Direct Detection of Malignant Mutations in Patients With Hypertrophic Cardiomyopathy

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We determined the prevalence of mutations considered malignant in the genes for beta-myosin heavy chain (*MYH7*, 11 mutations) and troponin T (*TNNT2*, 5 mutations) in 30 patients with hypertrophic cardiomyopathy aged 18 to 60 years, 83% of whom had familial antecedents of hypertrophic myocardiopathy or sudden death. Mutations were identified with polymerase chain reaction followed by restriction enzyme digestion and agarose gel electrophoresis.

Direct analysis identified 16 mutations in 2 of the 30 patients (7%): one women diagnosed at the age of 25 years as carrying the MYH7453cysteine mutation, and a 60-year-old women with the TNNT2278 cysteine mutation. These cases illustrate the considerable clinical heterogeneity that characterizes carriers of these mutations. Clinical manifestations can range from severe hypertrophy or early sudden death to the absence of symptoms up to advanced age.

Key words: *Hypertrophic cardiomyopathy. Sudden cardiac death. Beta-myosin. Troponin T. Mutations.*

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Detección directa de mutaciones malignas en pacientes con miocardiopatía hipertrófica

Hemos determinado la prevalencia de mutaciones consideradas malignas en los genes de la cadena pesada de la betamiosina (*MYH7*, 11 mutaciones) y la troponina T (*TNNT2*, 5 mutaciones) en 30 pacientes con miocardiopatía hipertrófica (MCH) menores de 60 años, de los que el 83% tenía antecedentes familiares de MCH y/o muerte súbita. Empleamos la reacción en cadena de la polimerasa (PCR) seguida de digestión con una enzima de restricción y electroforesis en geles de agarosa.

El análisis directo nos permitió identificar alguna de las 16 mutaciones en 2 de los 30 pacientes (7%): una mujer diagnosticada a los 25 años era portadora de la mutación MYH7-453 cisteína, y otra mujer, de 60 años, era portadora de la mutación TNNT2-278 cisteína. Estos casos ilustran la gran heterogeneidad clínica que caracteriza a los portadores, que van desde la hipertrofia grave o la muerte súbita temprana hasta la ausencia de síntomas en edades avanzadas de la vida.

Palabras clave: *Miocardiopatía hipertrófica. Muerte súbita. Cadena pesada de la betamiosina. Troponina T cardíaca. Mutaciones.*

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) affects one out of every 500 to 1000 individuals.[1] Even though the disease has a broad clinical spectrum, risk estimation is currently based on clinical criteria.[2-4]

Approximately half of all HCM patients have a family history of the disease, and therefore present a mutation in one of the two copies of a sarcomeric protein gene.[5] Mutations in the beta myosin heavy chain (*MYH7*) and cardiac troponin-T (*TNNT2*) genes would lead to severe HCM, with a high risk of sudden death.[5-7] Nevertheless, some carriers of these mutations present with only mild hypertrophy. Furthermore, certain *TNNT2* mutations do not cause evident hypertrophy on echocardiographic study, but do increase the risk of sudden death. In general, it has not been possible to establish a close relationship between a particular mutation and a specific prognosis.[8,9]

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TABLE 1. **Main characteristics of the 30 patients with hypertrophic cardiomyopathy**

Total	30
Men/women	16/14
Mean age at diagnosis	46 years (range, 18-60)
Left ventricular wall thickness	
13-16 mm	5 (16%)
7-19 mm	7 (23%)
>19 mm	18 (61%)
Concentric hypertrophy	11
Asymmetric septal asymmetry	19
Gradient (range, 16-130 mm Hg)	16
Family history	16 (54%)
Mean age	44 years (range, 24-60)
No family history	14 (46%)
Mean age	37 years (range, 20-56)
Family history of sudden death	9 (30%)

The purpose of the present study was to assess the value of direct screening for several malignant mutations, in order to determine its usefulness in clinical decision-making concerning individuals with HCM.

PATIENTS AND METHODS

Patients

Thirty patients, 18 to 60 years of age at the time of diagnosis, who presented with primary ventricular hypertrophy defined as left ventricular wall thickness of 13 mm, participated in the study. Twenty five patients had a family history of hypertrophic cardiomyopathy or sudden death (Table 1). The study was approved by the Research Ethics Committee of the Hospital Central de Asturias and all patients gave informed consent to participate.

