

MICROSATELLITE MARKER ANALYSIS IN THE TREATMENT AND DIAGNOSIS OF FAMILIAL HYPERTROPHIC CARDIOMYOPATHY

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Abstract: Familial hypertrophic cardiomyopathy (FHCM) is characterized by an autosomal dominant transmission, left ventricular hypertrophy and myocardial disorganization. So far, 13 genetic loci and more than 130 mutations in ten different genes have been identified. Recent study suggested impaired force production associated with inefficient use of ATP as the main disease mechanism. We performed haplotype analysis with the use of microsatellite markers linked with β -myosin heavy chain, troponin T, α -tropomyosin and cardiac myosin protein C genes in three Polish families with hypertrophic cardiomyopathy (23 individuals). This method is based on the analysis of distribution of the disease in the family and the alleles of chosen microsatellite markers. In two families, the disease was associated with β -myosin heavy chain gene. We also found a genetic carrier of the mutated gene among children of the patients. In one family the connection of the disease with the mutation in α -tropomyosin gene was confirmed, no sudden cardiac deaths were recorded and the degree of myocardial hypertrophy was small.

Keywords: familial hypertrophic cardiomyopathy, linkage analysis, microsatellites

Abbreviations: α -TPM – α -tropomyosin gene, β -MHC – β -myosin heavy chain gene, FHCM – familial hypertrophic cardiomyopathy, MyBPC3 – myosin binding cardiac protein C, TnT – cardiac troponin T gene

Hypertrophic cardiomyopathy (HCM) is a primary disease of myocardium with a genetic background. It can be defined as the left and/or right ventricle hypertrophy with unknown causes. It is familial in 70% of cases and has autosomal dominant inheritance. The development of molecular diagnostic techniques has led to defining etiopathogenesis of HCM. Familial hypertrophic cardiomyopathy (FHCM) may be caused by a mutation at any of the thirteen disease loci: β -myosin heavy chain gene (β -MHC, 14q11-q12) (1), α -tropomyosin gene (α -TPM, 15q2) (2), cardiac troponin T gene (TnT, 1q3) (3), myosin light chain genes (essential 3p21.3-p21.2 and regulatory 12q23-q24.3) (4), cardiac myosin binding protein C gene (MyBPC3, 11q11-p13) (5), troponin I gene (19q13.2-p13.2) (6), troponin C gene (7), cardiac actin gene (15q24) (8), titine gene (2q24.3) (9), α_2 subunit of AMP activated protein kinase (7q36) [10], human muscle LIM protein gene [11] and telethonin gene (12). Mutations in mitochondrial DNA were also detected. Recent findings indicate that 30% cases of HCM are caused by mutations in β -MHC gene, 15% cases are due to mutations in TnT or MyBPC3 and less than 5% result from mutations in α -TPM gene.

The most important step in genetic analysis of FHCM is the determination of the gene responsible for the disease in the respective family with the use of microsatellite markers linked with a candidate gene (located in the vicinity of the candidate gene). Microsatellite markers are tandemly repeats of simple sequences, which consist of about 10–50 copies of motifs of 1–6 bp and occur frequently in all eucaryotic genomes. Microsatellites are characterized by considerable polymorphism due to variation in the number of repeat units. This polymorphism can be easily detected by polymerase chain reaction (PCR) using a couple of primers hybridizing with adjacent DNA region. The length of PCR product depends on the number of repeat motives and it corresponds to the given allele. The use of few microsatellite markers minimizes the mistake, which could result from a division of gene marker and a gene responsible for the disease during crossing over. The arrangement of allele markers on the chromosome, that is haplotype, is very important in diagnostics, because it characterizes genetics of the disease [13]. Haplotype analysis with the use of microsatellite markers linked with β -MHC, MyBPC3, TnT and α -TPM genes in three Polish FHCM families was performed in the present study.

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MATERIALS AND METHODS

Three families (A,B,C) with hypertrophic cardiomyopathy were investigated. Twenty subjects aged from 13 to 76 years (8 females and 12 males) were examined. Specific information, especially pertaining to the time of disease detection, its course, sudden deaths, hypertension, and coexisting metabolic diseases, was obtained from all of the examined patients. The clinical examination included: physical examination, rest ECG, 24-h Holter monitoring, echocardiography (M-mode, 2-D and Doppler), which allowed us to estimate the thickness of muscle segments of the left and right ventricles and the inflow and outflow routes of the left ventricle. The value of interventricular gradient was estimated with the use of doppler echocardiography.

