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Usefulness of microsatellite markers in identification of family members carrying a mutation of the β-myosin heavy chain gene in families with hypertrophic cardiomyopathy

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Abstract. Familial hypertrophic cardiomyopathy (FHC) is characterised by autosomal dominant transmission, left ventricular hypertrophy and myocardial disarray. Genetic assessment is of special importance in this disease. Missense mutations of the gene coding for the β-myosin heavy chain (βMHC) have been identified as statistically the most important cause of the disease. Identification of specific mutations may be difficult, thus a simpler method of disease carrier identification is needed. We performed haplotype analysis of six Polish families (47 individuals) with three microsatellite markers located at the bMHC locus. Linkage of the disease locus to the bMHC gene was excluded in 4 out of the 6 families analysed. In 2 families particular haplotypes were coinherited with the disease phenotype. Microsatellite markers allowed identification of 2 carriers of the disease gene in these families among children of the patients.

Key words: familial hypertrophic cardiomyopathy, linkage analysis, microsatellites, myosin heavy chain gene.

Introduction

Hypertrophic cardiomyopathy (HCM) is a primary myocardial disease characterised by ventricular hypertrophy that may be associated with symptoms such as dyspnea, chest pain and arrhythmia. HCM is usually familial (70% of cases), with

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autosomal dominant inheritance. Different mutations causing a disease are connected with different prognostic implications: some mutations are associated with a high incidence of sudden cardiac death (SCD), others with benign prognosis (MARIAN, ROBERTS 1995). Familial hypertrophic cardiomyopathy (FHCM) may be caused by a mutation at any of the seven disease loci: β-cardiac myosin heavy chain gene (11q11-q12) (GEISTERFER-LOWRANCE et al. 1990), cardiac troponin T (1q3) and α-tropomyosin (15q2) (THIEREFELDER et al. 1994), cardiac myosin binding protein C (11q11-q13) (BONNE et al. 1995), essential (3p21.3-p21.2) or regulatory (12q23-q24.3) light chains of myosin (POETTER et al. 1996), cardiac troponin I (19p13.3-q13.2) (KIMURA et al. 1997), cardiac actin gene (15q24) (MOGENSEN et al. 1999) and titin gene (2q24.3) (SATOH et al. 1999). HCM with the Wolff-Parkinson-White syndrome was mapped to chromosome 7q3 in 1995. Recent findings indicate that 30% of cases of hypertrophic cardiomyopathy are caused by mutations in the β-cardiac myosin heavy chain gene, 15% of cases are due to mutations in cardiac troponin T gene, and less than 5% result from mutations in α-tropomyosin gene. Mutations in the cardiac binding protein C are responsible for 10-15% of cases of HCM (NIIMURA et al. 1998). Other genes that cause the disease (about 30% of cases) have not been identified yet (WATKINS et al. 1995, MOOLMAN et al. 1997, DAUSSE, SCHWARTZ 1993). The molecular basis of FHCM is very complex. The most important step is the determination of the gene responsible for the disease in the respective family. Some specific phenotypes reflect the mutation in the gene encoding cardiac troponin T and myosin light chain. Cardiac troponin T gene mutations are associated with a particularly poor prognosis (high indicidence of sudden cardiac death), but mild or undetectable hypertrophy (MOOLMAN et al. 1997). Rare mutations in the regulatory or essential light chain of myosin are associated with mid-cavity obstruction (POETTER et al. 1996). Missense mutations of the gene coding for the β-myosin heavy chain (bMHC), the most frequent cause of the disease, are not linked with any specific phenotype. Identification of specific mutations may be difficult, thus a simpler method of identification of the disease carriers is needed.

The aim of our study was to determine whether the disease locus is linked to β-MHC gene (14q11-q12) in six Polish families. We performed haplotype analysis with three microsatellite markers. Five microsatellite marker loci are located in the vicinity of the β-myosin heavy chain gene: AFM 199zf4, AFM 084ya1, AFM 079za5, AFM 291za5 and HAP1/HAP2. The intragenic markers located in the 5' end of the β-myosin heavy chain (MYH7PCR1, MYH7PCR2), and markers located near exon 19 (MYH7PCR3), in exon 21 (MYH21F212/MYH21R479), and in intron 24 (sCAW2, BEX23, MYOII), are of special diagnostic significance. In order to assess gene inheritance, at least two intragenic markers have to be analysed. In this study we chose three microsatellite markers: two intragenic (MYOII and MYH7PCR2) and one located near the β-myosin heavy chain gene (AFM 089ya1).

