 Human Molecular Genetics, 2001, Vol. 10, No. 11 **1215–1220**

Mutations in the γ_2 subunit of AMP-activated protein kinase cause familial hypertrophic cardiomyopathy: evidence for the central role of energy compromise in disease pathogenesis

Edward Blair[1,+], Charles Redwood[1,+], Houman Ashrafian[1], Marisa Oliveira[1], John Broxholme[2], Bronwyn Kerr[3], Anthony Salmon[4], Ingegerd Östman-Smith[5] and Hugh Watkins[1,§]

[1]Department of Cardiovascular Medicine and [2]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK, [3]Royal Manchester Children's Hospital, Manchester, UK, [4]Paediatric Cardiology, Southampton General Hospital, Southampton, UK and [5]Paediatric Cardiology, John Radcliffe Hospital, Oxford, UK

Received 2 March 2001; Revised and Accepted 2 April 2001

Familial hypertrophic cardiomyopathy (HCM) has been widely studied as a genetic model of cardiac hypertrophy and sudden cardiac death. HCM has been defined as a disease of the cardiac sarcomere, but mutations in the known contractile protein disease genes are not found in up to one-third of cases. Further, no consistent changes in contractile properties are shared by these mutant proteins, implying that an abnormality of force generation may not be the underlying mechanism of disease. Instead, all of the sarcomeric mutations appear to result in inefficient use of ATP, suggesting that an inability to maintain normal ATP levels may be the central abnormality. To test this hypothesis we have examined candidate genes involved in energy homeostasis in the heart. We now describe mutations in *PRKAG2*, encoding the γ_2 subunit of AMP-activated protein kinase (AMPK), in two families with severe HCM and aberrant conduction from atria to ventricles in some affected individuals (pre-excitation or Wolff–Parkinson–White syndrome). The mutations, one missense and one in-frame single codon insertion, occur in highly conserved regions. Because AMPK provides a central sensing mechanism that protects cells from exhaustion of ATP supplies, we propose that these data substantiate energy compromise as a unifying pathogenic mechanism in all forms of HCM. This conclusion should radically redirect thinking about this disorder and also, by establishing energy depletion as a cause of myocardial dysfunction, should be relevant to the acquired forms of heart muscle disease that HCM models.

INTRODUCTION

Familial hypertrophic cardiomyopathy (HCM) is a relatively common autosomal dominant disorder characterized by myocardial hypertrophy and a high incidence of sudden death in some affected families (1,2). HCM has come to be considered as a 'disease of the sarcomere', as multiple different mutant alleles have been described in nine genes encoding cardiac contractile proteins (3–5). Biochemical and biophysical analyses have, however, shown that there is no unifying abnormality of cardiac contractility resulting from these different mutations (for a review, see ref. 5); some mutant proteins appear to depress contractility (e.g. missense mutations in β-myosin heavy chain) (6) whereas others enhance calcium sensitivity and contractility (e.g. mutant α-tropomyosin or cardiac troponin I) (7,8). This suggests that the disease phenotype is not simply a direct consequence of altered contractility *per se* and that a more fundamental abnormality of myocardial function must be sought. One feature that the different classes of HCM-causing mutations do share is an inefficiency of ATP utilization (5,9,10). We predict that ATP wastage leading to a relative depletion of ATP in the cardiac myocyte may, in circumstances of increased demand, lead to a failure of energy-dependent homeostatic mechanisms. In particular, interference with calcium re-uptake in the myocyte would trigger calcium-dependent hypertrophic signalling as well as a tendency to lethal arrhythmias. Support for this hypothesis comes from the observation that a number of phenocopies of HCM have been defined at the molecular level recently and shown to be syndromes associated with abnormalities of ATP generation in the myocardium (e.g. mitochondrial mutations, Friedreich's ataxia and VLCAD deficiency) (11–13).

Pathogenic mutations in the known HCM disease genes are only found in ~60–70% of probands with familial HCM (unpublished data), suggesting that other disease genes remain to be identified. However, most of the remaining candidate sarcomeric protein genes have been screened directly and have yielded either no mutations in HCM (e.g. in troponin C) (14

