

Rapid Communication

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Role of Modifying Genes on the Severity of Rare Mutation of *MYH7* Gene in Hypertrophic Obstructive Cardiomyopathy

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Background

Hypertrophic Cardiomyopathy (HCM) is an autosomal dominant disorder due to mutations in sarcomeric genes with variable penetrance. Hypertrophic Obstructive Cardiomyopathy (HOCM) is a major complication of Hypertrophic Cardiomyopathy. Unexplained hypertrophy in the Left Ventricle (LV) or Intraventricular Septum (IVS) had been the diagnostic criterion for HCM which is more often confirmed by the echocardiography [1]. The frequency of HCM in general population is 1:500 and about 60-70% genetic predisposition is known [2]. It has been observed that mutations in the Cardiac myosin binding protein C (MYBPC3) gene causes late onset of disease with mild symptoms while mutations in the Beta Myosin Heavy chain (MYH7) gene leads to early onset with severe symptoms. Apart from Epigenetic and Environmental factors, modifier genes further complicate the situation leading to altered clinical outcome even among the same family members having identical mutation [3].

Aims

Present study was designed to evaluate genotype-phenotype correlation in the Index patient and family members harboring a rare mutation in *MYH7* (C → T at codon 923) gene and suffering from Hypertrophic Obstructive Cardiomyopathy (HOCM).

Materials and Methods

Total 8 individuals of a family of an index patient were included in this study in which father and youngest son diagnosed with hypertrophic Obstructive cardiomyopathy. All family members were screened with ECG, Echocardiography and clinical evaluation was done by the cardiologists as per the WHO criteria for diagnosis of HCM (1996) [4]. 5mL of intravenous blood was collected along with other physiological and physical parameters and DNA was extracted from blood by Phenol-chloroform method [5]. Suitable primers [6] were used to amplify exon 23 of *MYH7* gene, and the PCR product (390bp) was sequenced in an automated sequencer ABI 3700 by Sanger's method (Figure 1A). The study was ethically approved by institutional committee and informed written consent was taken from all participants.

Screening for other known mutations associated with HCM like E101K in Cardiac muscle Actin (*ACTC*) gene [7] and P77L in Cardiac Troponin T (*TNNT2*) gene [8] were done by RFLP method using

enzymes *Ava* I and *Sm*II respectively. 5bp deletion polymorphism in intron 3 of *TNNT2* gene was also analyzed by RFLP method using *Ear* I enzyme and genotyped in 8% Polyacrylamide gel (Figure 1B). 287bp Deletion polymorphism of Angiotensin Converting Enzyme (*ACE*) gene in the intron16 [9] and 25bp Deletion Polymorphism in Cardiac Myosin Binding Protein C (*MYBPC3*) gene in the intron 32 [10] were analyzed directly by genotyping the PCR product on 2% [10] and 3% Agarose gel respectively. To validate *MYH7* (C→T at codon 923) mutation, 20 HCM families including 21 affected and 46 unaffected family members were screened for exon 23 of *MYH7* gene by Sanger sequencing. All sequences were deposited in NCBI data base [GenBank accession nos. JX504729 - JX504803].

Results and Discussions

The rod region of alpha helical segment connecting myosin head to thick filament results in possible dysfunction in contraction indicating a functional role for variants in *MYH7* gene [11]. The rare *MYH7* mutation reported in the index family was not found in other 20 HCM families in our study.

The symptoms of HOCM (Hypertrophic obstructive cardiomyopathy) such as dyspnea and angina were observed in the patient at the age of 14 during sports activity performance. Preliminary ECG examination indicated signs of ischemic heart disease which was confirmed as HOCM by the echocardiographic examination.

