Br Heart J 1993;**69**:136–141

Identification of a mutation in the β cardiac myosin heavy chain gene in a family with hypertrophic cardiomyopathy

Sahar Al-Mahdawi, Susan Chamberlain, John Cleland, Petros Nihoyannopoulos, David Gilligan, Julie French, Lubna Choudhury, Robert Williamson, Celia Oakley

Abstract

***Objective*—To investigate the molecular genetic basis of the cause of disease in a family with hypertrophic cardiomyopathy.**

***Background*—Mutation within the β cardiac myosin heavy chain gene has been shown to be the pathogenetic mechanism underlying the disease in several families, though clear evidence of heterogeneity has been reported.**

***Patients*—A family with a history of hypertrophic cardiomyopathy.**

***Results and conclusion*—This paper reports a mutation at aminoacid position 908 within exon 23 of the β cardiac myosin heavy chain gene, resulting in a conversion of a leucine to valine. This base substitution was identified in an individual with a confirmed family history but with equivocal symptoms of the disease. Inheritance of the mutation by his symptom free juvenile offspring demonstrates the application of the technique to presymptomatic diagnosis.**

(*Br Heart J* 1993;**69**:136–141)

Department of Biochemistry and Molecular Genetics, St Mary's Hospital Medical School, Imperial College, London
S Al-Mahdawi
S Chamberlain
R Williamson

Department of Clinical Cardiology, Royal Postgraduate Medical School, Hammersmith Hospital, London
J Cleland
P Nihoyannopoulos
D Gilligan
J French
L Choudhury
C Oakley

Correspondence to:
Dr Sahar Al-Mahdawi, Department of Biochemistry and Molecular Genetics, St Mary's Hospital Medical School, London W2 1PG

Accepted for publication
27 August 1992

Hypertrophic cardiomyopathy (HCM) is a primary cardiac disease characterised by hypertrophy of the undilated left ventricle. The myocardial mass is increased and there is myocytic and myofibrillar disarray.[2] The condition is difficult to manage because those affected often do not have symptoms and are leading a healthy, normal life. The first indication of the disease can be sudden death[3] and the disease is the most common cause of sudden death in people under 30 years of age.[4] The disease is familial in at least 50% of cases and has an autosomal dominant mode of inheritance.[5–7]

The demonstration of linkage to chromosome 14q11-q12 in a large French-Canadian pedigree[8] led to the investigation of the β cardiac myosin heavy chain gene (MYH7), previously mapped to this region, as a potential candidate gene for the disorder. Identification of mutation within the coding sequence confirmed the role of this gene in the pathogenetic mechanism of the disease.[9 10] However, subsequent exclusion of the disease locus from the linked region on chromosome 14 in several well characterised HCM families is conclusive evidence of genetic heterogeneity.[11]

Current clinical diagnosis is based on physical examination (often unremarkable) and electrocardiography and echocardiography, where abnormalities may be subtle. Consequently, clinical diagnosis may be equivocal, particularly in those individuals who manifest clinical signs late in life.[12] The identification of mutation within the MYH7 gene provides a diagnostic tool for the investigation of correlation between genotype and phenotype, for determining the extent of locus heterogeneity, and for genetic counselling for affected families. It is particularly important in the presymptomatic individual where counselling on life style and clinical intervention may reduce the likelihood of sudden death, particularly in the young. Finally, characterisation of the genetic defect giving rise to this pathology should provide the basis for future therapy.

We report here a mutation in exon 23 of the coding sequence of MYH7 in an individual showing equivocal signs of the disorder. Detection of the mutation in a female offspring shows presymptomatic diagnosis in an apparently normal child.

Patients and methods

CLINICAL ANALYSIS

Family members were evaluated by physical examination, 12 lead electrocardiography, and echocardiography with an ultrasound scanner (Toshiba Sonolayer SSH-160A). Cross sectional images were obtained from a standardised series of cross sectional planes and were used to assess left ventricular systolic function. Integrated information from parasternal short and long axis views and apical two and four chamber views was used to define the presence and extent of ventricular hypertrophy at end diastole on cross sectional images. Continuous and pulsed wave Doppler echocardiography was used to evaluate left ventricular filling characteristics and outflow tract gradients.

