

Double heterozygosity for mutations in the β -myosin heavy chain and in the cardiac myosin binding protein C genes in a family with hypertrophic cardiomyopathy

Pascale Richard, Richard Isnard, Lucie Carrier, Olivier Dubourg, Yves Donatien, Bénédicte Mathieu, Gisèle Bonne, Françoise Gary, Philippe Charron, Albert Hagege, Michel Komajda, Ketty Schwartz, Bernard Hainque

Service de Biochimie
B, Groupe Hospitalier
Pitié-Salpêtrière, 47
Bld de l'Hôpital, 75651
Paris Cedex 13, France
P Richard
B Mathieu
B Hainque

Service de
Cardiologie, Groupe
Hospitalier
Pitié-Salpêtrière, 47
Bld de l'Hôpital, 75651
Paris Cedex 13, France
R Isnard
P Charron
M Komajda

INSERM U523, Institut
de Myologie, Groupe
Hospitalier
Pitié-Salpêtrière, 47
Bld de l'Hôpital, 75651
Paris Cedex 13, France
L Carrier
G Bonne
K Schwartz

Service de
Cardiologie, Hôpital
Ambroise Paré,
Boulogne, France
O Dubourg

Centre Hospitalier de
Fort de France,
Martinique
Y Donatien

URA CNRS 1922,
Généthon, Evry,
France
F Gary

Service de
Cardiologie, Hôpital
Boucicaut, Paris,
France
A Hagege

Correspondence to:
Dr Hainque.

Received 11 August 1998
Revised version accepted for
publication 1 March 1999

Abstract

Familial hypertrophic cardiomyopathy is a genetically heterogeneous autosomal dominant disease, caused by mutations in several sarcomeric protein genes. So far, seven genes have been shown to be associated with the disease with the β -myosin heavy chain (MYH7) and the cardiac myosin binding protein C (MYBPC3) genes being the most frequently involved. We performed electrocardiography (ECG) and echocardiography in 15 subjects with hypertrophic cardiomyopathy from a French Caribbean family. Genetic analyses were performed on genomic DNA by haplotype analysis with microsatellite markers at each locus involved and mutation screening by single strand conformation polymorphism analysis. Based on ECG and echocardiography, eight subjects were affected and presented a classical phenotype of hypertrophic cardiomyopathy. Two new mutations cosegregating with the disease were found, one located in the MYH7 gene exon 15 (Glu483Lys) and the other in the MYBPC3 gene exon 30 (Glu1096 termination codon). Four affected subjects carried the MYH7 gene mutation, two the MYBPC3 gene mutation, and two were doubly heterozygous for the two mutations. The doubly heterozygous patients exhibited marked left ventricular hypertrophy, which was significantly greater than in the other affected subjects.

We report for the first time the simultaneous presence of two pathological mutations in two different genes in the context of familial hypertrophic cardiomyopathy. This double heterozygosity is not lethal but is associated with a more severe phenotype.

(*J Med Genet* 1999;36:542-545)

Keywords: hypertrophic cardiomyopathy; genetics; β -myosin heavy chain gene; cardiac myosin binding protein C gene

Familial hypertrophic cardiomyopathy (FHC) is an autosomal dominant disease characterised by left ventricular hypertrophy (LVH) and myofibrillar disarray.^{1,2} It is genetically heterogeneous and can be caused by mutations in the

β -myosin heavy chain gene (MYH7)³ located on chromosome 14, the cardiac troponin T gene (TNNT2) on chromosome 1, the α -tropomyosin gene (TPM1)⁴ on chromosome 15, the cardiac myosin binding protein C gene (MYBPC3)^{5,6} on chromosome 11, the ventricular myosin essential (MYL3) and regulatory (MYL2) light chain genes⁷ respectively located on chromosomes 3 and 12, and the cardiac troponin I gene (TNNT3)⁸ located on chromosome 19. All these genes encode for proteins involved in the sarcomeric function, so hypertrophy might be the result of a compensatory phenomenon to quantitative or qualitative abnormalities of the contractile process. The pattern and degree of LVH show considerable variability, as does the clinical expression, ranging from no symptoms to heart failure and premature sudden death.¹ In our experience, in about one third of families, the FHC results from mutations in MYH7 and another third from mutations in MYBPC3. We report here the genetic and clinical data of a French Caribbean family with hypertrophic cardiomyopathy, which was first excluded from the chromosome 14, 1, 15, 11, and 12 loci by haplotype linkage analyses. A systematic screening of mutations in MYH7 and MYBPC3 allowed us to identify the segregation of two mutations, one in MYH7 and the other in MYBPC3, and we analysed the phenotype-genotype relationship.