Genomic DNA was extracted 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 markers linked with β -MHC gene, the following temperature condition were used for ten first cycles: denaturation at 93°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 of markers linked with MyBPC3, TnT, and α -TPM was performed at an annealing temperature of 61°C. Amplification was carried out in a total volume of 25 mL, containing 1–2 mg of genomic DNA per 100 mL, polymerase Tfl buffer (20 mM TRIS-HCL, 50 mM KCl), 2.5 mM MgCl₂, 200 mM of each dNTP, 20 pmoles of each primer. Primers used in the study are listed in

Table 1. PCR products were analyzed on 10% polyacrylamide gel with subsequent silver staining. Electropherograms were computed with the BASSYS 1D software (Biotec Fischer). To determine whether the DNA polymorphisms cosegregated with the locus for FHCM, the GENEHUNTER computer program to calculate LOD score value was used.

In families in which the β -MHC gene was responsible for the disease, the presence of mutations in the 13 exon associated with poor prognosis was evaluated by restriction fragment length polymorphism analysis with the use of Ava I and Sty restriction enzyme. In case of the presence of malignant mutation in the codon 403 of 13 exon, the PCR product is digested on the fragments 87 bp and 52 bp by the enzyme Sty, the wild phenotype will be digested by the enzyme Ava I giving the fragment lengths 88 bp and 51 bp.

RESULTS

In family A (9 members), four cases of hypertrophic cardiomyopathy with a similar phenotype were present (Fig. 1). All the affected had a small degree of the exercise tolerance impairment (NYHA I). ECG showed signs of the left ventricle hypertrophy in all of them (II-5, II-7, III-10, and III-12). The 24-h Holter monitoring did not reveal ventricular arrhythmias in anyone, but revealed bradycardia in all of these persons. Echocardiography revealed a similar type of hypertrophy in all of them. The interventricular septum (IVS) systolic thickness was, on the average, 2.2 cm, while the IVS diastolic thickness was 1.5 cm on the average. The average back

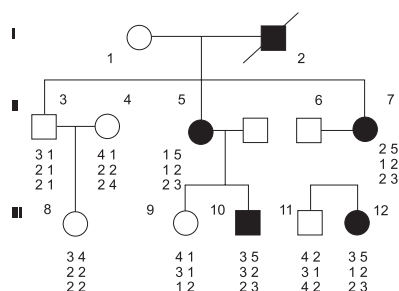


Figure 1. The diagram showing the family A with familial hypertrophic cardiomyopathy. Alleles are shown for microsatellite markers MYOII, MYH7PCR2 and AFM084ya1. ○ – unaffected female, ● – affected female, ■ – affected male, □ – unaffected male, / deceased

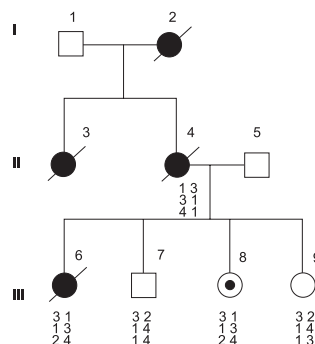


Figure 2. The diagram showing the family B with hypertrophic cardiomyopathy. Alleles are shown for microsatellite markers MYOII, MYH7PCR2 and AFM084ya1. ○ – unaffected female, ● – affected female, ■ – affected male, □ – unaffected male, / deceased

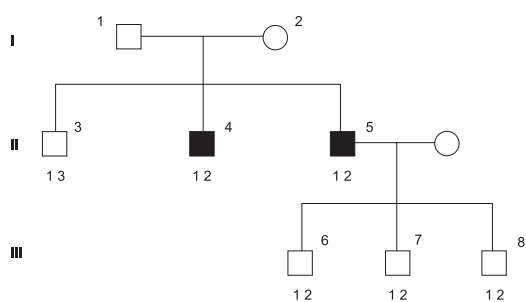


Figure 3. The diagram showing the family C with hypertrophic cardiomyopathy. Alleles are shown for microsatellite marker HTM(CA)_n. ○ – unaffected female, ● – affected female, ■ – affected male, □ – unaffected male

wall systolic thickness was 1.7 cm, and its average diastolic thickness was 1.2 cm. The other members of the family A had no clinical, electrocardiographic or echocardiographic signs of disease. No sudden cardiac deaths were recorded in family A. All affected persons (II-5, II-7, III-10 and III-12) received a haplotype 5-2-3 (for MYOII, MYH7PCR2 and AFM084ya1, respectively) so this haplotype was coinherited with the disease locus. The value of multipoint lod score was 2.1 ($q = 0.00$).