[+]These authors contributed equally to this work

[§]To whom correspondence should be addressed at: Department of Cardiovascular Medicine, University of Oxford, Level 5, John Radcliffe Hospital, Oxford OX3 9DU, UK. Tel: +44 1865 220257; Fax: +44 1865 768844; Email: hugh.watkins@cardiov.ox.ac.uk

and unpublished data) or only rare mutations (e.g. in titin and actin) (15,16), suggesting that mutations in contractile protein genes may not account for the shortfall. If the hypothesized role of inefficient ATP usage in 'sarcomeric' HCM is correct, genes encoding proteins involved in energy homeostasis in the myocyte could also be considered as strong candidates in this disorder. A particularly attractive candidate in this context is the AMP-activated protein kinase (AMPK) (17). AMPK, which has both protein kinase and transcriptional regulatory roles (18), is a heterotrimeric protein composed of a catalytic α subunit and two regulatory subunits (β and γ). It shows homology to the SNF1 transcription factor complex which has a central role in the regulation of glucose metabolism in yeast (19). When activated it functions to protect the cell from critical depletion of ATP by activating glycolysis and fatty acid uptake during hypoxic stress or extreme metabolic demand (17,20). The γ_2 protein is the dominant isoform of the regulatory γ subunit of AMPK in the heart. This is encoded by the gene *PRKAG2*, which has recently been mapped to human chromosome 7q36 (21). This chromosomal region includes a mapped locus for HCM associated with electrophysiological pre-excitation or Wolff–Parkinson–White (WPW) syndrome (22), a feature of HCM perhaps not likely to be explained by defects in structural contractile proteins. We now report *PRKAG2* mutations in two families with HCM associated with pre-excitation in some affected individuals.

RESULTS

Clinical findings

To test the hypothesis that mutations affecting the AMPK γ_2 subunit cause HCM we studied 23 families with HCM in which contractile protein mutations had not been identified (having screened the genes encoding β-myosin heavy chain, the regulatory myosin light chain, cardiac troponin T, myosin binding protein C and cardiac actin); amongst these families, one (WA) also showed features of pre-excitation. We subsequently selected family HA for study as members also manifested HCM with pre-excitation. Family WA (Fig. 1A and Table 1) shows autosomal transmission through three generations with five individuals affected by HCM, three of whom have died of the condition. Of note, individuals I:1 and II:2 died prematurely with early cardiac dilatation and III:2 has been referred for cardiac transplantation. Individual II:1 has a short PR interval on electrocardiogram (ECG) suggestive of pre-excitation, and individual III:1, who died suddenly at age 38 during the course of this study, had previously undergone an ablation of an accessory pathway for symptomatic WPW syndrome. In family HA (Fig. 1B and Table 1) the proband, I:1, presented early in life with HCM, which progressed to a dilated phase requiring cardiac transplantation at age 19. One affected child (II:1, 8 years) has HCM with pre-excitation on the resting ECG; the other (II:2, 4 years) was diagnosed clinically at birth after presenting with a murmur and pre-excitation and has since had symptomatic WPW syndrome.