MOLECULAR ANALYSIS

The MYH7 gene contains approximately 30000 nucleotides constituting 40 coding exons. Systematic screening of the MHY7 exonic sequence by direct sequencing in affected individuals from the Hammersmith Hospital series of families was undertaken to detect pathogenetic mutations.

Genomic DNA was isolated from peripher-

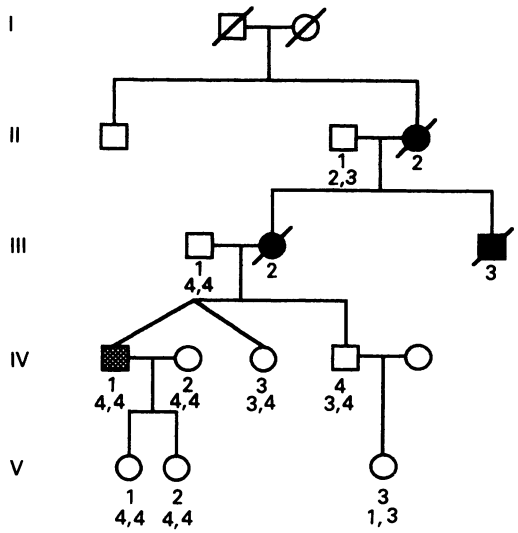

Figure 1 Pedigree of a family with hypertrophic cardiomyopathy transmitted as an autosomal dominant trait. Diagnosis in individual IV/1 is equivocal with electrocardiographic abnormalities detected. Solid symbols, individuals affected; open symbols, unaffected or unknown status; grey symbols, equivocal status; slashed symbols: dead. Genotypes generated from the analysis of a polymorphic microsatellite sequence identified in intron 24 of the MYH7 gene are indicated below the symbols (see fig 5 and text).

al blood samples, which were collected from family members including the grandchildren of the index case. Oligonucleotide primers used for polymerase chain reaction amplification of exon 23 of MYH7 are: 23F 5′ GCAAGAATGGAGGACCTTAC 3′ and 23R 5′ TGGGTCAAGGTCAGTATGGT 3′ with biotinylation of one primer for the subsequent direct sequencing of the polymerase chain reaction (PCR) product.

PCR amplification was carried out in a total volume of 50 μl containing 100–200 ng of genomic DNA, 10 pmol each of the forward and reverse primer, 250 μM each dNTP (nucleoside triphosphate) and 1 unit of Taq polymerase (NBL). Samples were initially denatured at 94°C for 10 min, followed by 40 cycles at 55°C for 1·5 min, 72°C for 4 min, and 92°C for 40 s. After purification of the PCR product (Geneclean II), a template was prepared with streptavidin magnetic beads (Dynal AS, Norway) and alkali denaturation.[13] Sequenase Version 2 was used for dideoxy sequencing.

To confirm that the observed base substitution was implicated in the disease pathology by its absence in the normal siblings, maternal inheritance of the disease allele in the two additional offspring of the index case was investigated by a (CA)n microsatellite sequence polymorphism identified in intron 24 of MYH7 (Al-Mahdawi, unpublished data). The oligonucleotide primers designed to amplify the polymorphism are I24F 5′ GTGAGTAGATTGAGAGTTGTGG 3′ and I24R 5′ TCAGAATTGATCACCAC-CTCTG 3′. PCR amplification was performed in a 25 μl reaction volume with [γ^{-32}P dATP] 5′ -end labelled reverse primer under the conditions described above. Alleles were resolved on standard 6% denaturing polyacrylamide DNA sequencing gels for 3 h and visualised by autoradiography.

Results

CLINICAL DESCRIPTION

Family 1 (fig 1) was investigated as part of a collaborative study to identify the genetic defect(s) giving rise to hypertrophic cardiomyopathy. A positive family history of the disease had been confirmed in two generations. The disease was transmitted as an autosomal dominant trait.

The index case, III/2, presented with murmur in 1952 at the age of 24 years, though hypertrophic cardiomyopathy was not diagnosed until 1965 at the time of cardiac catheterisation. A gradient of 35 mm Hg across the right ventricular infundibulum was noted.

