International Center For Science & high Technology and Environmental Sciences

چهارمین همایش ملی بیوتکنولوژی جمهوری اسلامی ایران
کرمان مردادماه ۱۳۸٤

The 4th National Biotechnology congress
Islamic Republic of Iran
Kerman august 2005

Investigations of hot spot regions in MYH7 genes in Iranian hypertrophic cardiomyopathy patients

Montazeri M*/,Houshmand , Mohamad Hossein Mandeger, Ghani Kakhki M, Estahbanati G, Nouhi F, Peyghambari M, Givtaj N, Paydar A.**

***,** National Institute for Genetic Engineering and Biotechnology, Tehran, Iran**
Tel: +98 21 4580390 Fax: +98 21 4580399 E-mail: maryam104@yahoo.com

Abstract

Background: Hypertrophic cardiomyopathy occurs approximately 1 in 500 people. It is the most common cause of sudden cardiac death in young people. It is the most common cause of sudden cardiac death in young people. The disease is characterized by hypertrophy of left and/or right ventricles and intrventricular septum. Patients could develop serious complications including heart failure, arrhythmias and sudden death. Familial hypertrophic cardiomyopathy is a single gene disorder and has autosomal dominant inheritance. Mutations in at least 11 genes (such as *MYH7, MYBPC3* and *TNNT2*) encoding sarcomeric proteins and possibly in one gene encoding a non-sarcomeric protein (*PRKG2*) have been associated with hypertrophic cardiomyopathy so far. In this study we focused on exons 13-15 and 19-21 of *MYH7* gene and introns located between them, which contain hotspots for so called "malignant mutations" that increase sudden cardiac death risk. Methods: Fifty unrelated Iranian patients with hypertrophic cardiomyopathy were selected sequentially and informed written consent was obtained from them. Genomic DNA was extracted from peripheral venous blood leukocytes by salting-out method. Exons 13-15 and 19-21 of *MYH7* gene and their related introns were amplified by polymerase chain reaction. Then PCR products were sequenced. Results: Mutations were detected in fourteen (28%) of the patients. Three mutations were found in exons. We have found a novel mutation, A10419C in exon 14, which was a missense mutation causing N444T substitution. Conclusion: Mutations in the *MYH7* gene can be found in patients without a family history of hypertrophic cardiomyopathy. Screening of already known mutations is not helpful and the analysis should be started systematically by testing *MYH7* and *MYBPC3* and then focus on *TNNI3*, *TNNT2*, and *MYL*. Also, in severe phenotypes, several mutations should be searched.

Keywords: Hypertrophic cardiomyopathy, *MYH7* gene, beta-myosin heavy chain, mutation, Iranian.

Introduction

Hypertrophic cardiomyopathy (HCM) is the most common cause of sudden cardiac death (SCD) in the young. It is characterized by left and/or right ventricular hypertrophy that is usually asymmetric and involves the intrventricular septum, in the absence of other loading conditions such as hypertension or hypertroidism (1-4). The disease occurrences is approximately 1 in 500 (5, 6). HCM is clinically heterogeneous and most patients have few or no symptoms while others develop serious complications including heart failure, arrhythmias and sudden death (5, 7, 8). The most common clinical symptoms of HCM are dyspnea and chest pain. Other manifestations include light-headedness, presyncope, syncope, tiredness, palpitation, orthopnea and SCD (1, 9). In most patients there are systolic murmurs due to left ventricular outflow tract obstruction and mitral valve incompetence. The ECG is often abnormal, showing the feature of left ventricular hypertrophy and nonspecific ST changes and arrhythmias (1, 4). Diagnosis is made by echocardiography showing hypertrophy with the septum or ventricular wall thickness of at least 13 mm without other cause (1, 10).

Familial hypertrophic cardiomyopathy (FHCM) is a single gene disorder inherited in an autosomal dominant pattern (10, 11, 12). Over 200 mutations in at least 11 genes (*MYH7, MYBPC3, TPM1, TNNT2, TNNI3, MYL2, MYL3, ACTC, TNNC1, TTN,* and *MYH6*) encoding proteins of the sarcomer (the basic contractile element of the heart) and possibly in one gene encoding a non-sarcomeric protein (*PRKG2*) have been identified. (7, 13, 14, 15) These genes are shown in Table-1. Mutations in beta-myosin heavy chain gene (*MYH7*), myosin binding protein C gene (*MYBPC3*) and troponin T (*TNNT2*) gene probably account for 70% to 80% of all cases of FHCM. The majority of the mutations are single-point missenes mutations. The

remainders are small deletions or insertions. In the human population, mutations in the *MYH7* gene account for ~30% of the documented HCM cases. (1, 13, 16)

MYH7 gene, which is the most common mutated gene in patients with HCM (17), is located on the long arm of chromosome 14 (14q1). (18) This gene contains 40 exons spread over ~25kb of genomic DNA. More than 100 disease-causing mutations have been defined in HCM patients, of which over 80 missense mutations have been identified in the globular head and neck domain of *MYH7*, encoded by exons 3 through 23, which shows a clustering in mutation points (17, 19, 20).

