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Türk Kardiyomiyopati Hastalarında Hipertrofik Kardiyomiyopati ile İlişkili Gen Paneli Kullanılarak Genotip ve Fenotip Analizi Yapılması

Genotype and Phenotype Analysis Using a Hypertrophic Cardiomyopathy-Associated Gene Panel in Turkish Cardiomyopathy Patients

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Öz

Giriş ve Amaç: Hipertrofik kardiyomiyopati (HCM), sarkomerik proteinlerdeki mutasyonların neden olduğu ve kalp kasının hipertrofisi ile karakterize otozomal dominant bir hastalıktır.
Gereç ve Yöntemler: Bu çalışmada, 21 HCM hastası ve bazılarının ebeveynleri, 17 genden oluşan hedeflenmiş bir panel kullanılarak yeni nesil dizileme aracılığıyla değerlendirilmiştir.
Bulgular: 6 hastada, MYH7 (p.R663C, p.A423V), MYBPC3 (p.P955fs*95, p.K301fs*31), TNNT2 (p.R154Q) ve TNNI3 (p.R204C) genlerinde patojenik veya yüksek olasılıkla patojenik varyantlar tespit edilmiştir.
Sonuç: Klinik bulgular literatür ile karşılaştırılarak bu varyantların genotip-fenotip korelasyonları tartışıldı. TNNI3 genindeki p.R204C varyantının literatürde ilk kez restriktif kardiyomiyopatiye neden olduğu saptanmıştır.

Anahtar Kelimeler: Hipertrofik kardiyomiyopati; MYH7; MYBPC3; TNNT2; TNNI3.

Abstract

Objective: Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disorder caused by mutations in sarcomeric proteins and characterized by hypertrophy of the heart muscle.
Materials and Methods: In the present study, 21 patients with HCM and some of their parents were evaluated via next-generation sequencing (NGS) using a targeted panel of 17 genes.
Results: Pathogenic or likely pathogenic variants were detected in six patients in the genes MYH7 (p.R663C, p.A423V), MYBPC3 (p.P955fs*95, p.K301fs*31), TNNT2 (p.R154Q), and TNNI3 (p.R204C).
Conclusion: The genotype-phenotype correlations of these variants were discussed by comparing the clinical findings with the literature. p.R204C variant in the TNNI3 gene was found to be caused restrictive cardiomyopathy for the first time in the literature.

Keywords: Hypertrophic cardiomyopathy; *MYH7*; *MYBPC3*; *TNNT2*; *TNNI3*.

1. Introduction

Hypertrophic cardiomyopathy (HCM) is a genetic disorder of cardiac myocytes that is characterized by unexplained left ventricular hypertrophy and a normal or increased ejection fraction. While asymmetric septal hypertrophy is the most common pattern of hypertrophy, the degree and location of hypertrophy are variable. Left ventricle (LV) hypertrophy can be concentric or confined to other walls or the LV apex [1]. Prevalence of HCM has been estimated at ≈ 1:500 in the general adult population [2]. HCM has a single genetic etiology with a locus heterogenity. While most cases are inherited in an autosomal dominant manner, autosomal recessive and X-linked modes of inheritance have been described but are rare [3]. Among the known causative genes, the two most common genes are MYH7 and MYBPC3, responsible for about half of patients with familial HCM [4]. Mutations

of TNNT2, TNNI3 (cardiac troponin I), and TPM1 (α-tropomyosin) are relatively uncommon causes of HCM and together are responsible for <10% of cases [5]. Mutations in some other genes have also been reported to be more rare for HCM, such as ACTC1 (cardiac α-actin), MYL2 (myosin light chain 2), MYL3 (myosin light chain3), and CSRP3 (cysteine- and glycine-rich protein 3) [6]. Unfortunately, patients with HCM can develop fatal complications, as therapeutic options are very limited, and patients develop progressive damage. Gene therapy is defined as the transfer of genetic material into cells for the treatment of a disease or to improve the clinical condition of a patient [7]. Since, hopeful results have been achived in the literature regarding gene-therapy for the hypertrophic cardiomyopathy, It is very importent to elucidate the causative mutation of the disease [8].In the present study, 21 patients with HCM and some of their parents were evaluated via next-generation sequencing (NGS) using a targeted panel of 17 genes (ACTC1, CALR3, DES, LAMP2, MYBPC3, MYH6, MYH7, MYL2, MYL3, PRKAG2, SLC25A4, TNNI3, TNNT2, RAF1, TPM1, TNNC1, TTN). This study aims to provide a genetic diagnosis of the patients in this cohort and discusses genotype-phenotype correlations of the patients according to the literature.