Material and methods

Six Polish FHCM families were studied. Clinical evaluation of family members was performed using case history, physical examination, electrocardiogram and echocardiogram. Genomic DNA was isolated from peripheral blood samples using DNA Genomic Prep Plus Kit (A&A Biotechnology). Polymerase chain reaction was performed in a Perkin Elmer 9600 thermal cycler by means of touchdown PCR. For the first 10 cycles the following temperature conditions were used: denaturation at 94°C for 40 s, annealing at 65°C to 55°C (2°C decrease after every 2 cycles) for 80 s and extension at 72°C. The following 25 cycles were carried out at an annealing temperature of 55°C, while other parameters were the same as in the first ten cycles. Amplification was carried out in a total volume of 25 µl containing 1-2 µg of genomic DNA per 100 µl, polymerase Tfl buffer (20 mM TRIS-HCl, 50 mM KCl), 2.5 mM $MgCl_2$, 200 µM of each dNTP, 20 pmoles of each primer: MYOIIF 5'-ATG CCA TGT CTA TCT GTG CC-3' and MYOIIR 5'-AAC ATC CTC TAA CCC TAC CCC-3' (DAUSSE et al. 1995); MYH7PCR2F 5'-GCA TCT GAG CAT ATG GGA CCA-3' and MYH7PCR2R 5'-TAT TTC TGT ATC AGT CAG TGAG-3' (ROGAEV et al. 1992); AFM084ya1F 5'-CCC CAA ATA TCA CTC CAA AT-3' and AFM084ya1R 5'-GAG TTG GCA ACC ACT TCT GT-3' (DIB et al. 1996), and 2.5 U of Tfl polymerase per 100 µl. Chromosomal locations and product sizes of microsatellite markers used in this study are listed in Table 1.

Table 1. Characteristics of microsatellite markers used in the study

Name of marker	Chromosomal location	Product size (bp)	References
MYOII	14q11-12	116-144	DAUSSE, SCHWARTZ 1993
MYH7PCR2	14q11-12	126-134	ROGAEV et al.1992
AFM084ya1	14q11.1-12	216-234	DIB et al.1996

The PCR products were analysed on a 10% polyacrylamide gel with subsequent silver staining. Electrophoregrams were computed with the BASSYS1D software (Biotec Fischer).

Results

The haplotype analysis of selected markers in six families (47 individuals) was performed. In our sample the MYOII marker was represented by four alleles, which were identified in six genotypes. MYH7PCR2 and AFM084ya1 markers

