A p.R870H mutation in the beta-cardiac myosin heavy chain 7 gene causes familial hypertrophic cardiomyopathy in several members of an Indian family

Murali D Bashyam PhD^{1,2}, Gorinabele R Savithri MSc¹,
Munimanda Gopikrishna MSc², Calambur Narasimhan MD³

MD Bashyam, GR Savithri, M Gopikrishna, C Narasimhan. A p.R870H mutation in the beta-cardiac myosin heavy chain 7 gene causes familial hypertrophic cardiomyopathy in several members of an Indian family. *Can J Cardiol* 2007;23(10):788-790.

Familial hypertrophic cardiomyopathy is an autosomal dominant genetic disorder characterized mainly by left ventricular hypertrophy and myocyte disarray; it is the most common cause of sudden death in otherwise healthy individuals. More than 270 mutations in genes encoding the cardiac sarcomere have been identified. Attempts to establish a genotype-phenotype correlation for each of the mutations have not been highly successful. It has been suggested that additional genetic loci, as well as nongenetic factors such as lifestyle, gender and age, may play a role in modulating the clinical presentation of the disease. The p.R870H mutation has been identified as the cause of familial hypertrophic cardiomyopathy in an Indian family. The results indicate that the disease phenotype varied among various affected members of the family, and the variation may be attributed to factors, such as gender and gene dosage.

Key Words: Angiotensin; Cardiomyopathy; Hypertrophy

Familial hypertrophic cardiomyopathy (FHC) is an autosomal dominant disease characterized mainly by left ventricular hypertrophy and myocyte disarray; it is the most common cause of sudden death in otherwise healthy individuals. More than 270 mutations in several genes encoding the cardiac sarcomere have so far been identified as causing FHC (1). Environmental factors, including gender, age, lifestyle and genetic factors, have been suggested to modulate clinical presentation in the patients harbouring the same mutation. A deletion-insertion (D/D versus D/I or I/I) polymorphism in intron 16 of the *ace* gene has been shown to affect the serum levels of angiotensin-converting enzyme and the pathophysiology of heart failure, as well as the clinical presentation of FHC.

Informed consent was obtained from all affected family members and control subjects. Approval from the local ethics committee was also obtained for the work, as per the guidelines of the Helsinki Declaration of 1975 <www.wma.net/e/policy/b3.htm>. Due to their poor socioeconomic condition and long distance from the hospital, several members did not present in person for

Une mutation p.R870H dans le gène 7 de la chaîne lourde bêta de la myosine responsable d'une myocardiopathie hypertrophique familiale chez plusieurs membres d'une famille indienne

La myocardiopathie hypertrophique familiale est un trouble génétique autosomique dominant caractérisé surtout par une hypertrophie ventriculaire gauche et une désorganisation des myocytes. C'est la principale cause de mort subite chez les personnes autrement en santé. On a trouvé plus de 270 mutations des gènes qui encodent le sarcomère cardiaque. Les tentatives pour établir une corrélation génotype-phénotype à chacune des mutations n'ont pas obtenu beaucoup de succès. On avance que des locus génétiques supplémentaires, de même que des facteurs non génétiques comme le mode de vie, le sexe et l'âge, peuvent participer à la modulation de la présentation clinique de la maladie. On a déterminé que la mutation p.R870H est responsable de la myocardiopathie hypertrophique d'une famille indienne. Selon les résultats, le phénotype de la maladie variait entre les divers membres de la famille qui étaient atteints, et cette variation peut être attribuée à des facteurs comme le sexe et le dosage génique.

clinical examination; however, they provided blood samples for genetic testing following informed consent. Family members who reported to the hospital were evaluated by physical examination, electrocardiogram and two-dimensional echocardiography. An interventricular septal diameter larger than 1.2 cm was taken as criteria for FHC. Genomic DNA was isolated from blood samples collected from the affected family members clinically diagnosed with FHC. The beta-cardiac myosin heavy chain 7 gene, the troponin T gene and the cardiac myosin-binding protein C gene were screened for the presence of mutations by polymerase chain reaction and single-strand conformation polymorphism using exon-specific primers using conditions described at <cardiogenomics.med.harvard.edu/home>. DNA sequencing (of both strands) for the 22nd exon of the beta-cardiac myosin heavy chain 7 gene was carried out using an ABI 3100A genetic analyzer (Applied Biosystems, USA) according to the manufacturer's instructions. The results revealed a one-base substitution in codon 870 (CGC to CAC), resulting in an amino acid change (arginine to histidine) (Figures 1A and B) in the 22nd exon of the gene. One hundred normal samples did

¹Laboratory of Molecular Oncology; ²National Genomics and Transcriptomics Facility, Centre for DNA Fingerprinting and Diagnostics, Nacharam; ³Care Hospital, Nampally, Hyderabad, India

Correspondence: Dr Murali D Bashyam, Laboratory of Molecular Oncology and National Genomics and Transcriptomics Facility, Centre for DNA Fingerprinting and Diagnostics, ECIL Road, Nacharam, Hyderabad 500076, India. Telephone 91-40-27151344, fax 91-40-27155610, e-mail bashyam@cdfd.org.in

Received for publication May 14, 2006. Accepted July 24, 2006

TABLE 1
Clinical and molecular genetic analyses of familial hypertrophic cardiomyopathy patients