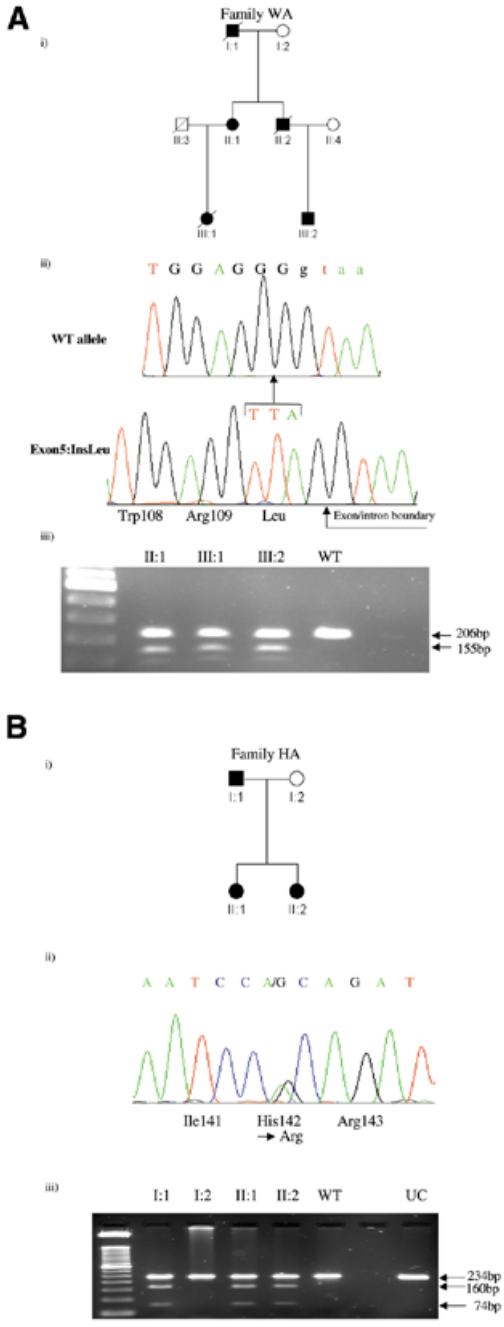

Figure 1. (**A**) (i) Pedigree of family WA, showing autosomal dominant transmission of HCM. Individuals I:1, II:2 and III:1 suffered premature sudden death. Individual III:2 is awaiting cardiac transplantation. (ii) Sequence of cloned wild-type (WT) and mutant alleles. Insertion of the three bases TTA in the mutant introduces a leucine residue into the protein product and a *Bsa*XI restriction site in the PCR amplimer. (iii) *Bsa*XI restriction digest of PCR products from genomic DNA templates in members of family WA and WT control. Note segregation of mutation with disease in this family. (**B**) (i) Pedigree of family HA. (ii) Sequence data from *PRKAG2* exon 7 showing an A→G transition. This results in a His142Arg amino acid substitution in the protein product and the introduction of an *Aci*I site in the PCR product from the mutant allele. (iii) *Aci*I digestion of exon 7 PCR products from family HA demonstrates cosegregation of the His142Arg mutation with disease.

Exon-intron sequences of *PRKAG2*

The published cDNA sequence and genomic structure (21) (GenBank accession no. AF087875) were compared with the genomic sequence obtained from two bacterial artificial chromosome clones in public domain DNA databases (accession

Table 1. Clinical features in families WA and HA

	Age/age at death/age at transplantation (years)	Symptoms	Conduction and ECG abnormalities	Max LV wall thickness (mm)	LV dilation
Family WA					
I:1	Sudden death at 51	Chronic cardiac failure	–	Massive hypertrophy, heart weight 980 g	Yes
II:1	70	Palpitations and syncope since childhood	Short PR interval, broad QRS, T wave inversion	32.5	Yes
II:2	Sudden death at 32	Chronic cardiac failure	–	–	–
III:1	Sudden death at 38	Symptomatic WPW, chest pain	WPW treated by ablation, broad QRS, AF	19	No
III:2	42	Chronic cardiac failure, awaiting transplant	AF, LBBB, pacemaker for AV block	21	Yes
Family HA					
I:1	Age 33, transplant at 19	Chronic cardiac failure	–	–	Yes
II:1	8	No	Pre-excitation, very large and bizarre QRS	9.7 (>2 SDs above normal for age)	No
II:2	Diagnosed at birth, now 4	Symptomatic WPW	Pre-excitation, very large and bizarre QRS	9.0 (>2 SDs above normal for age)	No

AF, atrial fibrillation; WPW, Wolff-Parkinson-White syndrome; LBBB, Left bundle branch block; AV block, atrio-ventricular block.

nos AC006358 and AC006966) to obtain additional intronic sequences. Intronic oligonucleotide primers were designed for the amplification of each of the 12 exons and flanking splice-sites of *PRKAG2*.

Identification of *PRKAG2* mutations

Individual *PRKAG2* exons amplified from genomic DNA of unrelated probands were screened for variants by heteroduplex analysis using a denaturing HPLC (DHPLC) apparatus. An abnormality on the DHPLC trace indicative of heteroduplex formation was identified in exon 5 from the proband of family WA. Direct sequencing of the PCR product, and later of the subcloned mutant allele, revealed the insertion of a TTA codon after the codon for arginine 109 (Fig. 1A). This mutation predicts the insertion of an additional leucine residue without disruption of the reading frame or of the splicing of exon 5 to exon 6 (the junction of which generates the normal codon for glutamate at residue 110); normal splicing was confirmed by RT–PCR. This mutation, which we denote Exon5:InsLeu, introduces a *Bsa*XI restriction enzyme site. This restriction fragment length polymorphism was used to confirm the identity of the mutation, its cosegregation with disease in the affected members of the family and its absence from over 240 normal control chromosomes (Fig. 1A).

A heteroduplex abnormality was also identified in exon 7 from the proband of family HA. Direct sequencing of this PCR product revealed an A→G transition predicting a His142Arg missense mutation (Fig. 1B). This variant introduces an *Aci*I restriction enzyme site, which was again used to confirm the identity of the mutation, its presence in each of the three affected individuals and its absence from over 240 normal chromosomes.

Additional support for the disease-causing role of these variants is provided by an analysis of evolutionary sequence conservation (Fig. 2). The leucine insertion is situated in a