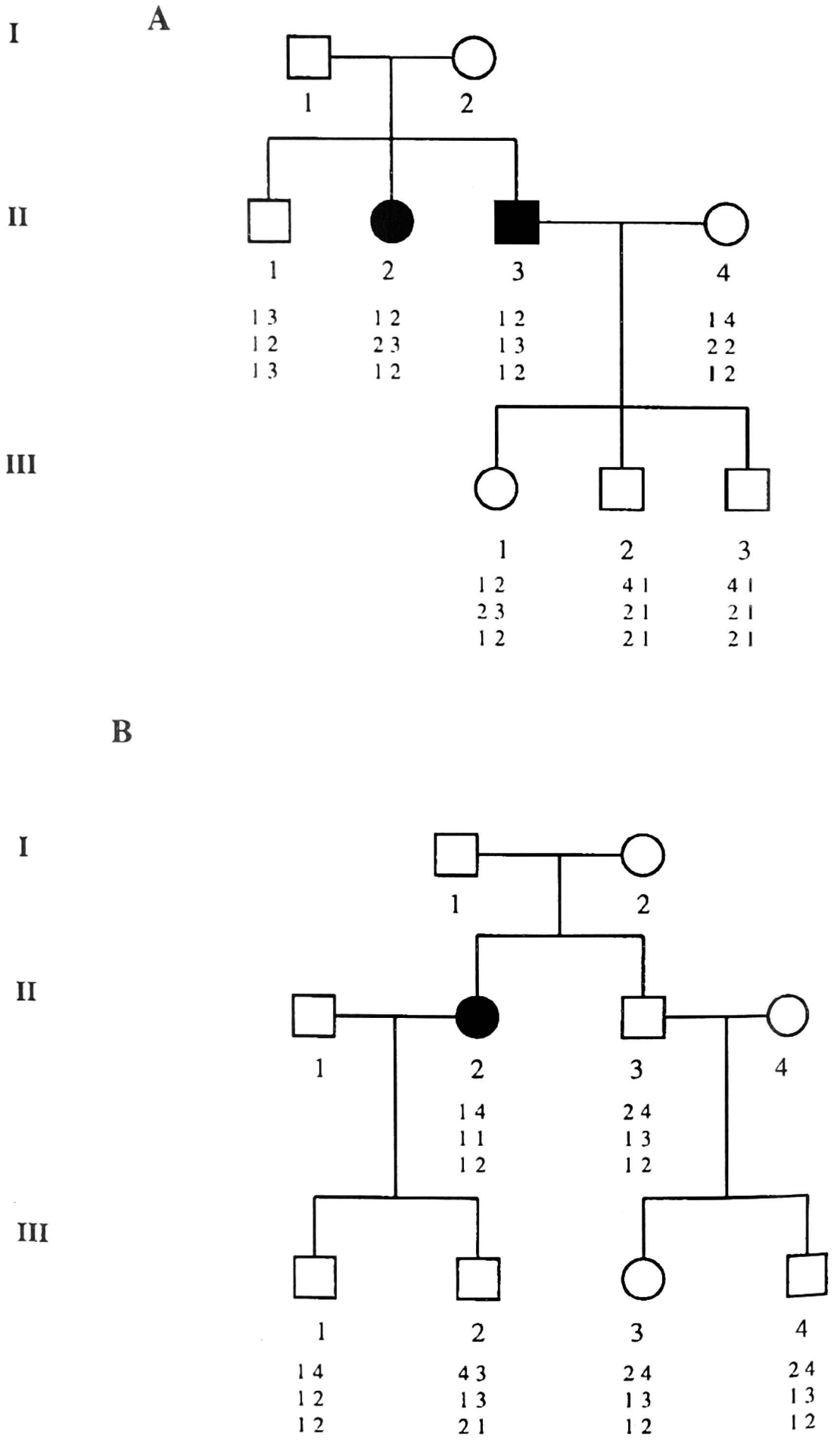

Figure 1a. Diagrams showing pedigrees of six Polish families (A-F) with familial hypertrophic cardiomyopathy. Alleles are shown for microsatellite markers MYOII, MYH7PCR2 and AFM084ya1. The length of the MYOII marker is 140 bpfor allele 1, 132 bp for allele 2, 126 bp for allele 3, 120 bp for allele 4, respectively. The length of allele 1 of the MYH7PCR2 marker is 130 bp, allele 2 – 128 bp, allel 3 – 126 bp, respectively. The length of allele 1 of the FM084ya1 marker is 225 bp, allele 2 – 221 bp, allele 3 – 217 bp, respectively. ○ – unaffected female; ● – affected female ❑ – unaffected male, ■ – affected male, ◤ – deceased affected male