Echocardiography in 1977 showed classic features of hypertrophic cardiomyopathy. The left ventricular cavity was small (end diastolic dimension 48 mm) and contracted well (fractional shortening 38%). There was symmetrical septal hypertrophy. The septum was 26 mm thick (normal < 11 mm) and the posterior wall was 11 mm thick (normal < 11 mm). There was no significant left ventricular outflow tract gradient, nor was there systolic anterior motion of the mitral valve. At

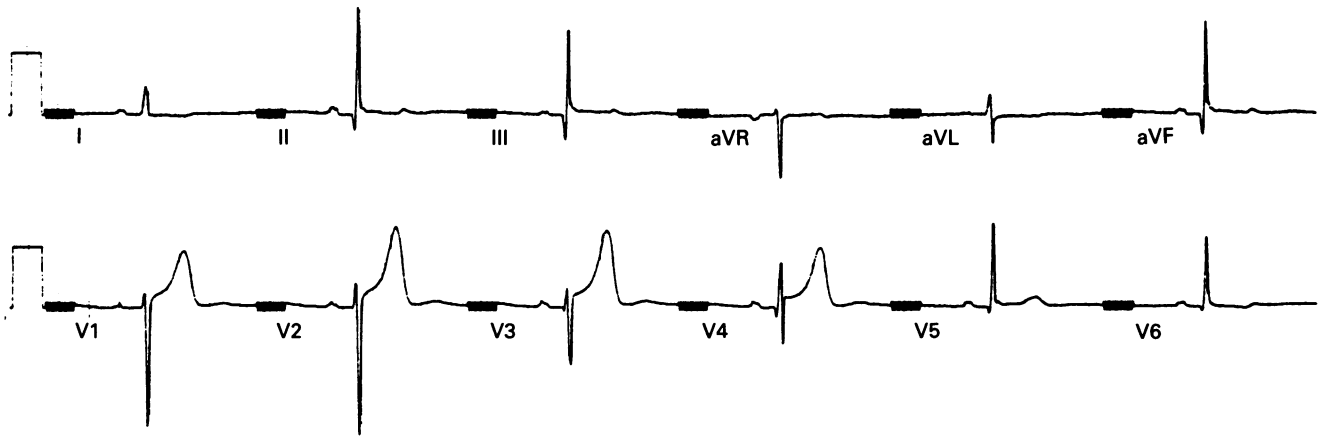

Figure 2 12 lead electrocardiogram of individual IV/1 showing Q waves in leads II,III, aVF (inferior), and V3 to V6 (anteroseptal) and inverted or biphasic T waves in the inferior and lateral leads (I, II, III, aVL and V6).

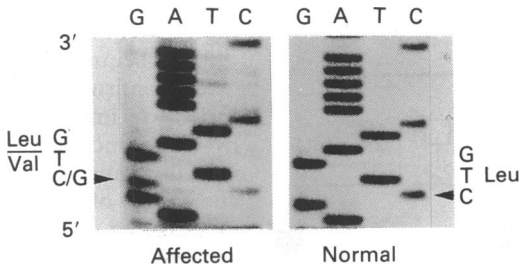

Figure 3 Direct sequencing after PCR amplification of exon 23 of the MYH7 gene. Sequence comparison between IV/1 and a normal unrelated individual showing a single base substitution from C to G at nucleotide 2808 which results in the conversion of leucine to valine.

this time the patient had chest pains and palpitation. No serious arrhythmia was detected on several 48 hour electrocardiographic recordings.

She was reviewed in 1989 because of an increasing diuretic requirement for progressive heart failure and was categorised as New York Heart Association class III. At the time the echocardiographic picture had changed. Although end diastolic dimensions were similar (49 mm) the end systolic dimensions had increased (38%) and fractional shortening had decreased to 22%. Ventricular septal thickness had also been reduced to 14 mm with the posterior wall remaining unchanged at 11 mm.

The patient died in intractable heart failure aged 61 years. A postmortem examination confirmed bi-ventricular hypertrophy (most prominent in the septum) and bi-ventricular dilatation. Histological examination showed cardiac myocyte hypertrophy and variation in nuclear size with a considerable increase in fibrous tissue. There was no evidence of myocardial fibre disarray, though only a small amount of myocardium was available for review. The intramural coronary arteries appeared normal. These findings are consistent with, but not diagnostic of, hypertrophic cardiomyopathy.