Genetic testing

DNA was obtained from the leukocytes in 10 mL of blood, using a salting-out method.[10] Exons were amplified by polymerase chain reaction (PCR) with primers designed from the sequences of the two genes (Table 2), followed by restriction endonuclease digestion (New England Biolabs or Boehringher Mannheim) and electrophoresis in 3% agarose gels to separate the fragments from each digestion. The fragments were then stained with ethidium bromide to identify bands corresponding to normal or mutated

TABLE 2. **MYH7 and TNNT2 mutations studied and primer sequences used to amplify the exons**

Mutations	Exon	Primers
	MYH7 gene	
R403Q*	13	S: 5'- CAGGCATGAACCACACACCTG -3'
R403L		A: 5'- TCTCATCCCACCATGCCAGT-3'
R403W		
R453C*	14	S: 5'- TCACTCTTCCCAACAACCCTG -3'
		A: 5'- AGAAATAGCTGTTGAATGTGGG -3'
V606M*	16	S: 5'- GCAGAATCCATGTCACCTGTGTGA-3'
		A: 5'- AATTGACCTGGCTCAGAACCTTG-3'
G716R*	19	S: 5'-CAGAACCCAGAACTTCAGTCCAGT-3'
		A: 5'-CCCACCTCTGCCGGAAGTCCG-3'
R719Q		
R719P		S: 5'-CAGAACCCAGAACTTCAGTCCAGT-3'
R719W*		A: 5'-CCCATTCCCATCAGGGCA-3'
R723G*	20	S: 5'-ACTCTGGAGACTTCCCTCCTCAGGA-3'
R723C		A: 5'-AGCATCAGAGGAGTCAATGGAA-3'
	TNNT2 gene	
I79N*	8	S: 5'-GCCCTGCCTGTCCTGGACAC-3'
		A: 5'-CCCACCTATGCTCTACCCCAG-3'
R92W*		
R92Q	9	S: 5'-GTCTAGCCCACCCATCTCTCCT-3'
R92L		A: 5'-GAGGTGGGGCCTCACAAAAG-3'
R278C*	16	S: 5'-CATGGTGACCTACTACCCTGC-3'
		A: 5'-GTGTGGGGGCAGGCAGGA-3'

*Mutations related to malignant forms of hypertrophic cardiomyopathy.

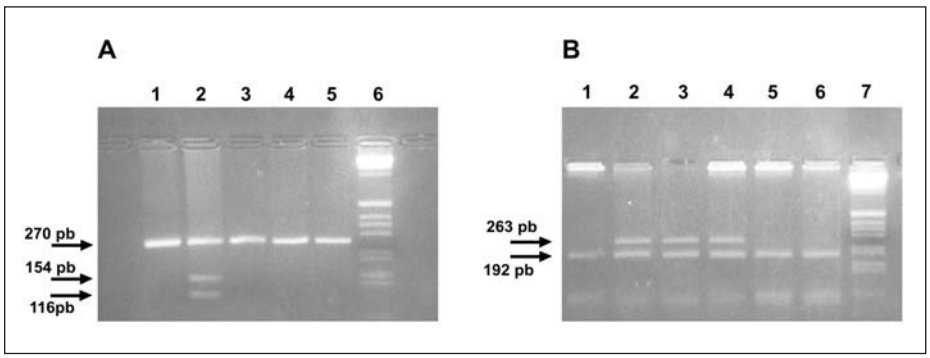

Fig. 1. Electrophoretic patterns for exon 14 of the MYH7 gene (A) and exon 16 of the TNNT2 gene (B). A: In the case of the MYH7 gene, digestion of the amplified fragment by the restriction enzyme NlaIII revealed a normal, undigested allele (270 bp band) of the mutation (154 and 156 bp bands) with an amino acid substitution (arginine to cysteine) at position 453. Lane 2 corresponds to the patient carrying the mutation, and lanes 1, 3, 4 and 5 to patients without the mutation. Lane 6 contains the size marker of the DNA fragments. B: BstUI digestion of the amplified fragment shows a mutation with an amino acid substitution at position 278 of the *TNNT2* gene (arginine to cysteine, 263 bp band) of the normal allele (192 and 71 bp band). Lanes 1, 5 and 6 correspond to patients without the mutation, and lanes 2, 3 and 4 to one patient with the mutation, her sister and her daughter. Lane 7 contains the size marker.

sequences. All mutations identified were verified by automatic sequencing using an ABI310 system (Applied Biosystems).

RESULTS

Two of the 16 mutations were found in two of the 30 patients analyzed: one had MYH7-R453C and the other had TNNT2-R278C (Figures 1 and 2).

Patient 1

Mutation R453C in exon 14 of the *MYH7* gene was found in a 44-year-old woman diagnosed as having HCM at age 25. She subsequently presented with symptoms consistent with the onset of atrial fibrillation that did not revert to sinus rhythm after electrical cardioversion. The patient is currently NYHA (New York Heart Association) Functional Class III/IV for dyspnea, with a blood pressure of 110/85 mm Hg, no murmurs and no signs of right-sided heart failure. The electrocardiogram demonstrated established atrial fibrillation. Echocardiography disclosed asymmetrical left ventricular and septal hypertrophy (17 mm septum and 12 mm posterior wall), with no gradient or systolic anterior motion of the mitral valve. Repeat Holter testing showed no ventricular arrhythmia. The patient´s only known family history of the disease was sudden death in a 17-year-old son.