Methods

SUBJECTS

After informed consent was obtained by applying the guidelines of the Comité d'Éthique du Centre Hospitalier Universitaire de la Pitié-Salpêtrière (Paris), all genotyped subjects underwent clinical and cardiovascular examination including a 12 lead electrocardiogram (ECG) and M mode, two dimensional, and Doppler echocardiography at the time of genotyping. The diagnosis of FHC was based on ECG and echocardiography as previously described⁹: briefly, major echo diagnostic criteria were defined by a left ventricular end diastolic maximal wall thickness >13 mm or the presence of major ECG abnormalities (LVH assessed by a Romhilt-Estes score ≥ 4 and/or Q waves >0.04 sec or >1/3R wave and/or significant ST-T changes). End diastolic left ventricular wall thickness measurements were obtained at different locations (anterior

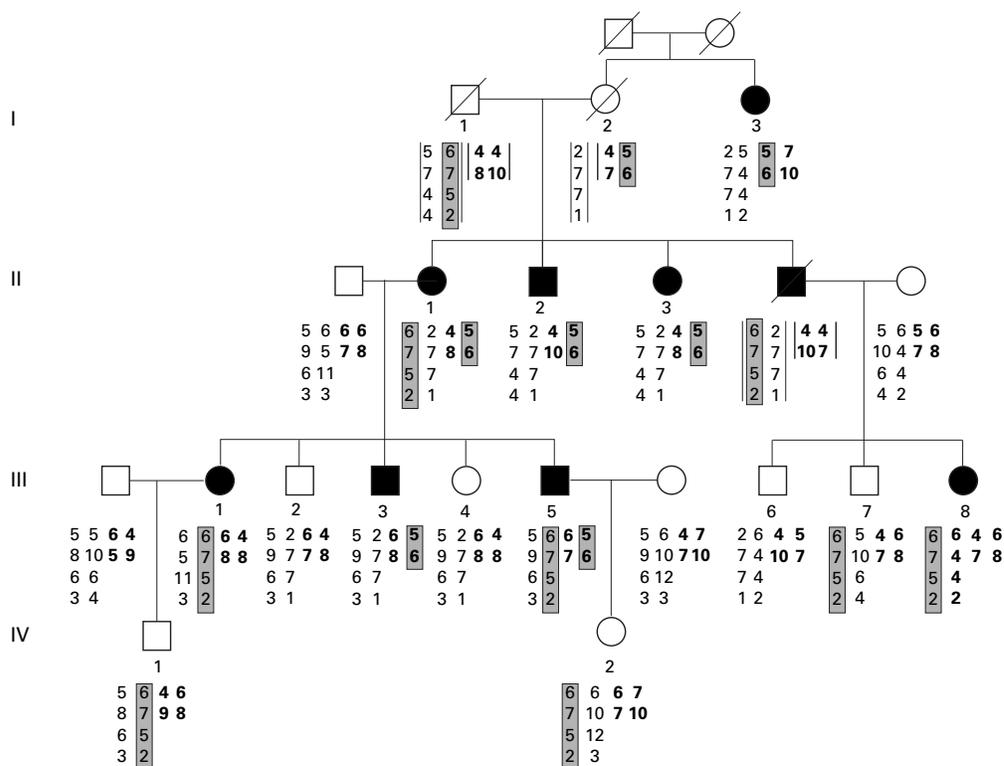


Figure 1 Inheritance of familial hypertrophic cardiomyopathy and haplotype analysis. Alleles shown below each family member are listed from top to bottom. On the left, chromosome 11 markers are indicated (D11S4133, D11S1344, D11S1350, D11S1326) and on the right the two MYO1, MYOII markers of chromosome 14. Data in brackets were deduced. Affected haplotypes are shaded in grey, 6-7-5-2 for the chromosome 11 locus and 5-6 for the chromosome 14 locus.

and posterior septum, lateral and posterior wall) from the parasternal short axis view, at both the mitral valve and the papillary muscle levels, and at the parasternal long axis view. The degree and extent of left ventricular hypertrophy were assessed by the maximal wall thickness and the scoring system proposed by Spirito and Maron.¹⁰ The distribution of hypertrophy was assessed according to the classification of Maron *et al.*¹¹