The brother of the index case (III/3) died suddenly after strenuous exercise in 1953 at the age of 18 years. Postmortem examination showed left ventricular hypertrophy. Unfortunately, a histological examination was not available for review. The patient's mother, II/2, died aged 71 years and a diagnosis of hypertrophic cardiomyopathy was made at necropsy.

The index case (III/2) had two sons and one daughter: the daughter and one son are twins. Electrocardiographic examination of individual IV/1 showed abnormal Q waves in leads V3 and V4 and inverted or biphasic T waves in the inferior and lateral leads (I, II, III, aVL, and V6) (fig 2). However, echocardiography was apparently normal with normal ventricular cavity dimensions and function. The interventricular septum was 11 mm and the posterior wall 10 mm (both within normal limits). These findings have not changed since 1989. This individual is currently symptom free: the abnormal electrocardiogram was detected in the course of routine screening of first degree relatives of confirmed HCM patients. His siblings show no clinical, electrocardiographic or echocardiographic evidence of the disease.

None of the grandchildren of the index case, the eldest of whom is 16 years old, show clinical signs of the disease. Case V/2 has had one syncopal episode related to hyperventilation. Her electrocardiogram and echocardiogram are entirely normal, as are those of the other grandchildren.

MOLECULAR EVALUATION

Direct sequencing of the PCR product corresponding to exons 9, 11, 13, 14, 17, and 23 of the MYH7 gene was undertaken in individual IV/1, in the knowledge that these exons have shown mutations in some HCM families.[14] This search resulted in the detection of a potential mutation in exon 23—the conversion of a cytosine[15] to a guanine residue at nucleotide position 2808 within exon 23 (fig 3). No mutations were detected in any of the other sequenced exons. This base substitution results in the conversion of leucine (*C*TG) to valine (*G*TG) at aminoacid position 908 without alteration in net charge. The mutation was not found in the siblings (IV/3 and IV/4).

The substitution had not been seen in the sequence analysis of exon 23 in 20 patients with confirmed HCM from unrelated families and five unrelated normal individuals. Analysis of the sequence in the immediate vicinity of the substitution showed that the mutation abolished *Pvu*II, *Alu*I *and Nsp*BII restriction sites normally present in exon 23. Direct digestion of the PCR product therefore provides an independent and rapid method of detection. The absence of the mutation in the analysis of an additional 200 normal and 20 HCM chromosomes argues strongly for the mutation being the pathogenetic mechanism in this family.

Figure 4 shows the digestion of the PCR amplified product of exon 23 with the restriction enzyme *Pvu*II. Digestion of normal DNA with *Pvu*II generates three fragments—251,

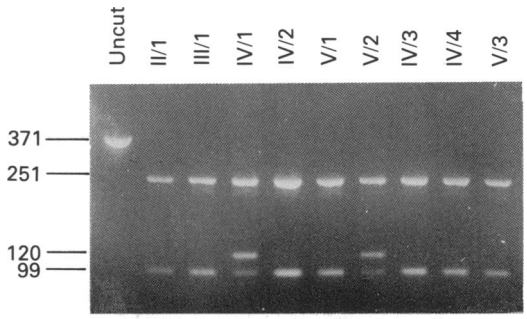

Figure 4 Digestion of the PCR amplification product of exon 23 with PvuII in the Pedigree. Products were digested at 37°C for 3 h and resolved on 2% agarose 2% NuSieve. Digestion of the PCR product from normal individuals results in the generation of three fragments - 251, 99 and 21bp. Abolition of a PvuII site by the mutation results in the detection of an additional 120 bp fragment (IV/1). This mutation has also been inherited by the symptom free female, V/2. The 21 bp fragment is not shown.

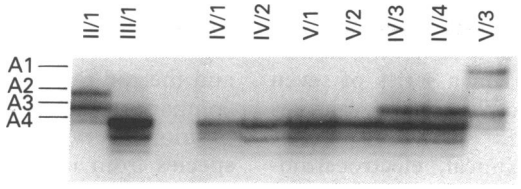

Figure 5 Segregation of a microsatellite sequence polymorphism identified within intron 24 of the MYH7 gene in the pedigree. The disease seems to segregate with allele A4 in this family (see fig 1). (A1 = 220; A2 = 216; A3 = 214; A4 = 212 base pairs).