2. Materials and Methods

2.1.Patients

Informed consent was obtained from all the patients. A total of 18 patients who presented with HCM as their primary complaint at their first visit to our hospital were recruited. Exclusion criteria included secondary conditions such as heart valve problems and long-term high blood pressure. Variants of the analyzed genes and patients' clinical data, including age, symptoms, cardiac imaging, and family history, were analyzed. Ethics Committee approval was obtained from our hospital's Ethics Board (Date: 21/10/2021, Decision: 970) and written informed consent was obtained from each patient.

2.2 Sample collection and DNA isolation

Genomic DNA was isolated from peripheral blood specimens of patients using the QIAamp DNA Blood Mini Kit (Qiagen), according to the manufacturer's instructions.

2.3 Next-generation sequencing

Multiple amplicon DNA libraries were obtained using an assay kit (QIAseq Targeted DNA Panel) according to the manufacturer's instructions. Genomic DNA samples were first fragmented, end-repaired, and A-tailed within a single controlled multi-enzyme reaction. The prepared DNA fragments were then ligated at their 5′ ends with a sequencing platform-specific adapter containing unique molecular identifiers (UMIs) and a sample index. For enrichment, the ligated DNA molecules were subjected to several cycles of targeted polymerase chain reaction (PCR)using one region-specific primer and one universal primer complementary to the adapter. Universal PCR amplified the library and added platform-specific adapter sequences and additional sample indices. The libraries were then aggregated and sequenced using the Illumina NGS system (MiniSeq®, Illumina MiniSeq). Table 1 shows the genes included in the panel.

Table 1. Analysed genes in the panel

Analysed genes in the panel
ACTC1, CALR3, DES, LAMP2, MYBPC3, MYH6, MYH7, MYL2, MYL3, PRKAG2, SLC25A4, TNNI3, TNNT2, RAF1, TPM1, TNNC1, TTN

2.4 Analysis of next-generation sequencing data

FASTQ files were downloaded from BaseSpace and uploaded to the QIAGEN Data Analysis Centre, and the variants were called and annotated using the QIAGEN Ingenuity® Variant Analysis software (QCI®-A). The variants were filtered for the following criteria: call quality of at least 30, read depth of at least 50, and mutant allele fraction of at least 30%.

3. Results and Discussion

3.1. Results

Of the 18 patients studied, six patients were found to carry pathogenic or likely pathogenic variants, with two in the MYH7 gene, two in the MYBPC3 gene, and two in the TNNT2 and TNNI3 genes, and three patients were found to carry a variant of unknown significance, with two in the TTN gene and 1 in the MYBPC3 gene. The ages of the patients ranged from three to 46 years, and their symptoms included palpitations, chest pain, fatigue, and shortness of breath (Table 2).

3.2.Discussion

A 41-year-old male patient presented with a two-year history of palpitations. Cardiac imaging showed hypertrophic obstructive cardiomyopathy. The patient's mother had a history of cardiovascular disease, and the patient was found to carry the NM_000257.4:c.1987C>T (p.R663C) pathogenic heterozygous variant in the MYH7 gene. This variant had been determined pathogenic in the Clinvar database and located in a hot-spot of length 17 amino acids. In addition, a 42-year-old male patient presented with a 10-year history of palpitations. Cardiac imaging showed asymmetric septal hypertrophy. The patient's mother had similar symptoms and findings in her family history. The patient was found to carry NM_000257.4:c.1268C>T (p.A423V), a likely pathogenic heterozygous variant located in a hot-spot of length 17 amino acids in the MYH7 gene. This variant was previously not reported in the literature. The MYH7 gene encodes beta myosin heavy chain, which is found in heart muscle and in type I skeletal muscle fibers. Mutations of this gene cause a variety of diseases, including familial hypertrophic cardiomyopathy; left ventricular noncompaction; cardiomyopathy, dilated, 1S myopathy, myosin storage; and Laing distal myopathy [9–12]. Both mutations reported in this study lead to the familial hypertrophic cardiomyopathy phenotype, also