Screening of all family members established presence of HOCM in father, who had very mild symptoms from the past 3yrs but avoided

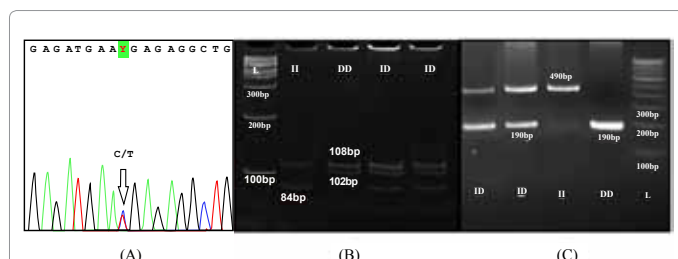


Figure 1: (A) Sequence region of exon 23 of *MYH7* showing heterozygous mutation C→T at codon 923. (B) PAGE showing homozygous wild (II) with band size 108bp and 84bp, heterozygous (ID) with band size 108bp, 102bp and 84bp and homozygous mutant 108bp and 102bp. (C) ACE genotyping in 2% Agarose Gel with homozygous wild (II) with band size 490bp, Heterozygous (ID) with band size 490bp and 190bp and homozygous mutant 190bp. (I: Insertion; D: Deletion; L: DNA Ladder).

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medical checkup. Figure 2 shows the dominant pattern of inheritance of the MYH7 (C→T at codon 923) mutation. The index patient and one of his elder brothers (20 years) inherited the same mutation from their father. The father's sisters, mother and his eldest brother was devoid of this mutation. This rare variant had been reported previously in an Indian HCM family and also in European cohort of 1000 genome project (www.ensembl.org).

The inference from the clinical parameters is depicted in table 1 in consonance with the genotypes. Abnormal ECG and 2D echocardiography revealed hypertrophied LV in index patient and his father only. The Blood Pressure and BMI of the index patient and the father were normal which excludes them from other hypertension which can also lead to hypertrophied LV. It was pertinent to note that the father had late onset of the disease (48yrs) while the youngest son (index patient) had early onset of the disease at the age of 14 years and the elder son (20 yrs) was asymptomatic though all three were having the rare MYH7 mutation.

The Cardiac Troponin T (TNNT2) protein, allows actomyosin interaction and contraction of muscle cell occur in response to Ca²⁺ [12] and ACE converts Angiotensin I to Angiotensin II, an important mediator in secondary myocardial hypertrophy, post-myocardial infarction remodeling and heart failure [13,14].

Screening of other known mutations associated with the HCM

was not found in any family members except for the TNNT2 and ACE polymorphism. The inheritance of TNNT2 and ACE polymorphisms revealed that the index patient was distinct from all other family members with respect to genotypes 5bp TNNT2 (II) and 287bp ACE (ID), while the elder brother and the father with the MYH7 mutation (C→T at codon 923) had similar genotypes i.e. 5bp TNNT2 (ID) and 287bp ACE (II) (Figure 2).

The Index patient with rare MYH7 (C→T at codon 923) mutation had severe symptoms and early onset of disease which could be explained due to co-occurrence of ACE (ID) and TNNT2 (II) genotypes. Since his father and elder brother having different ACE (II) and TNNT2 (ID) genotypes harboring same rare MYH7 mutation could have been protective, with father having mild symptoms with late onset of disease and elder brother had no clinical manifestation till now. Also it was reported that 'D' allele of 5bpTNNT2 gene polymorphism had higher frequency in Indian populations [15], suggesting it may have some selective advantage. So presence of 'D' allele in father and his elder brother could have been beneficial in counteracting pathogenic MYH7 mutation.

Conclusions

The present family study suggested that the combinational effect of two or more variants may modify the phenotypic expression of the pathogenic mutation. In HCM, several private mutations have been