99, and 21 base pairs in size (fig 4, individual II/1). In contrast, loss of a *Pvu*II site as a result of the mutation in the affected allele shows the presence of an additional fragment (120bp) (fig 4, individual IV/1). The absence of the mutation in the sibs of individual IV/1 was confirmed.

Members of generation V were also included in this analysis (fig 4). Individuals V/1 and V/2 are 13 and 12 years of age respectively and seem to be clinically normal. Digestion of the PCR product after amplification of exon 23 in V/2 showed that this individual has inherited the mutation from her father. Direct sequencing confirmed this result. Her sib showed no evidence of the mutation.

The amino acid, leucine, at position 908 in exon 23 of the human β cardiac myosin heavy chain gene[15] shows a high level of conservation across species as shown by evolutionary sequence comparison (table), emphasising the importance of this amino acid.

Additional evidence to support the role of this mutation as the underlying genetic defect in this family was sought by segregation analysis of the affected chromosome (fig 5). We used a highly informative microsatellite sequence polymorphism identified in intron 24 of the MYH7 gene to reconstruct the genotype of individual III/2. Though the locus was not fully informative in all members of the pedigree, we were able to establish that this individual was heterozygous for alleles 3 and 4 of the polymorphism. Analysis of the pedigree indicates that the disease mutation is segregating with allele 4; the subsequent inheritance of the normal maternal chromosome (allele 3) by individuals IV/3 and IV/4 is consistent with the absence of the mutation in these individuals.

Discussion

Hypertrophic cardiomyopathy is the commonest cause of sudden death in apparently healthy young people below the age of 30 years.[4] The disease seems to be present in most, if not all, racial groups with an incidence of approximately 1/5000 of the population.[16] In adults, the annual mortality rate is 2-3%, though children seem to be at greater risk with mortality as high as 6% per year.[17]

The family described in this study illustrates many of the most difficult clinical challenges in the diagnosis and management of HCM.

Sudden death may be the first manifestation of HCM, as in the case seen in this family (III/3). Unconfirmed postmortem data indicated evidence of HCM on gross pathology. This man was doing National Service and as a member of the armed forces should have had medical examination to confirm a general state of good health. It is highly likely that investigation by modern diagnostic techniques would have resulted in an unequivocal diagnosis. However, other individuals have been described who have died suddenly without clinical evidence of the disease and with a normal histological appearance. In these cases, only patchy myocardial fibre disarray and the family history attest to the presence of HCM.[18] Early diagnosis, facilitated by the identification of a pathogenetic mutation, would allow intervention with potentially life-saving advice and treatment.

Many cases with an equivocal HCM phenotype are discovered during screening of first degree relatives of confirmed patients and the course in these patients is not known. Case IV/1 is a typical example. At 40 years of age, despite a grossly abnormal electrocardiogram consistent with HCM, he does not show ventricular hypertrophy. In the absence of a confirmed family history of HCM this would leave the diagnosis in doubt. However, the lack of gross morphological evidence of the disease does not indicate that the patient is free from the risk of sudden death. A genetic analysis dispels doubts and allows more effective delivery of medical care.

Children may not show the classic clinical or echocardiographic features of HCM until their teens or early twenties. Young children are therefore largely symptom free,

Mutant	.....v.....
Cardiac β (human)	EQDNLADAEERCDQLIKNKIQLEAKVKEMNERLEDEEEMNAELTAKKRNVEDECSELKRDIDDLELTLAKVEKEKHATENK
Cardiac β (rat)	.....T.....KL.....
Cardiac α (rat)	.....T.....KL.....K.....
Cardiac β (rabbit)	.....KL.....
Embryonic, fast skeletal muscle (human)	.SE..L.....A.F.....I..VT..A.....I.....KL.....K.....
Skeletal muscle (human)	.....
Embryonic, fast skeletal muscle (rat)	.SE..L.....A.F.....I..VT..A.....I.....KL.....K.....
Skeletal muscle (rabbit)	.A.S.....Q.....T.....I..VT..A.....I.....KL.....K.....
Skeletal muscle (chicken)	.A.S.....T.....I..VT..A.....I.....KL.....K.....
Myosin β (*C elegans*)	KTSLFTNLSTKT..SDAEER.AKLEAQQKDASKQLS.L.DQADNEDRTA.VQRAK.KIEAEV.ALKKQIQDLEMSLRKA
Myosin II, non-muscle (*A castellanii*)	AEEDADKLKDLAALKLKILD.GEKADLE.DNALLQKKV.GEEELQEETSASNDILEQKRK.AEKGELKASLEEEERN