Patient 2

The R278C mutation of the *TNNT2* gene was found in a 60-year-old woman with no relevant family history. She had been diagnosed at age 49 with asymmetric septal hypertrophy and a history of syncope, dyspnea, dizziness and palpitations. Color Doppler ultrasound studies showed nonobstructive asymmetric septal hypertrophy (septum 22 mm and posterior wall 12 mm), no intraventricular gradient, normal diastolic mitral flow, delayed left ventricular relaxation pattern, and dilated left atrium. One sister and one daughter of this patient, aged 45 and 30 years old, respectively, were available for study. Both had the mutation, but function on Doppler study was normal and neither had left ventricular hypertrophy.

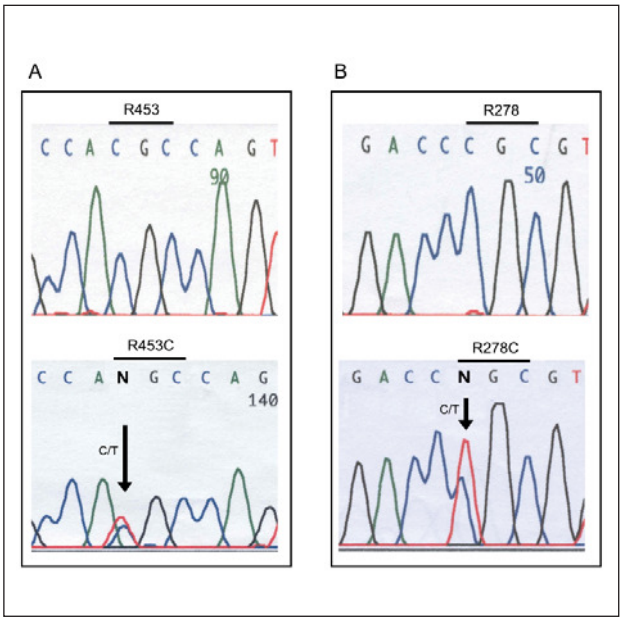

Fig. 2. Sequence fragments of the *MYH7* (A) and *TNNT2* (B) genes corresponding to the codons with mutations. In both cases the mutated nucleotide is indicated with an arrow. In the normal sequence, the nucleotide peak appears as a single color, whereas the carrier sequence is shown in two colors, as carriers have one normal copy and one mutated copy.

DISCUSSION

Hypertrophic cardiomyopathy is a genetically complex disease: more than 150 mutations have been identified in at least 11 genes that encode sarcomeric proteins.[5,11] An initial approach to genetic testing is to determine whether the patients carry any of the known mutations, particularly those associated with a poor prognosis. Ackerman et al. analyzed the *MYH7* mutations R403Q, R453C, G716R and R719W, and the *TNNT2* mutation R92W in 293 patients with HCM, but found that only three of these patients, all under 25 years of age, had any of these mutations.[12] Because only 1% of the patients showed mutations, the authors concluded that routine screening would not be useful. This percentage is lower than the figure found in the present study (7%), although Ackerman et al studied patients with a broader phenotypic spectrum than ours, including late-onset types (with patients up to age 89), whereas we included only patients younger than 60 years. Overall, these results suggest that direct screening for mutations would be most valuable in the severe or early onset forms of HCM. In most cases, however, the only way to identify mutations in these genes is to sequence all their exons.

No close correlation has yet been established between these mutations and the development of severe hypertrophy or the risk of sudden death, and this must be taken into account when making clinical decisions in carrier patients. One 44-year-old patient in our study was a carrier of the *MYH7* mutation R453C; her son died suddenly at age 17. Although we could not determine whether her son also had the mutation, we may assume he was a carrier. One patient with severe HCM was a carrier of the TNNT2-R278C mutation; her sister and daughter were also carriers, but had no signs of hypertrophy. These two cases illustrate the phenotypic heterogeneity that characterizes carriers of these mutations traditionally considered to be malignant. Clinical manifestations range from severe hypertrophy or early sudden death to the absence of symptoms even at an advanced age.

Limitations of the study

Our study had two limitations. First, we studied only a small number of patients. Furthermore, our results do not reflect the mutational spectrum of these genes, but rather the incidence of several previously described mutations. To determine the actual frequency of *MYH7* and *TNNT2* mutations, all exons of these genes should be sequenced.

CONCLUSIONS

In our experience, direct screening for 16 mutations in the *TNNT2* and *MYH7* genes revealed the genetic basis for HCM in approximately 7% of cases. This method could be useful for initial genetic screening.

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