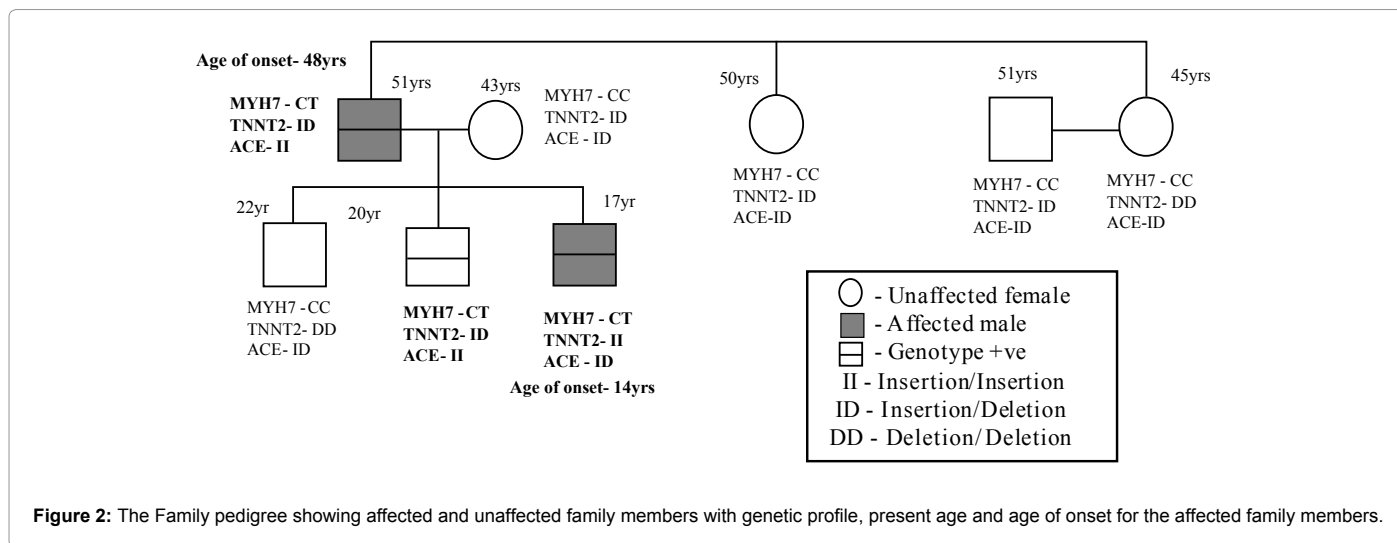


Figure 2: The Family pedigree showing affected and unaffected family members with genetic profile, present age and age of onset for the affected family members.

| Family Member & Relation with ego | Age, Sex and Age of onset of disease | NYHA Class | Echocardiographic findings | | ECG | Symptoms | BMI | BP (Sys/Dia) |
|-----------------------------------|--------------------------------------|------------|----------------------------|--------------------|-------------------------------------|---|------|--------------|
| | | | PW (LV) Thickness (mm) | IVS thickness (mm) | | | | |
| Proband | 17yrs, M, 14yrs | III | 17 | 23 | ST depression, Abnormal QRS complex | Severe symptoms like fatigue, chest pain, with mild SAM & MR. | 24.9 | 116/74 |
| Father | 51yrs, M, 48yrs | II | 19 | 31 | ST depression | Mild symptoms with mild SAM & MR | 31.4 | 108/72 |
| Brother | 20yrs, M, NA | - | 10 | 10 | Normal | No symptoms | 20.3 | 105/68 |
| Brother | 22 yrs, M, NA | - | 9 | 7 | Normal | No symptoms | 27.2 | 120/78 |
| Mother | 43 yrs, F, NA | - | 10 | 9 | Normal | No symptoms | 31.9 | 120/84 |
| Father's Sister | 50yrs, F, NA | - | 8 | 8 | Normal | No symptoms | 31.8 | 148/96 |
| Father's Sister | 45yrs, F, NA | - | 10 | 8 | Normal | No symptoms | 33.7 | 128/76 |

BMI: Body Mass Index; BP: Blood pressure; ECG:Electrocardiogram; MR:Mitral Regurgitation; SAM:Systolic Anterior Motion; PW: Posterior Wall; LV; left ventricular; mm;Milli meter; Sys:Systolic; Dia:Diastolic (Range for Normal BMI ≤ 25; Normal BP = 120/80).

Table 1: Clinical findings of the all family members.

reported, but cataloguing the same with clinical data will be a useful exercise for clinical practice with the larger picture of personalized genomic medicine in horizon.

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