Figure 6 Evolutionary sequence comparison of leucine at position 908 in exon 23 of the human β cardiac myosin heavy chain gene (P12883)[25] (SWISSPROT database). Conservation of this aminoacid across the species was seen in rat cardiac muscle, β (PO2564)[26]; rat cardiac muscle, α (PO2563)[26]; rabbit cardiac muscle, β (PO4461)[27]; human embryonic skeletal muscle (P11055)[28]; human skeletal muscle (P12882)[29]; rat embryonic skeletal muscle (P12847)[30]; rabbit skeletal muscle (P02562)[31]; chick skeletal muscle (P13538)[32]; Caenorhabditis elegans myosin heavy chain β isoform (P19706)[33]; and amoeba (Acanthamoeba castellanii) myosin II heavy chain non-muscle (P05659).[34]

though as a group they are at the highest risk of sudden death. However, evidence of abnormalities of the 12 lead electrocardiogram has been reported in a series of seven patients in whom echocardiographic features of HCM later developed.[19]

No evidence of a clinical, electrocardiographic, or echocardiographic abnormality was detected in individual V/2, apart from a single episode of syncope apparently precipitated by an episode of hyperventilation. Confirming the inheritance of the disease mutation by this individual supports previous data indicating that children with HCM do not necessarily show any cardiac abnormality.[10] Whether the absence of abnormality influences the risk of sudden death remains to be determined.

Despite a single genetic mutation the phenotypic variation in disease expression in the studied family is striking. Ventricular morphology varied from normal to the asymmetric septal hypertrophy that is typical of HCM. The natural history of the disease was also heterogeneous. Individuals II/2 and III/2 lived to the age of 71 and 61 years respectively and died of progressive ventricular dysfunction and heart failure. In contrast, III/3 died suddenly and unexpectedly at the age of 18 years without previous evidence of the disease. Apart from this young man, no other members of the family are known to have been involved in any competitive sport or activities involving strenuous physical exercise. This indicates that other genes or environmental factors may affect the expression and outcome of the disease.

The index case (III/2) showed the classic features of HCM in her youth but developed progressive systolic dysfunction and moderate ventricular dilatation leading to heart failure in the four years before she died. This is an uncommon occurrence in patients with HCM. Kawai and Fujiwara[20] described 11 similar patients and suggested that stenosis of small intramural coronary arteries leading to ischaemia and progressive myocardial fibrosis was the cause of death. Our case showed considerable myocardial fibrosis but no evidence of an abnormality of intramural coronary arteries. The relation between this observation and mutation in myocardial contractile proteins is not clear.

Though one member of the family died suddenly at an early age, the mutation causing HCM in this family seems to be associated with a relatively favourable prognosis. This is further supported by the findings in a recent preliminary report describing a similar mutation in a family in which sudden death was uncommon.[21 22] This contrasts with the high incidence of premature sudden death associated with mutations in other exons of MYH7 which alter the net charge.[14] The mutation reported here does not change the net charge and therefore suggests that such events may give rise to a more benign phenotype that can be expressed as sudden death as the result of environmental factors such as strenuous exercise.

The substitution does, however, reduce hydrophobicity and occurs at the boundary region between the globular head of myosin and the rod region.[23 24] Evolutionary sequence comparison has shown that the leucine at position 908 is highly conserved across species from man to amoeba (table), which supports the evidence for the deleterious effect of the mutation in the molecule.

The striking clinical heterogeneity among patients with HCM is reflected by the considerable regional differences in ventricular hypertrophy and disarray. The mechanism by which a genetic mutation could lead to abnormalities in only some regions of the myocardium remains to be resolved, as does the question of the variable clinical penetrance within families.

Demonstration of the genetic basis for the condition is important for many reasons. Elucidation of the molecular genetics of the disease will aid in understanding the function of the contractile proteins of the heart, and thus the myocardial fibre disarray and hypertrophy in HCM. In turn this may suggest explanations of other processes that lead to ventricular hypertrophy. Knowledge of the underlying molecular disorder could rationalise therapy by identifying patients at high risk through more lethal mutations and by suggesting specific treatments. Ascertaining the contribution of other genes and environmental influences on the expression of mutations of the MYH7 gene will also be important in future therapeutic developments.