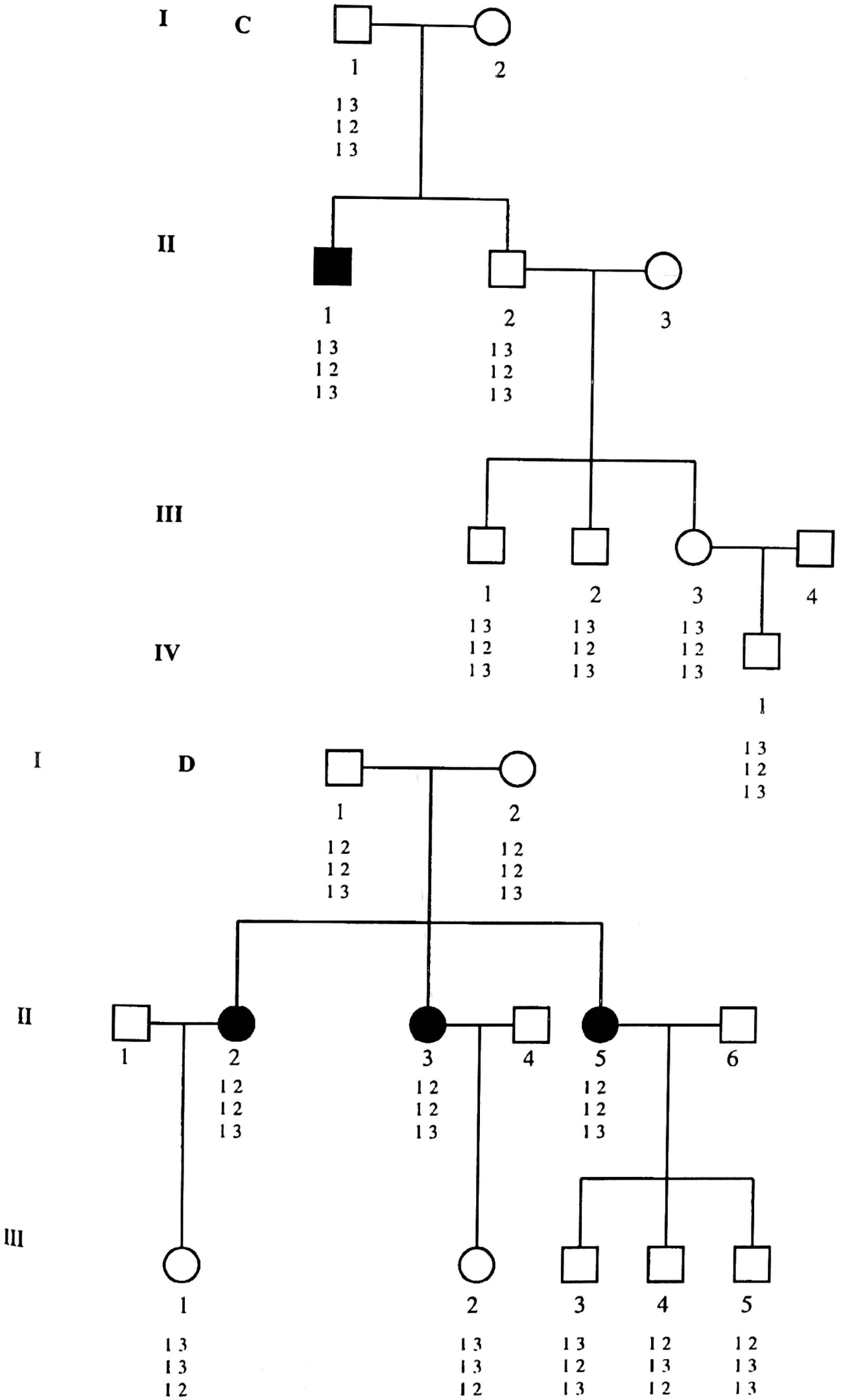

Figure 1b. Diagrams showing pedigrees of six Polish families with familial hypertrophic cardiomyopathy. Alleles are shown for microsatellite markers MYOII, MYH7PCR2 and AFM084ya1. Lengths of selected markers alleles are the same as in Figure 1a.