GENETIC ANALYSIS

Haplotype analyses were performed on different loci with microsatellite markers provided by the Généthon human genetic linkage map.¹² Linkage to chromosome 14 was carried out with two MYH7 intragenic microsatellites (MYO1 and MYOII), linkage to chromosome 1 with three extragenic markers (D1S306, D1S456, and D1S249), linkage to chromosome 15 with two extragenic and one intragenic microsatellite markers (D15S153, HTM α (CA), and D15S131), linkage to chromosome 11 with four extragenic markers (D11S4133, D11S1344, D11S1350, and D11S1326), and linkage to chromosome 12 with six extragenic markers (D12S84, D12S1645, D12S1339, D12S1344, D12S1583, and D12S354).¹³

Screening of mutations was performed after amplification of each MYH7 and MYBPC3 exon with oligonucleotide primers determined in the flanking intronic sequences and single strand conformation analysis as previously described.^{6 14}

Then, the PCR products were sequenced on both strands according to the method of Sanger *et al.*¹⁵ using rhodamine fluorescent dideoxynucleotides. The reaction products were then electrophoresed on an automated laser fluorescent DNA sequencer (Applied Biosystems).

STATISTICAL ANALYSIS

Values were expressed as mean (standard deviation (SD)); comparison between groups was performed with the Mann-Whitney test. Differences were considered significant at $p < 0.05$.

Results

The pedigree of the family is shown in fig 1. Fifteen subjects were studied and eight were clinically affected. Clinical and echocardiographic characteristics of affected subjects are shown in table 1. All clinically affected subjects were adult and had a maximal left ventricular end diastolic wall thickness >13 mm. None had isolated major ECG abnormalities and none had significant intraventricular obstruction.

Preliminary haplotype analyses showed no linkage with any of the loci tested. This finding suggested the presence of an unknown locus. Despite this possibility, we performed a mutation detection analysis of the 35 MYBPC3 exons in the proband (II.1). This analysis by SSCP showed an abnormal profile in exon 30, and sequence analysis subsequently showed a G to T mutation at codon 1096 leading to a TAA termination codon. Analysis of all the

Table 1 Clinical, ECG, and echocardiographic characteristics of affected subjects

No	Age (y)	Sex (M/F)	ECG	Max IVS (mm)	Spirito score (mm)	Maron type	Gene mutation
I.3	84	F	Ventricular pacing	25	67	III	MYH7
II.1	56	F	LVH, negative T waves	32	85	III	MYH7/MYBPC3
II.2	58	M	Normal	15	49	II	MYH7
II.3	62	F	Normal	21	61	III	MYH7
III.1	30	F	Negative T waves (V2-4)	20	63	III	MYBPC3
III.3	31	M	Negative T waves (V2-4)	19	63	III	MYH7
III.5	33	M	LVH, negative T waves	28	75	III	MYH7/MYBPC3
III.8	27	F	Normal	17	52	III	MYBPC3

Max IVS: maximal end diastolic interventricular wall thickness. Maron type: type II hypertrophy involving both the anterior and posterior segments of the interventricular septum, type III hypertrophy involving both the interventricular septum and the left ventricular free wall.

family members showed that only the clinically affected subjects III.1, III.5, and III.8 carried this mutation. Microsatellite marker analysis at the MYBPC3 locus showed that the haplotype 6-7-5-2 cosegregated with the mutation (fig 1) but seemed to be transmitted by the dead subject I.1. The finding that subject I.3 was clinically affected but did not bear either the affected haplotype or the MYBPC3 mutation led us to postulate that another mutation could be involved in the family. Therefore we analysed the other locus haplotype cosegregations taking into account the first localisation. We found that the particular haplotype 5-6 on the chromosome 14 locus containing MYH7 showed cosegregation in the remaining affected subjects. This haplotype was found in affected subject I.3 and could have been transmitted by the dead female I.2. The SSCP screening of the first 24 MYH7 exons followed by sequencing of the abnormal profiles allowed us to identify a G to A mutation in exon 15 leading to a Glu483Lys mutation. This missense mutation was found in subjects I.3, II.2, II.3, and III.3 and also in subjects II.1 and III.5, who thus carried the two mutations (fig 1). To check that the MYH7 Glu483Lys mutation is not a polymorphism, SSCP analysis of 200 unrelated normal chromosomes was performed and failed to detect this abnormal profile. In addition, this amino acid is highly conserved in different species and isoforms.