Patient	Age/sex	Interventricular septal diameter, cm	Left ventricular posterior wall thickness, cm	Left ventricular outflow tract gradient, mmHg	Left ventricular ejection fraction, %	Symptoms
1	30/female	1.2	0.8	—	73	Palpitations, angina, presyncope
2	13/female	0.7	0.9	—	75	Palpitations, angina, dyspnea, syncope
3	40/female	1.2	1.0	—	65	Palpitations, angina, dyspnea
4	20/male	2.1	1.0	16	86	Dyspnea, syncope
5	48/male	1.1	1.1	108	75	Angina, dyspnea, syncope
6*	36/male	2.0	—	—	55	Palpitations, syncope
7	44/male	1.6	1.1	40	68	Palpitations, angina, dyspnea
8	29/male	2.3	1.3	64	76	Dyspnea, syncope
9	66/male	—	—	—	—	Syncope
10	55/female	—	—	—	—	Palpitations, angina, dyspnea
11	24/female	—	—	—	—	Palpitations, angina, presyncope
12	16/female	—	—	—	—	Dyspnea, presyncope
13	25/female	—	—	—	—	Presyncope
14	14/male	—	—	—	—	Angina

*Patient 6 harboured a homozygous mutation and received an implant during the study; however, the patient died within a few months of the implant

Figure 1) DNA sequencing results. Panels **A** and **B** show electro-pherograms of sequencing reactions performed using the forward primer for exon 22 of the beta-cardiac myosin heavy chain 7 gene on patient samples 6 and 1, respectively. The arrows indicate the homozygous mutation in patient 6 and the heterozygous mutation in patient 1

not harbour this mutation, indicating that the nucleotide change was not a single-nucleotide polymorphism.

Figure 2) Pedigree chart for the Indian family with familial hypertrophic cardiomyopathy. All 29 members who were screened for the mutation are indicated. Sample 6 harboured a homozygous mutation

DISCUSSION

The p.R870H mutation was reported previously by Nishi et al (2), but the proband was a compound heterozygote (the affected members harboured a nonsense mutation in codon 54 of the MYH7 gene in addition to the p.R870H mutation); therefore, the present study is the first report of FHC caused exclusively by the p.R870H mutation. Based on analyses of 29 family members, 14 members who harboured the p.R870H mutation were identified (Table 1). One affected member (patient 6) harboured a homozygous mutation. A pacemaker was implanted in the patient during the study; however, within a few months of the implant, the patient died at the young age of 36 years. The parents of patient 6, who had undergone a consanguineous marriage (Figure 2), harboured a heterozygous mutation (the father, sample 9, was 66 years of age, and the mother, sample 10, was 55 years of age). There were no other FHC-related deaths among the affected members of the family, indicating that the prognosis of patients with two abnormal alleles for the p.R870H mutation may be worse than for those with only

one abnormal allele. Such a gene dosage effect has also been observed in a few other mutations (3).

Abnormalities in the ventricle were undetected in younger patients (0.7 cm in patient 2, a 13-year-old female patient), as expected (Table 1). Most affected members presented with asymmetrical septal hypertrophy, with more abnormalities in the septal diameter than in the left ventricular posterior wall thickness (Table 1). It is interesting to note that all affected individuals had a normal to near normal left ventricular ejection fraction (Table 1), as reported in the previous study on the same mutation (2). None of the patients exhibited pulmonary arterial hypertension, and an abnormal left ventricular outflow tract gradient was evident only in a few patients (Table 1). Almost all affected members exhibited clinical symptoms characteristic of hypertrophic cardiomyopathy, including dyspnea, angina, syncope and presyncope.

Interestingly, the interventricular septal diameter exhibited a great degree of variation among the members who harboured the mutation (Table 1). Despite the small sample size, it was evident that almost all male patients exhibited a larger interventricular septal diameter than the female patients, irrespective of age (Table 1). However, due to the small number of affected patients who presented for a clinical examination, it was difficult to come to a firm conclusion in this regard, although the trend was similar to those obtained earlier for other mutations.

Based on our molecular diagnosis of FHC in this family, we have been able to identify young affected members, which will facilitate better management before the onset of symptoms. In

addition, the p.R870H mutation appears to result in a benign form of the disease (in a heterozygous condition), which is also consistent with previous physiological studies that had revealed a milder effect on in vitro motility activity of beta-cardiac myosin chain harbouring the p.R870H mutation compared with other mutations such as the p.R403G, p.Y162C, p.V606M and the p.L908V (4).

ACKNOWLEDGEMENTS: The authors thank the participating patients, their family members and control subjects for their cooperation in this study. This study was supported by a core grant from the Department of Biotechnology, Government of India, to the Centre for DNA Fingerprinting and Diagnostics.

REFERENCES

1. Bashyam MD, Savithri GR, Kumar MS, Narasimhan C, Nallari P. Molecular genetics of familial hypertrophic cardiomyopathy (FHC). *J Hum Genet* 2003;48:55-64.
 2. Nishi H, Kimura A, Harada H, et al. A myosin missense mutation, not a null allele, causes familial hypertrophic cardiomyopathy. *Circulation* 1995;91:2911-5.
 3. Ho CY, Lever HM, DeSanctis R, Farver CF, Seidman JG, Seidman CE. Homozygous mutation in cardiac troponin T: Implications for hypertrophic cardiomyopathy. *Circulation* 2000;102:1950-5.
 4. Cuda G, Fananapazir L, Epstein ND, Sellers JR. The in vitro motility activity of beta-cardiac myosin depends on the nature of the beta-myosin heavy chain gene mutation in hypertrophic cardiomyopathy. *J Muscle Res Cell Motil* 1997;18:275-83.
-