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A new missense mutation, Arg719Gln, in the β-cardiac heavy chain myosin gene of patients with familial hypertrophic cardiomyopathy

Michael W.Consevage, Grant C.Salada, Barry G.Baylen, Roger L.Ladda and Peter K.Rogan

Department of Pediatrics, Milton S.Hershey Medical Center, Pennsylvania State University, Hershey, PA 17036, USA

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Familial hypertrophic cardiomyopathy (FHCM) is an autosomal dominant disorder of unexplained myocardial hypertrophy and microscopic myocellular disarray associated with a spectrum of ventricular dysfunction and variable clinical outcome including sudden death (1). The clinical diagnosis of this condition may be difficult since many affected individuals are leading a healthy, normal life. Current diagnosis relies upon echocardiographic measurements of maximal left ventricular diastolic wall diameter (LVDWD). Due to variable expressivity and age-dependent onset, many affected individuals do not present with symptoms of FHCM until early adulthood, while others manifest subtle echocardiographic findings. Although the etiology of FHCM is genetically heterogeneous, genetic testing, when informative, can be more sensitive and detect FHCM earlier than conventional echocardiography.

Mutations in the β-cardiac heavy chain myosin gene (MYH7, chromosome 14q11-q12), and three other, as yet undetermined loci assigned to chromosomes 1q31, 11q11, and 15q2 appear to cause FHCM (2–6). Up to half of all cases of FHCM may result from mutations at MYH7 (2). Most of the defects in MYH7 are point mutations in the head domain that arose independently, however, rearrangements involving carboxy-terminal coding regions of the gene also occur (6). Sixteen different missense mutations have been described, all of which are predicted to alter evolutionary conserved amino acid residues (8,9). Identification of new mutations is of clinical import, since the type and location of these mutations may be prognostic for penetrance, age of onset, and the characteristic cardiac events that are likely to occur in undiagnosed, affected family members (2,7,8,9).

This report describes a new mutation in exon 19 of the MYH7 gene in a family of Hispanic origin. Family members were initially evaluated by physical examination, by 12 lead electrocardiogram and by 2D-echocardiography in the standard views (10). Only one of the five preadolescents (III-2, Fig. 1) displayed echocardiographic characteristics of FHCM. The remaining 4 children (III-1,3,4, Fig. 1) were assigned an unknown affection status. Maximal LVDWD was 16 mm in the 5 y. old affected child and ranged between 20–27 mm in the three affected adults. Maximal LVDWD in the unaffected adults ranged between 8–11 mm and was within normal limits for age in the 4 children without symptoms of FHCM. The four affected individuals have no history of syncope or presyncope, demonstrate a normal EKG, exercise stress test, and ambulatory Holter monitors. Three of the individuals (second and third generation) were diagnosed with FHCM by 3 y. of age, which is consistent with an early age of onset of FHCM in this pedigree. An extended family history of 64 family members at risk (ages ranging from 4 to 80 years) is negative for sudden death.

Multipoint likelihood analysis of genetic loci adjacent to and within MYH7 (TCRD, D14S50, MYH7, and D14S54; references 11–13) was inconclusive because of the limited number of family members available ($\hat{\theta} = 0.0$; $Z_{max} = 1.2$). Southern analysis with probe pSC14 (kindly provided by H.P.Vosberg) demonstrated no gross rearrangements in the 3′ terminal domain of MYH7 in this family (results not shown). Exons 3 through 25 and the corresponding splice-junctions of MYH7 were then screened with the polymerase chain reaction (PCR; 14) and single stranded conformation polymorphism analysis on MDE gels (AT Biochem) to identify sequence variants that were present only in affected individuals. A unique set of conformers derived from exon 19 and introns 18 and 19 cosegregated with FHCM in this family; none were noted in the four asymptomatic preadolescents or in 42 unrelated, normal control individuals (results not shown).

This variant was identified by cloning and sequencing of this exon from four affected and two unaffected family members. PCR products from two affected and two unaffected individuals were cloned into pCR™II (Invitrogen) and the sequences of both strands were determined from 12 different subclones. A G→A transition on the coding strand at nucleotide position 13463 of MYH7 (GenBank locus HUMBMYH7) introduces a missense