Table 2. Patients and Detected Variants

Patient no.	gene	variant	zygosity	Clinical significance	phenotype
1	-	-			36-year-old male with chest pain, palpitations, shortness of breath, hypertension; cardiac imaging found increased aneurysmatic convexity in the posterior lateral part of the right ventricle, pericardial and pleural effusion, movement defect in the interventricular septum, and mildly increased trabeculation in the left ventricle.
2	-	-			10-year-old female with fatigue and attention deficit; cardiac imaging found asymmetric septal hypertrophy.
4	*TNNT2*	NM_000364.4 c.461G>A p.R154Q	Het	LP	31-year-old female whose infant son had died because of dilated cardiomyopathy, white matter intensities in brain MRI, and electrolyte imbalance.
7	*TNNI3*	NM_000363.5:c.610C>T p.R204C	Het	P	17-year-old female with fatigue and shortness of breath; cardiac imaging found pericardial effusion, dilation of right atrium and right ventricle, and right heart failure.
8	*MYBPC3*	NM_000256.3:c.1008C>T p.I336I	Hom	VUS	3-year-old male with hypertrophic cardiomyopathy, cryptorchidism, hypotonia.
9	*TTN*	NM_003319.4:c.41015C>T p.A13672V	Het	VUS	50-year-old female with palpitations, chest pain during exercise, and history of heart attack in 3 generations on mother's side.
10	-				60-year-old female with palpitations, hypertrophic obstructive cardiomyopathy, and mitral regurgitation.
11	*MYH7*	NM_000257.4:c.1987C>T p.R663C	Het	P	41-year-old male with palpitations; cardiac imaging showed hypertrophic obstructive cardiomyopathy.
12	*TTN*	NM_003319.4:c.48893G>A p.R16298H	Het	VUS	Father of patients 13 and 15, healthy.
13	*TTN*	NM_003319.4:c.48893G>A p.R16298H	Het	VUS	13-year-old female with shortness of breath, palpitations; cardiac imaging was normal.
14	-				Mother of patients 13 and 15, healthy.
15	*TTN*	NM_003319.4:c.48893G>A p.R16298H	Het	VUS	six-year-old female with fatigue; cardiac imaging was normal.
16	-				13-year-old female with palpitations; cardiac imaging found mitral valve prolapse; family history of HCM in father.
17	-				Mother of 16, healthy.
18	*MYBPC3*	NM_000256.3:c.2864_2865 delCT p.P955fs*95	Het	P	46-year-old male with myocardial infarction; cardiac imaging found septal hypertrophy.
19	*MYBPC3*	NM_000256.3:c.901_902del AA p.K301fs*31	Het	P	20-year-old male with chest pain during exercise; cardiac imaging found septal hypertrophy.
20	*MYH7*	NM_000257.4:c.1268C>T p.A423V	Het	P	42-year-old male with palpitations; cardiac imaging found asymmetric septal hypertrophy, tricuspid regurgitation; patient's mother has same findings.
21	*TTN*	NM_003319.4:c.52225C>G p.P17409A	Het	VUS	17-year-old male with hypertension; cardiac imaging found hypertrophic obstructive cardiomyopathy.
22	-				38-year-old male with chest pain; cardiac imaging was normal.
23	-				7-year-old female; cardiac imaging found hypertrophic obstructive cardiomyopathy.
24	-				16-year-old male with palpitations; cardiac imaging found hypertrophic obstructive cardiomyopathy.