In family B (6 members), two cases of HCM with a similar phenotype were found (II-4, III-6), but II-4 died during genetic and clinical diagnosis (Fig. 2). Both affected persons had a similar degree of the exercise tolerance impairment and the ECG showed signs of the left bundle branch block in both. The 24-h Holter monitoring revealed ventricular arrhythmias in both persons. Echocardiography showed a similar type of asymmetric left ventricle hypertrophy without signs of outflow route constriction in both of them. Medium interventricular septum diastolic thickness was 2.8 cm, and medium systolic thickness was 1.3 cm. Medium back wall diastolic thickness was 1.3 cm and medium systolic thickness was 0.9 cm. In this family, three cases of sudden cardiac death involving members of I-2, II-3, II-4 about 30 year old were recorded. In the rest of the family, no clinical, electrocardiographic, or echocardiographic signs of disease were found. In family B, a haplotype 1-3-4 (for MYOII, MYH7PCR2 and AFM084ya1, respectively) was coinherited with the disease and it was present in the affected persons (II-4 and III-6) and in healthy one III-8. The value of multipoint LOD score in this family was 0.9 ($q = 0.00$). Restriction fragment length polymorphism with the use of Ava I and Sty enzymes excluded the presence of malignant mutation in the codon 403 of 13 exon of β -MHC gene in families A and B.

In family C (6 members), two cases of HCM with a similar phenotype and NYHA functional class (NYHA I) were found (Fig. 3). In both affected persons, a similar type of myocardial hypertrophy was found by echocardiographic examination and similar arrhythmias were revealed by Holter monitoring. In the rest of the family, no clinical, electrocardiographic, or echocardiographic signs of disease were found. Medium interventricular septum diastolic thickness was 2.4 cm, and medium systolic thickness was 2.0 cm. Medium back wall diastolic thickness was 1.2 cm, and medium systolic thickness was 0.9 cm. The other members of family C had no clinical, electrocardiographic, or echocardiographic signs of disease. No sudden cardiac deaths were recorded in family C. Genetic analysis with the use of microsatellite marker linked with β -MHC, MyBPC3 and TnT genes excluded these loci as responsible for the HCM in family C. The analysis of microsatellite marker linked with α -TPM gene showed that this loci was responsible for the disease in this family.

DISCUSSION AND CONCLUSION

The classic approach to determine the gene which causes a disease involves identifying a biochemically affected protein which is a carrier of a function related to disorder. Then the gene of the affected protein can be identified, allowing designation of the mutation responsible for the condition. In recent years, reverse genetic examination or positional cloning have been developed to determine causes of inherited diseases. The goal is the identification of genomic marker, which is coinherited together with the disease phenotype. Coinheritance is taken as evidence for the disease gene being located in the vicinity of the marker.

The aim of this study was to present the use and clinical usefulness of microsatellite markers analysis in genetic diagnosis of familial hypertrophic cardiomyopathy. Molecular basis describing 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 genes coding for TnT, MyBPC and α -TPM. Mutations in gene for cardiac troponin T are connected with a small, often undetectable, myocardial hypertrophy, but one with a very bad prognosis. Among persons with these mutations, frequent sudden cardiac deaths were observed [14]. Mutations in gene for cardiac protein C are associated with a benign phenotype, late onset of the disease and good prognosis [15]. Mutations in α -tropomyosin gene are associated with a small degree of myocardial

hypertrophy and with good prognosis [16].

In family C, in which the association of the disease with the mutation in this gene was confirmed, no sudden cardiac deaths were recorded and the degree of myocardial hypertrophy was also low. Mutations located in the β -myosin heavy chain gene are associated not only with a different phenotype, but also with a different prognosis. Mutations with a good, medium and bad prognosis were found among them [17]. In families A and B, the connection of the disease with β -myosin heavy chain gene was confirmed. In family A, the disease seems to have a good prognosis because so far, no sudden cardiac deaths occurred among the affected members, in contrast to family B, in which all affected persons died before 30 years of age. In family A, the founder of the mutation was the patient I-2. The affected children III-10 and III-12 received an associated with the disease haplotype 5-2-3 from their affected mothers (II-5 and II-7). The other children of affected parents (III-9 and III-11) received the haplotypes 1-1-2 and 2-1-2, respectively, which are not associated with the mutated gene.

In family B, the founder of the mutation was patient I-2. The affected mother II-4, transferred haplotype 1-3-4, associated with the disease, to her daughters III-6 and III-8. The other siblings, III-7 and III-9, received from their mother haplotype 3-1-1, thus they do not carry the disease gene. Daughter III-8 is a genetic carrier of the mutated gene. So far, she has shown no signs of the disease.

Analysis of microsatellite markers linked with candidate genes is well suited for routine use in clinical laboratories for carrier detection and in diagnosis of FHCM families. The possibility of performing diagnosis before eventual appearance of the signs of the disease allows not only to introduce prophylaxis, but also to be aware of the risk of sudden death. Different types of missense mutations related to candidate genes 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 affected families.

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