Mutation in *MYH7* have been associated with greater disease peneterance, more sever hypertropic, and high risk of SCD in comparison with mutations in other sarcomeric genes (16, 19, 21). In the *MYH7* gene several mutations are associated with a high risk of SCD (so-called malignant mutations): Arg403Gln, Arg719Trp, Arg453Cys, Arg723Gly and Gly716Arg. In contrast, patients with Asp232Ser, Gly256Glu, Phe513Cys, Val606Met, Arg719Glu and Leu908Val mutations have a good prognosis regarding arrhythmic events (being mutation) (7, 22, 23, 24).

As malignant mutations are located in exons 13-15 and 19-21 of *MYH7* gene, we focused on these exons and their interlocated introns in this study.

Materials and Methods

Patients

Fifty unrelated Iranian patients attended to Rajayi Heart Center (RHC) in Tehran with a clinical diagnosis of hypertrophic cardiomyopathy (HCM), confirmed by echocardiography were selected sequentially. Informed written consent was obtained in accordance to approved protocol by RHC medical ethics committee. Clinical examination and paraclinical tests including ECG, echocardiography, and chest X-ray were performed. Diagnostic criteria for HCM were left ventricular wall thickness >13 mm on echocardiography and absence of other confounding diagnoses (1, 10, 11).

Genetic Analysis

A 5ml venous blood sample from each HCM patient was collected in an EDTA containing tube, and genomic DNA was extracted from peripheral leukocytes by standard salting-out method previously described. Two primer pairs were designed for *MYH7* gene exons 13-15 (b MHC 13 F: 5′– agt cat ctc ttt acc aac ttt gct a– 3′ and

bMHC 15 R: 5′ – gaa ttc agg tgg taa ggc caa aga g– 3′) and for exons 19-21 (bMHC 19F: 5′ -aca aag cca gga tca gaa ccc aga- 3′ and bMHC 21R: 5′ -gcc tct gac cct gtg act gca gtg-3). PCR was performed by Primus Thermocycler (MWG-BIOTECH) in a 25µl volume using Taq enzyme (Roche). Annealing temperatures of 62°C were used. Each of the denaturation, annealing, extension segments was for 45 sec, and 32 cycles were performed. Fragments sizes were determined by comparison with 1-kb ladder DNA size marker.

Two 909 and 1022 bp amplified fragments were visualized on 1.5% agarose gel electrophoresis of PCR products and ethidium bromide staining. PCR products were eluded from agaros gels using Nucleospin Extract kit (MACHEREY-NAGEL) and sequenced on an ABI 3700 automatic sequencer machine (GenefanAvaran, Macrogen, Korea). Resulted sequences were compared with reference sequence of the *MYH7* gene using blast program accessed through NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/)

Results

DNA extracted from probands was screened for mutations in exons 13-15 and 19-21 of *MYH7* gene. Mutations were detected in fourteen (28%) of these patients (Table-2). Three mutations were found in exons: G10195A in exon 13, A10419C in exon 14, and C13430T in exon 19. Other mutations occurred in introns: A10230C in intron 13 in one patient, T10630A in intron 14 in 4 patients, C10663T in intron 14 in 3 patients, A13573G in intron 19 in one patient, C13879G in intron 20 in one patient, C13978A in intron 20 in one patient.

Discussion

This report describes the screening a population of 50 Iranian unrelated index cases with HCM for mutations in exons 13-15 and 19-21 of *MYH7* gene and introns located between them that contain hot spots.

