomyopathy (dyspnoea, syncope, complete right bundle branch block (RBBB), and pronounced septal hypertrophy (21 mm)) underwent a genetic analysis for the detection of mutations in the β-MHC gene. Both clinical and molecular screening was extended to his children and first degree relatives. His 16 year old son was clinically asymptomatic, but a cross sectional echocardiogram suggested the presence of myocardial hypertrophy. The proband's parents and other relatives were entirely normal.

The genetic analysis was performed as described elsewhere.[6] Briefly, genomic DNA was phenol-chloroform extracted from peripheral blood lymphocytes and each of the 40 exons of the β-MHC gene was amplified by radioactive polymerase chain reaction (PCR). Intronic primers were used in order to avoid the coamplification of the highly homologous α-MHC gene. The PCR-amplified exons were subjected to single-stranded conformational polymorphism (SSCP) analysis followed by direct DNA sequencing of the PCR-SSCP product. This technique allowed us to identify an abnormal pattern of migration of the PCR-amplified exon 13 from the proband and his son. Sequencing of this product, performed on both coding and non-coding strands, revealed an inserted-deleted guanine at nucleotides 8823 and 8850 of the β-MHC gene, respectively. This mutation, which occurs at a critical site of the myosin molecule (actin binding domain of myosin subfragment-1) results in a dramatic change of the nucleotide and aminoacid sequence of β myosin (table) and generates a new Bsp1286 restriction site (figure). Genetic analysis of the proband's parents was negative, suggesting that the mutation had arisen de novo: this accorded with the clinical appearance of the disease. Paternity and the parental origin of the chromosome bearing the de novo mutation were assessed by a demonstration of the inheritance of appropriate alleles at eight polymorphic dimeric short tandem repeats sequences (STRs) and by haplotype analysis of the β-MHC gene,[7] (data not shown). The mutation was not detected in 100 chromosomes from unrelated, unaffected individuals.

Comparison of nucleotide and amino acid sequences of β-MHC

		Nucleotide sequence												
Wild type	8819	GAC	CTG	CTC	AAG	GGG	CTG	TGC	CAC	CCT	CGG	GTG	AAA	8854
Mutant	8819	GAC	CGT	GCT	CAA	GGG	GCT	GTG	CCA	CCC	TCG	GGT	AAA	8854
		Aminoacid sequence												
Wild type	394	D	L	L	K	G	L	C	H	P	R	V	K	405
Mutant	394	D	*R*	*A*	*Q*	*G*	*A*	*V*	*P*	*P*	*S*	*G*	K	405



A Bsp1286 digest of PCR fragments amplified fropm genomic DNA, encompassing the 395–404 amino acid residues in the β myosin heavy chain gene of the proband and one control. The Bsp1286 site is generated by the mutation and upon digestion yields two aberrant 147 and 124 bp fragments in the affected individuals together with the predicted normal 271 bp fragment, P, proband; C, control; MW, molecular weight marker.

Discussion

This study provides the first description of a de novo insertion-deletion mutation in the β-MHC gene in familial hypertrophic cardiomyopathy. Our finding is particularly interesting because the nature of this mutation is quite different from the missense mutations reported by us and others in patients with β-MHC-linked hypertrophic cardiomyopathy. The site of the mutation, near the actin-binding interface of myosin S-1, as demonstrated by the amino acid and the three-dimensional structural similarities between chicken skeletal muscle and human β-cardiac myosin, may be causing a dramatic impairment of the protein function,[8] and it could be responsible for the development of cardiac hypertrophy in the affected individuals. The genetic background may play a major part in the appearance of this mutation. Calabria, a region of south Italy where the study was performed, has a very homogeneous genetic background for historic and geographic reasons. As shown by others,[9] ethnic origin can be responsible for particular forms of this disease arising and it should be taken in account when genetic screening is done.

We thank Dr Angelo Lamberti for the valuable technical assistance.

This study was supported by the Italian Telethon Foundation (grant 474).

1 Geisterfer-Lowrance AA, Kass S, Tanigawa G, Vosberg HP, McKenna W, Seidman CE, *et al.* A molecular basis for familial hypertrophic cardiomyopathy: a β-cardiac myosin heavy chain gene missense mutation. *Cell* 1990;**62**:999–1006.

2 Thierfelder L, Watkins H, MacRae C, Lamas R, Williams M, McKenna W, *et al.* α-Tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: a disease of the sarcomere. *Cell* 1994;**77**:701–12.

3 Watkins H, Conner D, Thierfelder L, Jarcho JA, MacRae C, McKenna WJ, *et al.* Mutations in the cardiac myosin binding protein-C gene on chromosome 11 cause familial hypertrophic cardiomyopathy. *Nature Genet* 1995;**11**: 434–7.

4 Bonne G, Carrier L, Bercovici J, Cruaud C, Richard P, Hainque B, *et al.* Cardiac myosin binding protein-C gene splice acceptor site mutation is associated with familial hypertrophic cardiomyopathy. *Nature Genet* 1995;**11**: 438–40.

5 Cuda G, Fananapazir L, Zhu WS, Sellers JR, Epstein ND. Skeletal muscle expression and abnormal function of β-myosin in hypertrophic cardiomyopathy. *J Clin Invest* 1993;**91**:2861–5.

6 Epstein ND, Cohn GM, Cyran F, Fananapazir L. Differences in clinical expression of hypertrophic cardiomyopathy associated with two distinct mutations in the β-myosin heavy chain gene: a $908^{Leu-Val}$ mutation and a $403^{Arg-Gln}$ mutation. *Circulation* 1992;**86**:345–52.

7 Watkins H, Thierfelder L, Hwang D-S, McKenna W, Seidman G, Seidman CE. Sporadic hypertrophic cardiomyopathy due to de novo myosin mutations. *J Clin Invest* 1992;**90**:1666–71.

8 Rayment I, Holden H, Sellers JR, Fananapazir L, Epstein ND. Structural interpretation of the mutations in the β-cardiac myosin that have been implicated in familial hypertrophic cardiomyopathy. *Proc Natl Acad Sciences USA* 1995;**92**:3864–8.

9 Nishi H, Kimura A, Harada H, Adachi K, Koga Y, Sasazuki T, *et al.* Possible gene dose effect of a mutant cardiac β-myosin heavy chain gene on the clinical expression of familial hypertrophic cardiomyopathy. *Biochem Biophys Res Commun* 1994;**200**:549–56.