We thank Dr Francois Chartier, Mr Jaime Carvajal, Mr Christopher Doudney and Mr David Wilkes for their computation skills and Dr Jacqui Shaw for useful comments during the course of this work. S Al-M is supported by CORDA.

1 Maron BJ, Bonow RO, Cannon RO III, Leon MB, Epstein SE. Hypertrophic cardiomyopathy: Interrelations of clinical manifestations, pathophysiology and therapy. *N Engl J Med* 1987;**316**:780-9, 844-52.
2 Davies MJ. The current status of myocardial disarray in hypertrophic cardiomyopathy. *Br Heart J* 1984;**51**:361-3
3 Maron BJ, Roberts WE, Epstein SE. Sudden death in hypertrophic cardiomyopathy: a profile of 78 patients. *Circulation* 1982:**65**:1388-94.
4 McKenna WJ, Deanfield JE. Hypertrophic cardiomyopathy: an important cause of sudden death. *Arch Dis Child* 1984:**59**:971-5.
5 Clark CE, Henry WL, Epstein SE. Familial prevalence and genetic transmission of idiopathic hypertrophic cardiomyopathy *N Engl J Med* 1973:**289**:709-14.
6 Maron BJ, Nicholas PF III, Pickle LW, Wesley YE, Mulvihill JJ. Patterns of inheritance in hypertrophic cardiomyopathy: Assessment by M-mode and two-dimensional echocardiography. *Am J Cardiol* 1984;**53**: 1087-94.
7 Greaves SC, Roche AH, Neutze JM. Inheritance of hypertrophic cardiomyopathy: a cross sectional and M mode electrocardiographic study of 50 families. *Br Heart J* 1987;**58**:259-66.
8 Jarcho JA, McKenna W, Pare JAP, Solomon SD, Holcombe RF, Dickie S, *et al.* Mapping a gene for familial hypertrophic cardiomyopathy to chromosome 14 ql. *N Engl J Med* 1989;**321**:1372-8.
9 Geisterfer - Lowrance AA, Kass S, Tanigawa G, Vosberg, H-P, McKenna W, Seidman CE and Seidman JG. A molecular basis for familial hypertrophic cardiomyopathy: A beta cardiac myosin heavy chain gene missense mutation. *Cell* 1990;**62**:999-1006.
10 Rosenzweig A, Watkins H, Hwang D-S, Miri M, McKenna W, Traill TA, *et al.* Preclinical diagnosis of familial hypertrophic cardiomyopathy by genetic analysis of blood lymphocytes. *N Engl J Med* 1991:**325**: 1753-60.
11 Solomon SD, Jarcho JA, McKenna W, Geisterfer-Lowrance, A Germain R, Salerni R *et al.* Familial hypertrophic cardiomyopathy is a genetically heterogeneous disease. *J Clin Invest* 1990:**86**:993-9.
12 Lever HM, Karem RF, Currie PJ, Healy BP. Hypertrophic cardiomyopathy in the elderly. *Circulation* 1989;**79**:580-9.