About 55% of HCM cases are familial and 45 % are sporadic. About 75% of the familial form of HCM reveal autosomal dominant pattern of inheritance (25). Hypertrophic cardiomyopathy is a genetically complex disease: more than 150 mutations have been identified in at least 11 genes that encode sarcomeric proteins. These are; β-myosin heavy chain (*MYH7*); α-myosin heavy chain (*MYH6*); cardiac troponin T (*TNNT2*); cardiac troponin I (*TNNI3*); α-tropomyosin (*TPM1*); myosin binding protein C (*MYBPC3*); essential myosin light chain (*MYL3*); regulatory myosin light chain (*MYL2*); cardiac titin (*TTN*) and α-cardiac actin (*ACTC*). The recently identified *PRKA2* gene encodes the γ_2 subunit of protein kinase activated by AMP (AMPK) (15, 20, 26); and *MLP* gene encodes the major nuclear regulator of myogenic differentiation, which is human muscle LIM protein (27). These all indicate the genetic heterogeneity of HCM. In 35% of patients, HCM occurs as a result of mutations in *MYH7* gene. *MYBPC3, TNNT2* and *TPM1* genes also may have mutations and end up with the disease, with a frequency of 20%, 15% and 3%, respectively (26). Distribution of the disease genes of the full 197 case series reported by Marian AJ and Roberts R was as follows: *MYBPC3*, 26%; *MYH7*, 25%; *TNNT2*, 4%; *TNNI3*, 4%; *MYL2*, 2.5%; and *MYL3*, <0.5%. These results differ from previously reported estimates in which *MYH7* was the most frequent and then *TNNT2* and *MYBPC3* (28). Subsequent to mutations in a single gene, diverse phenotypes with various clinical appearances and various degrees of hypertrophy occur. Some mutations in *MYH7* gene have poor prognosis, while some have moderate and some have good prognosis. Mutations in the *MYH7* gene can also be found in patients without a family history of hypertrophic cardiomyopathy (29). Charron P. and colleagues in their study illustrate the extreme phenotypic heterogeneity in carriers of *MYH7* mutation. They imply screening of already known mutations is not helpful and the analysis should be started systematically by testing *MYH7* and *MYBPC3* and then focus on *TNNI3*, *TNNT2*, and *MYL2*. Also, in severe phenotypes, several mutations should be searched. And finally, sporadic cases can be successfully screened. The development of genotyping in HCM based on this more accurate approach, along with the increasing knowledge about relations between the genotype and the phenotype, should lead to improved genetic counseling and better clinical management in families with HCM. (29).

Three hot spots for mutations have been identified, codons 403, 719 and 741 (30, 31, 32). Our initial approach to genetic testing is to determine whether the patients carry any of the known mutations, particularly those associated with a poor prognosis in regions, which contain hotspots for malignant mutations that increase SCD risk. We found three mutations in exons 13, 14 and 19. First, a previously reported showed V411I substitution in exon 13. We found a novel mutation, N444T in exon 14. Third mutation was a synonymous mutation in R703 in exon 19 with no amino acid change, which will have no effect.

Since our study was the first in Iranian population, and it is a limited preliminary one, we cannot conclude that these are important mutations or just they are SNPs. We are preparing for

extended more comprehensive studies on HCM in Iranian patients to elucidate genetic factors of HCM more precisely.