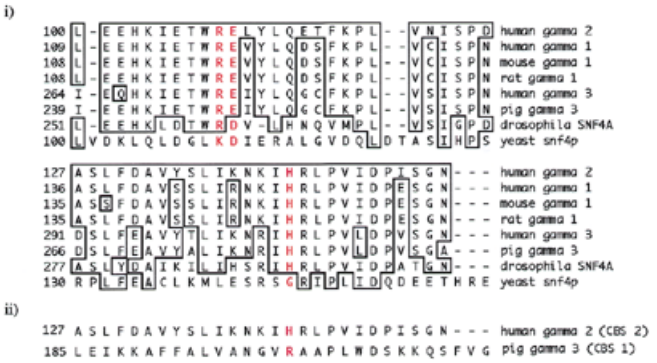

Figure 2. (i) Protein sequence alignment of human AMPK γ_2 subunit with other AMPK isoforms and cross-species homologues. Residues identical to the human γ_2 sequence are boxed. Conservation of the amino acid residues (Arg109 and Glu110) flanking the leucine insertion in family WA, and the His142 mutated in family HA, is found in all mammalian species and isoforms examined. The amino acid characteristic (basic or acidic) at each of these three positions (highlighted in red) is conserved between mammalian sequences and those of invertebrates and yeast. (ii) Pig AMPK γ_3 subunit CBS1 domain sequence aligned with respect to the human γ_2 CBS2 sequence (as above) according to Bateman (26). The residue mutated in porcine skeletal myopathy has been highlighted in red. Note that this residue is in an equivalent position to His142 within the human γ_2 CBS2 domain structure, three amino acids N-terminal of an invariant proline.

highly conserved region of the protein, between an invariant basic residue (arginine or lysine in all homologues including the yeast Snf4p protein) and an invariant acidic residue (glutamate in all γ AMPK isoforms; aspartate in *Drosophila* and yeast Snf4 proteins). Similarly, His142 is conserved as far back as *Drosophila* Snf4 and the disease-causing mutation replaces it with arginine, which has a more basic side chain. Strikingly, the His142Arg missense mutation affects a residue

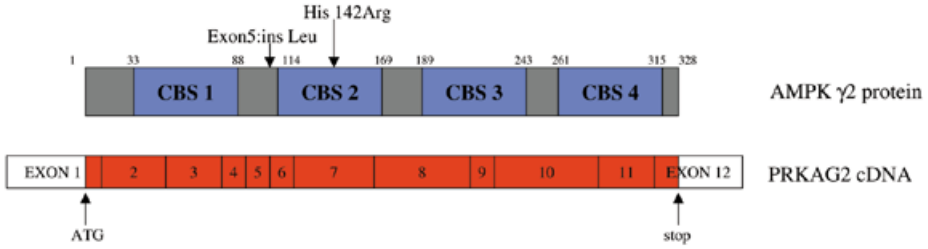

Figure 3. Schematic of AMPK γ_2 protein showing the four CBS domains and the relative positions of the mutations in families WA and HA. The exonic contributions to each CBS domain are illustrated with respect to the cDNA sequence shown below.

in the second cystathionine-β-synthase (CBS) domain, which is in the same position as Arg200 in the first CBS motif of the AMPK γ_3, mutation of which has recently been shown to cause a skeletal myopathy with glycogen storage abnormalities in the pig (23) (Fig. 2). The position of these mutations in the AMPK γ_2 primary amino acid sequence is illustrated in Figure 3.

DISCUSSION

We believe that these data are sufficient to confirm the causal role of the mutations in the *PRKAG2* gene in the two families. Each mutation results in a significant change within a conserved region of the protein sequence, cosegregates with disease with complete penetrance, and is absent from the control population. Further, the residue affected by the missense mutation occupies a position of demonstrated importance in the CBS domain structure of other proteins. Finally, while implication of a gene with a primary role in energy homeostasis as a cause of HCM is entirely novel, it does in fact fit with prior observations regarding the pathophysiology of this condition.

The cardiac phenotype is extremely similar in the two families and is notable in a number of ways. Firstly, the cardiomyopathy is severe, with early onset and poor prognosis, with symptomatic presentation in childhood in family HA and multiple sudden deaths in early adult life in family WA. Secondly, massive hypertrophy is present in some individuals (WA I:1 and WA II:1) and markedly increased wall thickness persists despite cavity dilation in others. Thirdly, there is an unusually marked propensity towards early dilatation of the ventricle; although this complication usually occurs in only a minority of individuals with HCM, the majority of adults in these families either died of heart failure or required cardiac transplantation at an early age. Fourthly, as was described in the family previously mapped to this locus on chromosome 7, pre-excitation indicated by short PR interval on the ECG and, in some instances, by symptomatic supra-ventricular tachycardias, is present in some individuals in both families (Fig. 4). Formal electrophysiological studies confirmed an accessory pathway in WA III:1, and the association of pre-excitation with conduction disease in individual WA III:2 is also highly characteristic of the WPW and HCM phenotype (24). Our data to date suggest that *PRKAG2* mutations may be expected where HCM and WPW co-exist but may not be a frequent cause of HCM where no features of pre-excitation are found in affected individuals.