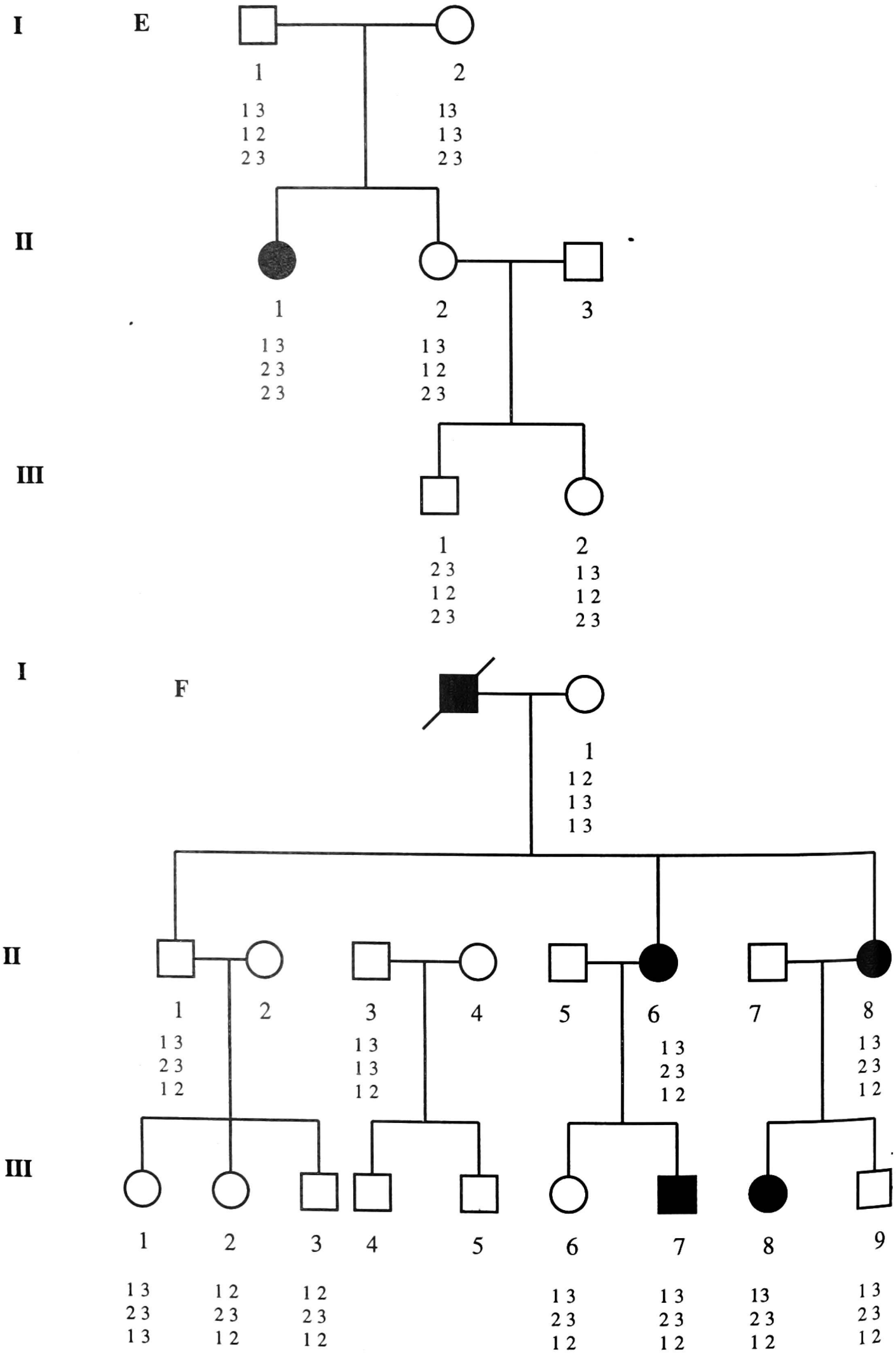

Figure 1c. Diagrams showing pedigrees of six Polish families with familial hypertrophic cardiomyopathy. Alleles are shown for microsatellite markers MYOII, MYH7PCR2 and AFM084ya1. Lengths of selected markers alleles are the same as in Figure 1a.

Table 2. Genotypes of markers MYOII, MYH7PCR2 and AFM084ya1 and their incidence in the analysed families

Marker MYOII		Marker MYH7PCR2		Marker AFM084ya1	
genotype	frequency %	genotype	frequency %	genotype	frequency %
1 3	48.9	1 2	46.2	1 2	51
1 2	27.6	1 3	29.8	1 3	36.1
1 4	10.6	2 3	19.2	2 3	12.9
2 4	6.4	2 2	21.0		
2 3	4.2	1 1	2.1		
3 4	2.2				

Table 3. LOD scores between the FHCM locus and three polymorphic markers (MYOII, MYH7 and AFM084ya1) in 6 families (A-F) with FHCM. at $\theta = 0.01$ (θ = recombination fraction)

Marker	A	B	C	D	E	F
MYOII	1.67	1.18	0.07	–0.30	0.12	0.47
MYH7PCR2	1.27	0.89	0.11	–0.27	0.19	0.39
AFM084ya1	1.42	0.81	0.37	–0.37	0.31	0.43

show three distinct alleles, which were identified in five and three genotypes, respectively (Table 2). Such a high polymorphism of the MYOII locus makes it an extremely valuable marker for genetic studies. The alleles obtained for each pedigree with the myosin gene markers are shown in Figure 1.

The LOD score was calculated (Table 3) and we were able to exclude linkage of disease locus to β-MHC gene in four of the six analysed pedigrees. Only in families A and B the haplotypes were coinherited with the disease phenotype. LOD scores for the families C, D, E and F provided little or no evidence of linkage to the β-MHC locus.