References

1. Roberts R, Sigwart U. New concepts in hypertrophic cardiomyopathies, Part I. *Circulation* 2001;104: 2113-2116.
2. Semsarian C, Maron BJ. Sudden cardiac death in the young. *The Med J Australia* 2002;176(4):148- 9.
3. Maron BJ, Shirani J, Poliac LC, et al. Sudden death in young competitive athletes: clinical, demographic, and pathological profiles. *JAMA* 1996; 276:199 –204.
4. Bonne G, Carrier L, Richard P, Hainque B, Schwartz K. Familial hypertrophic cardiomyopathy from mutations to functional defects. *Circulation* 1998; 83:580-593.
5. Spirito P, Seidman CE, McKenna WJ, Maron BJ. The management of hypertrophic cardiomyopathy. *N Engl J Med* 1997; 336:775-785.
6. Maron BJ, Gardin JM, Flack JM, et al. Prevalence of hypertrophic cardiomyopathy in a general population of young adults: echocardiographic analysis of 4,111 subjects in the CARDIA study. *Circulation* 1995;92:785–789.
7. Van Driest SL, Ackerman MJ, Ommen SR, Shakur R, Will ML, Nishimura RA, et al. Prevalance and severity of "benign" mutations in the β-myosin heavy chain, cardiac troponin T, and α-tropomyosin genes in hyperthrophic cardiomyopathy. *Circulation* 2002; 106:3085-3090.
8. Maron BJ, Olivotto L, Spirito P, Casey SA, Bellone P, Gohman TE, et al. Epidemiology of hypertrophic cardiomyopathy-related death. *Circulation* 2000; 102:858-864.
9. Elliott PM, Poloniecki J, Dickie S, et al. Sudden death in hypertrophic cardiomyopathy: identification of high risk patients. *J Am Coll Cardiol* 2000; 36:2212–2218.
10. Erdmann J, Daehmlow S, Wischke S, Senyuva M, Werner U, Raible J, et al. Mutation spectrum in a large cohort of unrelated consecutive patients with hypertrophic cardiomyopathy. *Clin Genet* 2003; 64:339-349.
11. Niimura H, Patton KK, McKenna WJ, Soults J, Maron BJ, Seidman JG, et al. Sarcomer protein gene mutations in hypertrophic cardiomyopathy of the eldery. *Circulation* 2002; 105:446-451.
12. Fananapazir L, Dalakas MC, Cyran Francis, Cohn G, Epstein ND. Missense mutations in the β-myosin heavy chain gene cause central core disease in hypertrophic cardiomyopathy. *Proc Natl Acad Sci* 1993;90:3993-3997.
13. Waldmüller S, Sakthivel S, Saadi AV, Selignow C, Rakesh PG, Golubenko M, et al. Novel deletions in *MYH7* and *MYBPC3* identified in Indian families with familial hypertrophic cardiomyopathy. *J Mol Cell Cardiol* 2003; 35:623-636.
14. The Familial Cardiomyopathy Mutation Database. Available at: http://www.angis.org.au/atabases/Heart/dasearch.html. Accessed March 16 2004.
15. Richard P, Charron P, Carrier L, Ledeuil C, Cheav T, Pichereau C, et al. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation* 2003; 107:2227-2232.
16. Havndrup O, Bundgaard H, Andersen PS, Larsen LA, Vuust J, Kjeldsen K, et al. Outcome of clinical versus genetic family screening in hypertrophic cardiomyopathy with focus on cardiac β-myosin gene mutations. *Cardiovasc Res* 2003; 57:347-57.
17. Blair E, Redwood C, Oliveira MJ, Moolman-Smook JC, Brink P, Corfield VA, et al. Mutations of the light meromyosin domain of the β-myosin heavy chain rod in hypertrophic cadiomyopathy. *Circ Res* 2002; 90:263-9.
18. García-Castro M, Reguero JR, Batalla A, Díaz-Molina B, González P, Alvarez V, et al. Hypertrophic cardiomyopathy: low frequency of mutations in the β-myosin heavy chain (*MYH7*) and cardiac troponin T (*TNNT2*) genes among Spanish patients. *Clin Chemistry* 2003; 49(8):1279-85.
19. Van Driest SL, Jaeger MA, Ommen SR, Will ML, Gersh BJ, Tajik AJ, et al. Comprehensive analysis of the beta-myosin heavy chain gene in 389 unrelated patients with hypertrophic cardiomyopathy. *J Am coll cardiol* 2004;44(3):602-10.
20. Arad M, Seidman JG, Seidman CE. Phenotypic diversity in hypertrophic cardiomyopathy. *Human Molecular Genetics* 2002;11(20):2499-506.
21. Kok WEM, Baars MJH. Genetics of hypertrophic cardiomyopathy. *Heart Metabol* 2003;(21):37-41 .
22. Keller DI, Carrier L, Schwartz K. Genetics of familial cardiomyopathies and arrhythmias. *Swiss med wkl* 2002; 132:401-7.
23. Blair E, Price SJ, Baty CJ, Östman-Smith I, Watkins H. Mutations in *cis* can confound genotype-phenotype correlations in hypertrophic cardiomyopathy. *J med genet* 2001-385-7.

24. García-Castro M, Reguero JR, Batalla A, Catalán F, Mayordomo J, Coto E. Direct detection of malignant mutations in patients with hypertrophic cardiomyopathy. *Rev Esp Cardiol* 2003; 56(10)1022-5.
25. Sasson Z, Rakowski H, Wigle D. Hypertrophic cardiomyopathy. Cardiol Clin 1988; 6: 233-88.
26. Fatkin D, Graham RM. Molecular mechanisms of inherited cardiomyopathies. *Physiol Rev* 2002; 82: 945-80.
27. Geier C, Perrot A, Ozcelik C, et al. Mutations in the human muscle LIM protein gene in families with hypertrophic cardiomyopathy. *Circulation* 2003; 107; 1390-5.
28. Marian AJ, Roberts R. The molecular genetic basis for hypertrophic cardiomyopathy. *J Mol Cell Cardiol* 2001; 33: 655–670.
29. Charron P, Heron D, Gargiulo M, et al. Genetic testing and genetic counselling in hypertrophic cardiomyopathy: the French experience. *J Med Genet* 2002; 39: 741–746.
30. Dausse E, Komajda M, Fetler L, Dubourg O, Dufour C, Carrier L, Wisnewsky C, Bercovici J, Hengstenberg C, al-Mahdawi S, et al. Familial hypertrophic cardiomyopathy. Microsatellite haplotyping and identification of a hot spot for mutations in the beta-myosin heavy chain gene. *J Clin Invest* 1993;92(6):2807-13.
31. Anan R, Greve G, Thierfelder L, Watkins H, McKenna WJ, Solomon S, Vecchio C, Shono H, Nakao S, Tanaka H, et al. Prognostic implications of novel beta cardiac myosin heavy chain gene mutations that cause familial hypertrophic cardiomyopathy. *J Clin Invest* 1994;93(1):280-5.
32. Schwartz K, Carrier L, Guicheney P, Komajda M. Molecular basis of familial cardiomyopathies. Circulation 1995; 91:532-40.