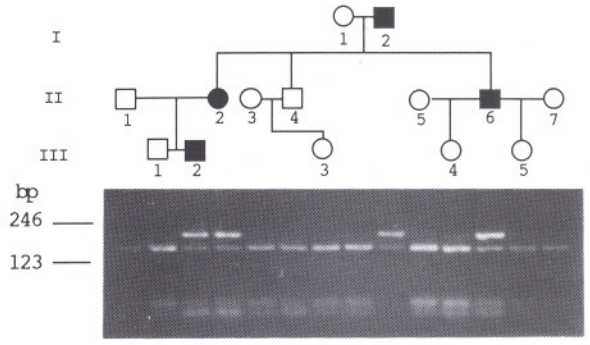

Figure 1. Loss of a diagnostic *Msp*I restriction site at position 13461 in exon 19 of the MYH7 gene in affected family members. Genomic DNA (isolated from lymphocytes) was amplified with the polymerase chain reaction using oligonucleotide primers spanning exon 19 as well as the adjacent acceptor and donor splice junctions (positions 13306–13512; GenBank locus HUMBMYH7). The sequences of the intron 18 and 19 primers are respectively: 5′ CAGTCC-AGTTCTCACAGACTCC and 5′ GGCTCCCCCTGTTCTATGAG. A 20 μl reaction (consisting of 50 ng genomic DNA, 10 mM Tris pH 8.3, 1.5 mM $MgCl_2$, 200 mM each dNTP and 0.5 units *Taq*I polymerase) was amplified under the following conditions: 93°C for 10 min, 35 cycles of 93°C for 30 s., 59°C for 45 s., 72°C for 90 s. followed by 10 min at 72°C. One half of the reaction volume was then ethanol precipitated, cleaved with *Msp*I (10 u, 30') and electrophoresed on a 4% NuSieve agarose gel (SeaKem). After digestion, the predicted 207 bp PCR product releases fragments of 156 and 51 bp in normal individuals. In affected members of the pedigree (filled symbols), this restriction site is ablated in one allele due to a G→A transition at position 13463 of the coding strand (HUMBMYH7).

* To whom correspondence should be addressed

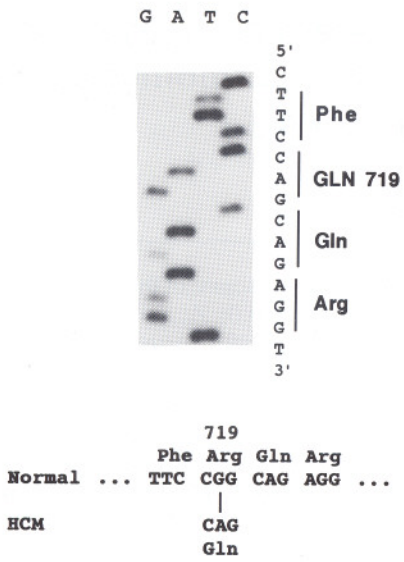

Figure 2. Representative sequence showing the G13463A missense mutation (in capitals) in the MYH7 gene of individual II-2. PCR-amplification was performed according to the conditions given in Figure 1. Reaction products from both affected and unaffected family members were cloned into pCR™II (Invitrogen). Transformants were screened for the presence of the single stranded conformer found in affected individuals. Dideoxy DNA sequencing with Sequenase, Version 2.0 (US Biochem) was performed for each strand using the oligonucleotide primers given in the legend to Figure 1.

mutation at residue Arg719 of the coding sequence: Arg719Gln (Fig. 2; EMBL Accession number X77053). The mutation results in the loss of a diagnostic *Msp*I restriction site in exon 19 of affected family members, but not in unaffected relatives (Fig. 1). Homozygosity for this *Msp*I site was demonstrated in 42 unrelated normal individuals (data not shown). It is therefore unlikely that this variant is a non-disease related genetic polymorphism.

Arg719 resides in a domain that has been proposed to interact with the myosin light chains. This domain is adjacent to the invariant SH1/SH2 domain in the skeletal and cardiac myosin coding sequences of humans and other organisms (15). Arg719 is invariant in adult mammalian muscle heavy chain myosin sequences, and embryonic isoforms of some vertebrates and the avian muscle contain lysine at this position. A positively charged side chain would appear to be important for normal myosin function at this site.

Greve *et al.* (1993) have identified a different point mutation at the same codon, Arg719Trp, which would also result in the loss of a positive charge under physiological conditions (16). The Arg719Gln and the Arg719Trp mutations presumably occur by C→T transitions on different strands of the same CpG dinucleotide (17, 18). Very different prognoses are expected for individuals inheriting these two mutations. The Arg719Trp mutation has been associated with a high incidence of premature death, whereas Arg719Gln does not appear to exhibit either life-threatening arrhythmia or cause sudden death. Watkins *et al.* (19) and Anan *et al.* (8) have postulated a malignant phenotype for missense mutations within MHY7 that alter charge. However, our results are more consistent with those of Fananapazir and Epstein (9) who have reported other charge-altering mutations in MYH7, that are associated with near normal survival. Conversely, these same investigators also identified missense substitutions that conserve charge which are associated with sudden death (9). Other biochemical properties of mutations in MHY7 appear to be important in predicting clinical outcome. Perhaps the indole ring in Arg719Trp perturbs the structure of the myosin head domain to a greater extent than does the substitution of an ethyl amide side chain in Arg719Gln.

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