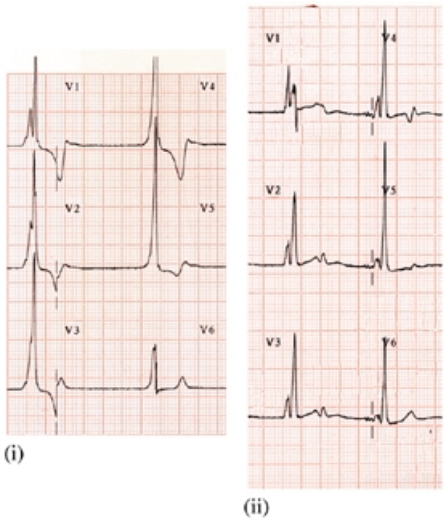

Figure 4. Sample ECGs from (i) HA II:1, recorded at 5 mm/mV (i.e. half normal scale) and (ii) HA II:2, recorded at 10 mm/mV. The very short PR interval in HA II:2 is indicative of ventricular pre-excitation, with P waves merging with a delta wave and aberrant QRS as the ventricle is depolarized through an accessory pathway; in HA II:1 the rhythm is nodal and P waves are not clearly seen. In both, the QRS voltages are dramatically enlarged, especially in II:1, indicating the presence of left ventricular hypertrophy. Re-polarization abnormalities are also prominent.

The dramatic phenotypic impact of these mutations confirms the importance of the AMP-activated kinase as the 'cellular fuel gauge' (17). The precise impact of these mutations on the structure and function of the AMPK γ_2 subunit, and the subsequent effect on the heterotrimer, will need further evaluation. Presumably the autosomal dominant phenotype reflects a dominant negative disruption of AMPK activity whereby mutant γ subunits are incorporated but then alter the activity and/or regulation of AMPK in the myocardium. Comparable dominant negative actions have been described for an engineered α subunit mutant (25). Structurally, the γ subunits of AMPK consist primarily of four consecutive CBS domains (20). The Exon5:InsLeu mutation inserts an amino acid in the link between CBS1 and CBS2, and His142Arg alters a residue within CBS2 (Fig. 3). The latter mutation alters the same position within the CBS consensus motif (26) as the Arg200Gln missense mutation in the pig AMPK γ_3 CBS1 domain, which causes skeletal myopathy (23), and the Asp444Asn mutation in human CBS, which leads to homocystinuria (27). We conclude that this position has particular

importance within the CBS motif and note that it lies towards the end of the β2 sheet in the CBS domain of the inosine monophosphate dehydrogenase structure (28).

AMPK activity is suppressed in the absence of AMP due to an autoinhibitory region on the α subunit blocking the catalytic site. As ATP is consumed the level of AMP is increased by the conversion of 2ADP↔ATP + AMP by adenylate kinase, such that a climbing AMP/ATP ratio is an extremely sensitive signal of energy depletion in times of stress (17). AMP activates the enzyme by competing with the kinase domain for binding to the autoinhibitory region and stimulates further activation by an upstream kinase (AMPKK). In the model of Cheung *et al.* (29), AMP is bound via interactions with both the autoinhibitory region and the γ subunit. Thus, mutations in γ may act to weaken AMP binding and hence reduce activation of AMPK. Although this remains unproven, the disease-causing Arg200Gln mutation in the pig γ_3 subunit has been found to give decreased AMPK activity in skeletal muscle (23). Thus the mutations in families WA and HA may lead to a decreased AMP-activated level of kinase activity and hence a reduced responsiveness to ATP depletion.

In addition, mammalian AMPK subunits have a close homology with transcription factors involved in repression/derepression of genes encoding glucose-metabolizing enzymes in yeast (19) and localize to the cell nucleus as well as the cytoplasm (18). The γ subunit homologue in yeast is the Snf4p component of the SNF1 transcription factor complex. It is therefore likely that AMPK also has transcriptional roles in man that may similarly be perturbed by these mutations in the γ_2 subunit.

Perhaps the most important implication of these findings is that they offer substantial support to the hypothesis that inability to maintain adequate levels of ATP is the unifying abnormality in familial HCM and, indeed, related phenotypes. Just as ATP wastage through inefficient chemo-mechanical transduction in the sarcomere or ATP deficiency due to β-oxidation or mitochondrial electron transport chain defects can leave the cardiomyocyte exposed to critical energy insufficiency, so, presumably, can failure of the AMPK system to initiate protective metabolic compensation during periods of excess demand. Once activated, AMPK inhibits enzymes in biosynthetic and ATP-consuming pathways (e.g. creatine kinase and HMGco-A reductase) and activates rate-limiting enzymes in glycolytic and fatty acid metabolism pathways to promote ATP production. We postulate that in this and other forms of HCM, periods of increased cardiac demand render the myocyte unable to maintain highly energy-dependent homeostatic pathways, particularly the SERCA2 calcium re-uptake pump (10), resulting in increased intracellular calcium leading to hypertrophy and vulnerability to arrhythmia. The marked progression to dilation (which is the end result of myocyte death) suggests that the energetic defect may be more severe with AMPK γ_2 mutations than other forms of HCM, such that cardiomyocytes are exposed to sufficiently disordered metabolism and calcium homeostasis as to initiate apoptosis, resulting ultimately in loss of contractile function and heart failure. The particular phenotype of pre-excitation suggests the presence of aberrant conduction pathways bypassing the atrioventricular node. This may perhaps be a consequence of disruption of AMPK's role as a transcription factor regulating an as yet unknown gene. Alternatively, it is possible that deficiencies in energy homeostasis *per se* can cause pre-excitation, as the WPW syndrome has been described in a number of instances of mitochondrial mutation (30–32).