In summary, five subjects carried the MYBPC3 mutation including three clinically unaffected members, two children and one 23 year old adult, who had normal ECG and echocardiogram. Four subjects carried the MYH7 mutation, and all were clinically affected. Two patients, a woman and her son (II.1 and III.5) aged 56 and 32 years, were double heterozygotes for the two mutations (table 1). No difference in the degree and pattern of LVH was observed between patients bearing MYH7 or MYBPC3 mutations. In contrast, the degree and extent of LVH was significantly higher in the two double heterozygotes (maximal interventricular septum thickness 28 and 32 mm) than in the other affected

patients (maximal interventricular septum thickness between 15 mm and 25 mm) (table 2).

Discussion

This is the first report of the presence of two mutations in two distinct genes, a missense mutation in the MYH7 gene and a nonsense mutation in the MYBPC3 gene, in a family with hypertrophic cardiomyopathy, in which two members carried both mutations. Based on the prevalence of hypertrophic cardiomyopathy in the general population, which is 1/500,¹⁶ these findings should occur in fewer than 1/250 000. Until now, only two compound heterozygotes for mutations in the MYH7 gene have been described, one patient carrying both a missense and a nonsense mutation in a Japanese family¹⁷ and the other carrying two missense mutations.¹⁸ In each of these two families, one of the mutations seemed to be recessive, as it was phenotypically silent in the heterozygous carriers with an unaffected allele. However, only the compound heterozygote patients were phenotypically affected, with onset at an early age. In the present family, each of the two distinct mutations was responsible for the FHC phenotype. As the two described mutations are new ones, the number of genotyped subjects carrying a mutation is not sufficient to determine the penetrance. There was no difference in wall thickness between patients with the β -myosin heavy chain mutation and patients with the myosin binding protein C mutation, as previously observed.¹⁹ Nevertheless, the degree and extent of hypertrophy were more pronounced in the two patients bearing both mutations, although the age of onset in these patients is unknown.

The MYBPC3 Glu1096 termination codon mutation is predicted to produce a truncated protein without the C-terminal domain,⁶ which binds to titin and myosin. The MYH7 Glu483Lys mutation is likely to affect a protein domain involved in actin fixation. These findings are consistent with previously described mutations in which most of the MYH7 mutations are missense and most of the MYBPC3 mutations are nonsense ones.^{20,21} As β -myosin heavy chain and myosin binding protein C interact together in sarcomeric structure and function, we can hypothesise that the presence of quantitative or qualitative abnormalities at both levels results in an increased compensatory hypertrophy owing to additional or synergistic negative effects on the contractile

Table 2 Comparison of the degree and extent of LVH assessed by the maximal interventricular wall thickness (max IVS) and the Spirito score in the double heterozygous and single heterozygous patients

	Double heterozygous MYH7/ MYBPC3 (n=2)	Single heterozygous MYH7 or MYBPC3 (n=6)
Max IVS (mm)	30.5 \pm 3.5	19.5 \pm 2.1*
Spirito score (mm)	80 \pm 7	59 \pm 7*

*p<0.05.

function. In conclusion, clinical and genetic analysis of the present family showed that two distinct mutations responsible for a FHC phenotype may be simultaneously present in patients without being responsible for a lethal phenotype. Nevertheless, the degree and extent of left ventricular hypertrophy were more pronounced in the doubly heterozygous patients. Because of the limited number of doubly heterozygous patients in our report, future studies will be needed to confirm this finding and assess its prognostic implications.

PR, RI, LC, BM, GB, PC, MK, KS, and BH are members of the Institut Fédératif de Recherche "Physiopathologie et Génétique Cardiovasculaire" No 14. This work was supported by INSERM (Réseau de Recherche Clinique No 4R009B), the Association Française contre les Myopathies, the Fédération Française de Cardiologie, and the Délégation à la Recherche Clinique de l'Assistance Publique-Hopitaux de Paris (Crédits EMUL et IFR Physiopathologie et Génétique Cardiovasculaire). We wish to thank Dr Jacques Beckmann from Généthon for his helpful collaboration.