13 Hultman T, Stahl S, Hornes E, Uhlen M. Direct solid phase sequencing of genomic and plasmid DNA using magnetic beads as solid support. *Nucleic Acids Res* 1989; **17**:4937-46.
14 Watkins H, Rosenzweig A, Hwang D-S, Tatjana L, McKenna W, Seidman CE, Seidman JG. Characteristics and prognostic implications of myosin missense mutations in familial hypertrophic cardiomyopathy. *N Engl J Med* 1992;**326**:1108-14.
15 Jaenicke T, Diederich KW, Hass W, Schleich J, Lichter P, Vosberg H-P. The complete sequence of the human beta myosin heavy chain gene and a comparative analysis of its product. *Genomics* 1990;**8**:194-206.
16 Codd MB, Surgue DD, Gersh BJ, Melton LJ. Epidemiology of idiopathic dilated and hypertrophic cardiomyopathy: A population based study in Olmsted county, Minnesota, 1975-1984. *Circulation* 1989:**80**: 564-72.
17 McKenna WJ, Franklin RCG, Nihoyannopoulos P, Robinson KC, Deanfield JE, Dickie S, et al. Arrhythmia and prognosis in infants, children and adolescents with hypertrophic cardiomyopathy. *J Am Coll Cardiol* 1988: **11**:147-53.
18 McKenna WJ, Stewart JT, Nihoyannopoulous P, McGinty F, Davies MJ. Hypertrophic cardiomyopathy without hypertrophy: two families with myocardial disarray in the absence of increased myocardial mass. *Br Heart J* 1990;**63**:287-90.
19 Panza JA, Maron BJ. Relation of electrocardiographic abnormalities to evolving left ventricular hypertrophy in hypertrophic cardiomyopathy during childhood. *Am J Cardiol* 1989;**63**:1258-65.
20 Kawai C, Fujiwara H. Intramyocardial small arteries. In: Zipes DP, Roland DJ, eds. Progress in Cardiology 2/2. Philadelphia: Lea and Febiger, 1989;33-4.
21 Epstein N, Cohn G, Cyran F, Fananapazir L. Heterogeneity in hypertrophic cardiomyopathy demonstrated by beta myosin heavy chain markers. *Circulation* 1991;**84**(Suppl II):419.
22 Epstein N, Cohn G, Cyran F, Zhu W-S, Fananapazir L. Identification of two mutations in the beta myosin heavy chain gene in hypertrophic cardiomyopathy: a 403 mutation associated with a high incidence of sudden death and a 908 mutation in a family with infrequent sudden death [abstr]. *J Am Coll Cardiol* 1992;**19**:271.
23 Harrington WF. On the origin of the contractile force in skeletal muscle. *Proc Natl Acad Sci USA* 1979;**76**: 5066-70.
24 Harrington WF, Rogers ME. Myosin. *Ann Rev Biochem* 1984;**53**:35-73.
25 Liew C-C, Sole MJ, Yamauchi-Takihara K, Kellman B, Andreas DH, Lin L, Liew JC. Complete sequence and organization of the human cardiac beta myosin heavy chain gene. *Nucleic Acids Res* 1990;**18**:3647-51.
26 Kraft R, Bravo-Zehnder M, Taylor D, Leinwand LA. myosin heavy chain. *Nucleic Acids Res* 1989;**17**:7527-8.
27 Kavinsky CJ, Umeda PK, Levin JE, Sinha AM, Nigro JM, Jakovcic S, *et al.* Analysis of cloned mRNA sequences encoding subfragment 2 and part of subfragment 1 of alpha - and beta myosin heavy chains of rabbit heart. *J Biol Chem* 1984;**259**:2775-81.
28 Eller MS, Stedman HH, Sylvester JE, Fertels SH, Rubinstein NA, Kelly AM, *et al.* Nucleotide sequence of full length human embryonic myosin heavy chain cDNA. *Nucleic Acids Res* 1989;**17**:3591-2.
29 Saez L and Leinwand LA. Characterization of diverse forms of myosin heavy chain expressed in adult human skeletal muscle. *Nucleic Acids Res* 1986;**14**:2951-69.
30 Strehler EE, Strehler-Page M-A, Perriard JC, Periasamy M, Nadal-Ginard B. Complete nucleotide and encoded amino acid sequence of a mammalian myosin heavy chain gene: Evidence against intron dependent evolution of the rod. *J Mol Biol* 1986;**190**:291-317.
31 Maeda K, Sczakiel G and Wittinghofer A. Characterization of cDNA coding for the complete light meromyosin portion of a rabbit fast skeletal muscle myosin heavy chain. *Eur J Biochem* 1987;**167**:97-102.
32 Maiata T, Hayashida M, Tamiokal Y, Komine Y, Matsuda G. The primary structure of the myosin head. *Proc Natl Acad Sci USA* 1987;**84**:416-20.
33 Karn J, Brenner S, Barnett L. Protein structural domains in the *Caenorhabditis elegans* unc-54 myosin heavy chain gene are not separated by introns. *Proc Natl Acad Sci USA* 1983;**80**:4253-7.
34 Hammer J A III, Bowers B, Paterson BM, Korn ED. Complete nucleotide sequence and deduced polypeptide sequence of a non muscle myosin heavy chain gene from Acanthamoeba: Evidence of a hinge in the rod like tail. *J Cell Biol* 1987;**105**:913-25.