In conclusion, we believe that the identification of AMPK γ_2 mutations in HCM strongly supports the proposal that the unifying pathophysiology in this condition is energy compromise. Demonstration of the central role of abnormalities of ATP homeostasis suggests novel avenues for potential therapy, which would be equally applicable in all forms of HCM. For example, the requirement to protect the heart from excess energy demand lends support to the proposed protective effect of high-dose beta blockade in HCM (33). Equally, it may be possible to enhance energy production or to directly manipulate components of the AMPK system. In this regard, we believe that understanding the pathogenesis in those families in which HCM results from AMPK γ_2 mutations may be of wide relevance to all forms of the disease. Potentially of even greater importance is that progressive abnormalities in cardiac energetics are found in acquired forms of dilated cardiomyopathy and heart failure and have been shown to predict prognosis (34). Thus a more complete understanding of the protective mechanisms in the normal heart is an important goal and one which should be facilitated by further study of AMPK mutant alleles and their phenotypes.

MATERIALS AND METHODS

Family members were ascertained through our clinical practice or the practice of referring physicians, and evaluated by physical examination, ECG and echocardiography, allowing the diagnosis of FHC to be made in those clinically affected. Blood or mouth wash samples were collected in each affected individual and other available members of the families and processed for genomic DNA preparation using standard protocols.

Mutation analysis

Oligonucleotides were designed from flanking intronic sequence for all exons of the *PRKAG2* gene. Amplifications were performed with 'touchdown' PCR using high fidelity polymerases from 50 ng of genomic DNA using standard conditions. Annealing temperatures were optimized for each exon and touched down from 7.5°C above the final annealing temperature in 0.5°C decrements. Products of each PCR reaction were checked on 1.5% agarose gels. Mutation analysis was undertaken using temperature-modulated heteroduplex analysis (TMHA) on an automated HPLC instrument equipped with a DNASep column (Transgenomic). Mobile phase gradients and melting temperatures for TMHA of each amplimer were calculated using the Wavemaker software package. Crude PCR products were denatured at 95°C and gradually re-annealed before application to the TMHA apparatus. Exons with an abnormal TMHA profile were sequenced using an ABI377 (Applied Biosystems), following product purification by QIAquick PCR purification (Qiagen) or, in the case of family WA, subcloned prior to sequencing, and compared with published genomic sequence of the *PRKAG2* gene (GenBank accession no. AF087875). Mutations were confirmed by restriction enzyme digestion of PCR-amplified exons prior to separation on an agarose gel.

ACKNOWLEDGEMENTS

We thank the families for their participation in this study, Dr Graham Goode for providing clinical information, the Clinical Genetics Laboratory at the Churchill Hospital, Oxford and Katherine Lygate and Philip Townsend for help in manuscript preparation. This work was supported by the British Heart Foundation (programme grant RG/97008 and project grant PG/99196) and the Wellcome Trust.