VUS: variant of unknown significance, LP: likely pathogenic, P: pathogenic, Het: heterozygous, Hom: homozygous, HCM: hypertrophic cardiomyopathy

known as asymmetric septal hypertrophy. In early stages, hereditary ventricular hypertrophy produces a presystolic gallop due to an atrial heart sound and EKG changes of ventricular hypertrophy.

Progressive ventricular outflow obstruction may cause palpitation associated with arrhythmia, congestive heart failure, and sudden death [13].

A 46-year-old male patient presented with a history of myocardial infarction since the age of 40. Cardiac imaging showed asymmetric septal hypertrophy. His cousin had a history of myocardial infarction at the age of 21. The patient was found to carry the NM_000256.3:c.2864_2865delCT (p.P955fs*95) pathogenic heterozygous variant in the *MYBPC3* gene. This variant was previously determined as a common mutation in the Netherlands causing HCM [14]. In addition, a 20-year-old male patient presented with chest pain during exercise. Cardiac imaging showed asymmetric septal hypertrophy. The patient was found to carry the NM_000256.3:c.901_902delAA (p.K301fs*31) pathogenic heterozygous variant in the *MYBPC3* gene; this variant was previously not reported in the literature. The *MYBPC3* gene encodes the myosin-binding protein C found in heart muscle. Mutations of this gene cause a variety of diseases, including cardiomyopathy, hypertrophic, 4; cardiomyopathy, dilated, 1MM; and left ventricular noncompaction 10 [13–17]. Both mutations reported in this study lead to HCM. Mutations in the *MYBPC3* gene are a common cause of familial hypertrophic cardiomyopathy, accounting for up to 30% of all cases. This condition is characterized by thickening (hypertrophy) of the cardiac muscle. Although some people with familial hypertrophic cardiomyopathy have no obvious health effects, all affected individuals have an increased risk of heart failure and sudden death [18].

A 17-year-old female patient presented with a three-year history of fatigue and shortness of breath during exercise. She also had a history of cardiac arrhythmia during tympanic membrane surgery. Cardiac imaging showed pericardial effusion, dilation of the right atrium and right ventricle, and right heart failure. Cardiac MRI result was compatible with restrictive cardiomyopathy. The results of *MEFV* gene sequence analysis were normal. On physical examination, facial and upper extremity muscles were atrophic. Muscle strength was normal, but areflexia was detected. The results of electromyography were normal The patient was found to carry the NM_000363.5:c.610C>T (p.R204C) pathogenic heterozygous variant in the *TNNI3* gene. Mutations in the *TNNI3* gene are associated with restrictive, dilated, and hypertrophic cardiomyopathies. Two HCM mutations are known to occur at *TNNI3*, arginine 204, R204C and R204H, and both mutations are associated with a poor clinical prognosis. Of the mutations on *TNNI3*, the mutation at the arginine residue at position 204 is the most common mutation associated with familial HCM. The R204H mutation is also associated with restrictive cardiomyopathy (RCM) [19]. In this study, it was reported for the first time that the R204C mutation can also lead to the RCM phenotype.

A 31-year-old female presented without symptoms herself, but she had a son who died in infancy and had dilated cardiomyopathy ,electrolyte imbalance, and white matter hyperintensities on brain MRI. The mother was found to carry the NM_000364.4:c.461G>A (p.R154Q) likely pathogenic heterozygous variant in the *TNNT2* gene. This variant is a novel missense change at an amino acid residue where a different missense change (p.R154W) determined to be pathogenic has been seen before [20]. Although it is not known whether there is evidence of cardiomyopathy in the heart since echocardiography could not be performed on the woman, this variant was considered to be a likely pathogenic variant according to the ACMG criteria.

Conflict of interest: None declared.

No financial support was received for this study.

4. Conclusion

In this study, pathogenic variants obtained with targeted gene panel in patients with hypertrophic cardiomyopathy and clinical findings of patients were analyzed. The R204C mutation was found that it can also lead to the RCM phenotype.

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