- 1 Maron BJ, Bonow RO, Cannon RO, Leon MB, Epstein SE. Hypertrophic cardiomyopathy: interrelations of clinical manifestations, pathophysiology, and therapy. *N Engl J Med* 1987;316:780-9, 844-52.
- 2 Wigle AD, Rakowski H, Kimball BP, Williams WG. Hypertrophic cardiomyopathy: clinical spectrum and treatment. *Circulation* 1995;92:1680-92.
- 3 Geisterfer-Lowrance AAT, Kass S, Tanigawa G, et al. A molecular basis for familial hypertrophic cardiomyopathy: a β cardiac myosin heavy chain gene missense mutation. *Cell* 1990;62:999-1006.
- 4 Thierfelder L, Watkins H, MacRae C, et al. α -tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: a disease of the sarcomere. *Cell* 1994;77:701-12.
- 5 Bonne G, Carrier L, Bercovici J, et al. Cardiac myosin binding protein-C gene splice acceptor site mutation is associated with familial hypertrophic cardiomyopathy. *Nat Genet* 1995;11:438-40.
- 6 Carrier L, Bonne G, Bährend E, et al. Organization and sequence of human cardiac myosin binding protein C gene (*MYBPC3*) and identification of mutations predicted to produce truncated proteins in familial hypertrophic cardiomyopathy. *Circulation Res* 1997;80:427-34.
- 7 Poetter K, Jiang H, Hassanzadeh S, et al. Mutations in either the essential or regulatory light chains of myosin are associated with a rare myopathy in human heart and skeletal muscle. *Nat Genet* 1996;13:63-9.
- 8 Kimura A, Harada H, Park JE, et al. Mutations in the cardiac troponin I gene associated with hypertrophic cardiomyopathy. *Nat Genet* 1997;16:379-82.
- 9 Charron P, Dubourg O, Desnos M, et al. Diagnostic value of electrocardiography and echocardiography for familial hypertrophic cardiomyopathy in a genotyped adult population. *Circulation* 1997;96:214-19.
- 10 Spirito P, Maron BJ. Relation between extent of left ventricular hypertrophy and diastolic filling abnormalities in hypertrophic cardiomyopathy. *J Am Coll Cardiol* 1990;15:808-13.
- 11 Maron BJ, Gottdiener JS, Epstein S. Patterns and significance of distribution of left ventricular hypertrophy in hypertrophic cardiomyopathy. A wide angle, two dimensional echocardiographic study of 125 patients. *J Am Acad Cardiol* 1981;48:418-28.
- 12 Dib C, Fauré S, Fizames C, et al. A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 1996;380:152-4.
- 13 Flavigny J, Richard P, Isnard R, et al. Identification of two novel mutations in the ventricular regulatory myosin light chain gene (*MYL2*) associated with familial and classical forms of hypertrophic cardiomyopathy. *J Mol Med* 1998;76:208-14.
- 14 Liew CC, Sole MJ, Yamauchi-Takihar K, et al. Complete sequence and organization of the human cardiac β -myosin heavy chain gene. *Nucleic Acids Res* 1990;18:3647-51.
- 15 Sanger F, Nicklen S, Coulson A. DNA sequencing with chain terminating inhibitors. *Proc Natl Acad Sci USA* 1977;74:5460-7.
- 16 Maron BJ, Gardin JM, Flack JM, Gidding SS, Kurosaki TT, Bild DE. Prevalence of hypertrophic cardiomyopathy in a general population of young adults: echocardiographic analysis of 4111 subjects in the CARDIA study. *Circulation* 1995;92:785-9.
- 17 Nishi H, Kimura A, Harada H, et al. A myosin missense mutation, not a null allele, causes familial hypertrophic cardiomyopathy. *Circulation* 1995;91:2911-15.
- 18 Jeschke B, Uhl K, Weist B, et al. A high risk phenotype of hypertrophic cardiomyopathy associated with a compound genotype of two mutated β -myosin heavy chain genes. *Hum Genet* 1998;102:299-304.
- 19 Charron P, Dubourg O, Desnos M, et al. Clinical features and prognostic implications of familial hypertrophic cardiomyopathy related to cardiac myosin binding protein C gene. *Circulation* 1998;97:2230-6.
- 20 Vikstrom KL, Leinwand LA. Contractile protein mutations and heart disease. *Curr Opin Cell Biol* 1996;8:97-105.
- 21 Rottbauer W, Gautel M, Zehelein J, et al. Novel splice donor site mutation in the cardiac myosin-binding protein-C gene in familial hypertrophic cardiomyopathy. Characterization of cardiac transcript and protein. *J Clin Invest* 1997;100:475-82.