REFERENCES

1. Maron, B.J., Gardin, J.M., Flack, J.M., Gidding, S.S., Kurosaki, T.T. and Bild, D.E. (1995) Prevalence of hypertrophic cardiomyopathy in a general population of young adults. Echocardiographic analysis of 4111 subjects in the CARDIA Study. Coronary Artery Risk Development in (Young) Adults. *Circulation*, **92**, 785–789.
2. Spirito, P., Seidman, C.E., McKenna, W.J. and Maron, B.J. (1997) The management of hypertrophic cardiomyopathy. *N. Engl. J. Med.*, **336**, 775–785.
3. Thierfelder, L., Watkins, H., MacRae, C., Lamas, R., McKenna, W., Vosberg, H.P., Seidman, J.G. and Seidman, C.E. (1994) α-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: a disease of the sarcomere. *Cell*, **77**, 701–712.
4. Bonne, G., Carrier, L., Richard, P., Hainque, B. and Schwartz, K. (1998) Familial hypertrophic cardiomyopathy: from mutations to functional defects. *Circ. Res.*, **83**, 580–593.
5. Redwood, C.S., Moolman-Smook, J.C. and Watkins, H. (1999) Properties of mutant contractile proteins that cause hypertrophic cardiomyopathy. *Cardiovasc. Res.*, **44**, 20–36.
6. Lankford, E.B., Epstein, N.D., Fananapazir, L. and Sweeney, H.L. (1995) Abnormal contractile properties of muscle fibers expressing β-myosin heavy chain gene mutations in patients with hypertrophic cardiomyopathy. *J. Clin. Invest.*, **95**, 1409–1414.
7. Bottinelli, R., Coviello, D.A., Redwood, C.S., Pellegrino, M.A., Maron, B.J., Spirito, P., Watkins, H. and Reggiani, C. (1998) A mutant tropomyosin that causes hypertrophic cardiomyopathy is expressed *in vivo* and associated with an increased calcium sensitivity. *Circ. Res.*, **82**, 106–115.
8. Elliott, K., Watkins, H. and Redwood, C.S. (2000) Altered regulatory properties of human cardiac troponin I mutants that cause hypertrophic cardiomyopathy. *J. Biol. Chem.*, **275**, 22069–22074.
9. Sweeney, H.L., Feng, H.S., Yang, Z. and Watkins, H. (1998) Functional analyses of troponin T mutations that cause hypertrophic cardiomyopathy: insights into disease pathogenesis and troponin function. *Proc. Natl Acad. Sci. USA*, **95**, 14406–14410.
10. Spindler, M., Saupe, K.W., Christe, M.E., Sweeney, H.L., Seidman, C.E., Seidman, J.G. and Ingwall, J.S. (1998) Diastolic dysfunction and altered energetics in the αMHC403/+ mouse model of familial hypertrophic cardiomyopathy. *J. Clin. Invest.*, **101**, 1775–1783.
11. Puccio, H. and Koenig, M. (2000) Recent advances in the molecular pathogenesis of Friedreich ataxia. *Hum. Mol. Genet.*, **9**, 887–892.
12. Merante, F., Tein, I., Benson, L. and Robinson, B.H. (1994) Maternally inherited hypertrophic cardiomyopathy due to a novel T-to-C transition at nucleotide 9997 in the mitochondrial tRNA (glycine) gene. *Am. J. Hum. Genet.*, **55**, 437–446.
13. Bonnet, D., Martin, D., De Lonlay, P., Villain, E., Jouvet, P., Rabier, D., Brivet, M. and Saudubray, J.M. (1999) Arrhythmias and conduction defects as presenting symptoms of fatty acid oxidation disorders in children. *Circulation*, **100**, 2248–2253.
14. Kimura, A., Harada, H., Park, J.E., Nishi, H., Satoh, M., Takahashi, M., Hiroi, S., Sasaoka, T., Ohbuchi, N., Nakamura, T. *et al.* (1997) Mutations in the cardiac troponin I gene associated with hypertrophic cardiomyopathy. *Nat. Genet.*, **16**, 379–382.
15. Satoh, M., Takahashi, M., Sakamoto, T., Hiroe, M., Marumo, F. and Kimura, A. (1999) Structural analysis of the titin gene in hypertrophic cardiomyopathy: identification of a novel disease gene. *Biochem. Biophys. Res. Commun.*, **262**, 411–417.
16. Olson, T.M., Doan, T.P., Kishimoto, N.Y., Whitby, F.G., Ackerman, M.J. and Fananapazir, L. (2000) Inherited and *de novo* mutations in the cardiac actin gene cause hypertrophic cardiomyopathy. *J. Mol. Cell. Cardiol.*, **32**, 1687–1694.
17. Hardie, D.G. and Carling, D. (1997) The AMP-activated protein kinase—fuel gauge of the mammalian cell? *Eur. J. Biochem.*, **246**, 259–273.
18. Salt, I., Celler, J.W., Hawley, S.A., Prescott, A., Woods, A., Carling, D. and Hardie, D.G. (1998) AMP-activated protein kinase: greater AMP dependence, and preferential nuclear localization, of complexes containing the α2 isoform. *Biochem. J.*, **334**, 177–187.