Discussion

The classic approach to determination of the gene that causes a disease involves identification of an affected protein whose function is somehow related to the disorder pathomechanism. Then, the gene of the affected protein can be identified, allowing the identification of the mutation responsible for the condition. In recent years reverse genetic examination or positional cloning have been developed to

determine the causes of inherited diseases. The goal is to identity the genetic marker which is coinherited together with the disease phenotype. Coinheritance is taken as an evidence for the disease gene being located in the vicinity of the marker.

The basic feature of genetic markers is their polymorphism, i.e. a large number of different alleles existing in a population. Two indices are commonly used to characterise marker usefulness: heterozygosity (H) and polymorphism information content (PIC). H and PIC are defined as:

$$H = \sum p_i^2 \qquad \text{and} \qquad PIC = 1 - \sum p_i^2 - \sum \sum 2p_i^2 p_j^2,$$

where p_i and p_j are the frequencies of the i-th and j-th allele at the locus, and n is the total number of alleles. The values of H and PIC range from 0 to 1. If these values are close to one (more than 0.8), the markers are especially useful for linkage analysis (HEARNE et al. 1992, LITT, LUTY 1992).

Mini- and microsatellites are DNA markers of special value for modern genetics. These sequences contain a variable number of repeats of a particular sequence. The number of repeats may vary from one to several hundred and each of these possible numbers of repeats represent a different allele. The number of different alleles at such loci is therefore very large, making them ideal for linkage analysis (their values of PIC and heterozygosity are high). Minisatellites consist of a motive from 9 to 80 bp; they are usually less then 20 kbp long. Microsatellites consist of 10-50 copies of motives from 1 to 6 bp; they are usually less then 100 bp long. Minisatellites are highly polymorphic but they are less common then microsatellites, less amenable to PCR analysis, and appear to be clustered in the proterminal regions of human chromosomes (HEARNE et al. 1992, MARCZEWSKI 1995).

As mentioned above, we chose the three most often used microsatellite markers linked to the β-MHC gene: two intragenic (MYOII and MYH7PCR2) and one located near the β-MHC gene – AFM084ya1. It was shown by DIB et al. (1996) that the marker MYOII consists of 15 alleles with heterozygosity values of at least 86%. The other two markers, MYH7PCR2 and AFM084ya1, are represented by 5 alleles with 75% heterozygosity and 8 alleles with 72% heterozygosity, respectively. Since microsatellites occur frequently and randomly in all eukaryotic DNA and can be easily amplified in vitro using polymerase chain reaction, we decided to choose this method for further genetic analysis. Alleles of dinucleotide microsatellite markers show characteristic additional bands (HEARNE et al. 1992). Using touchdown PCR we ensured that the first primer template hybridisation events involved only the reactants with the greatest complementarity. Even though the annealing temperature may eventually drop down to the Tm of non-specific hybridisation, the target amplicon is already in a position to outcompete any nonspecific PCR products during remaining cycles. We calculated the melting temperature of primers used in the reaction and proposed the value of annealing temperature 55°C. The length of PCR products depends on

the number of repeat motives and corresponds to the given allele. The application of few microsatellite markers decreases the error rate, possibly as a result of a separation of the marker locus and the gene responsible for the disease during crossing over. Each marker is represented by one dominating allele present in about 50% of the analysed patients. The LOD score values eliminated 4 out of the 6 families analysed (Table 3). The low values for families A and B reflect the rather low number of family members of. In our study only in families A and B particular haplotypes were coinherited with the disease locus. In family B a haplotype 1-1-1 (for AFM084ya1, MYH7PCR2, MYOII markers, respectively) was coinherited with the disease locus. The son III-1 received this haplotype from his affected mother (II-2), thus he carries a disease gene. In family A the siblings II-2 and II-3 had left ventricular hypertrophy, therefore they should carry the same haplotype. This is haplotype 2-3-2 (for markers AFM084ya1, MYH7PCR2 and MYOII, respectively) which is absent in their healthy brother (II-1). The daughter III-1 received haplotype 2-3-2 from her sick father and haplotype 1-1-2 from her mother, thus she carries the disease gene. Two sons (III-2 and III-3) received haplotype 1-1-1 from their father, and 4-2-2 from the mother thus they do not carry the disease gene.

Conclusions

Microsatellite markers analysis within the β-myosin heavy chain gene is well suited for routine use in clinical laboratories for carrier detection and diagnosis in HCM families. Different types of missense mutations related to β-MHC may have different prognostic implications; linkage analysis alone is not intended to replace identification of a single mutation, but rather to facilitate genetic screening of the affected families.

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