19. Wilson, W.A., Hawley, S.A. and Hardie, D.G. (1996) Glucose repression/derepression in budding yeast: SNF1 protein kinase is activated by phosphorylation under derepressing conditions, and this correlates with a high AMP:ATP ratio. *Curr. Biol.*, **6**, 1426–1434.
20. Kemp, B.E., Mitchelhill, K.I., Stapleton, D., Michell, B.J., Chen, Z.P. and Witters, L.A. (1999) Dealing with energy demand: the AMP-activated protein kinase. *Trends Biochem. Sci.*, **24**, 22–25.
21. Lang, T., Yu, L., Tu, Q., Jiang, J., Chen, Z., Xin, Y., Liu, G. and Zhao, S. (2000) Molecular cloning, genomic organization, and mapping of *PRKA*G2, a heart abundant γ_2 subunit of 5′-AMP-activated protein kinase, to human chromosome 7q36. *Genomics*, **70**, 258–263.
22. MacRae, C.A., Ghaisas, N., Kass, S., Donnelly, S., Basson, C.T., Watkins, H.C., Anan, R., Thierfelder, L.H., McGarry, K., Rowland, E. *et al.* (1995) Familial hypertrophic cardiomyopathy with Wolff-Parkinson-White syndrome maps to a locus on chromosome 7q3. *J. Clin. Invest.*, **96**, 1216–1220.
23. Milan, D., Jeon, J.T., Looft, C., Amarger, V., Robic, A., Thelander, M., Rogel-Gaillard, C., Paul, S., Iannuccelli, N., Rask, L. *et al.* (2000) A mutation in *PRKAG3* associated with excess glycogen content in pig skeletal muscle. *Science*, **288**, 1248–1251.
24. Khair, G.Z., Soni, J.S. and Bamrah, V.S. (1985) Syncope in hypertrophic cardiomyopathy. II. Coexistence of atrioventricular block and Wolff-Parkinson-White syndrome. *Am. Heart J.*, **110**, 1083–1086.
25. Woods, A., Azzout-Marniche, D., Foretz, M., Stein, S.C., Lemarchand, P., Ferre, P., Foufelle, F. and Carling, D. (2000) Characterization of the role of AMP-activated protein kinase in the regulation of glucose-activated gene expression using constitutively active and dominant negative forms of the kinase. *Mol. Cell. Biol.*, **20**, 6704–6711.
26. Bateman, A. (1997) The structure of a domain common to archaebacteria and the homocystinuria disease protein. *Trends Biochem. Sci.*, **22**, 12–13.
27. Kluijtmans, L.A., Boers, G.H., Stevens, E.M., Renier, W.O., Kraus, J.P., Trijbels, F.J., van den Heuvel, L.P. and Blom, H.J. (1996) Defective cystathionine β-synthase regulation by S-adenosylmethionine in a partially pyridoxine responsive homocystinuria patient. *J. Clin. Invest.*, **98**, 285–289.
28. Colby, T.D., Vanderveen, K., Strickler, M.D., Markham, G.D. and Goldstein, B.M. (1999) Crystal structure of human type II inosine monophosphate dehydrogenase: implications for ligand binding and drug design. *Proc. Natl Acad. Sci. USA*, **96**, 3531–3536.
29. Cheung, P.C., Salt, I.P., Davies, S.P., Hardie, D.G. and Carling, D. (2000) Characterization of AMP-activated protein kinase γ-subunit isoforms and their role in AMP binding. *Biochem. J.*, **346**, 659–669.
30. Aggarwal, P., Gill-Randall, R., Wheatley, T., Buchalter, M.B., Metcalfe, J. and Alcolado, J.C. (2001) Identification of mtDNA mutation in a pedigree with gestational diabetes, deafness, Wolff-Parkinson-White syndrome and placenta accreta. *Hum. Hered.*, **51**, 114–116.
31. Yamagata, K., Tomida, C., Umeyama, K., Urakami, K., Ishizu, T., Hirayama, K., Gotoh, M., Iitsuka, T., Takemura, K., Kikuchi, H. *et al.* (2000) Prevalence of Japanese dialysis patients with an A-to-G mutation at nucleotide 3243 of the mitochondrial tRNA(Leu(UUR)) gene. *Nephrol. Dial. Transplant.*, **15**, 385–388.
32. Mourmans, J., Wendel, U., Bentlage, H.A., Trijbels, J.M., Smeitink, J.A., de Coo, I.F., Gabreels, F.J., Sengers, R.C. and Ruitenbeek, W. (1997) Clinical heterogeneity in respiratory chain complex III deficiency in childhood. *J. Neurol. Sci.*, **149**, 111–117.
33. Ostman-Smith, I., Wettrell, G. and Riesenfeld, T. (1999) A cohort study of childhood hypertrophic cardiomyopathy: improved survival following high-dose β-adrenoceptor antagonist treatment. *J. Am. Coll. Cardiol.*, **34**, 1813–1822.
34. Neubauer, S., Horn, M., Cramer, M., Harre, K., Newell, J.B., Peters, W., Pabst, T., Ertl, G., Hahn, D., Ingwall, J.S. *et al.* (1997) Myocardial phosphocreatine-to-ATP ratio is a predictor of mortality in patients with dilated cardiomyopathy. *Circulation*, **96**, 2190–2196.