The variations of BOP gene in hypertrophic cardiomyopathy

Hipertrofik kardiyomiyopatide BOP geni varyasyonları

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ABSTRACT

Objective: The observation that Bop null allele mice show underdeveloped right ventricle and excessive development of left ventricle, suggests the possible relationship between human BOP gene and hypertrophic cardiomyopathy (HCMP). In our study, we investigated this possible relationship between BOP gene variations and QT dispersion, a noninvasive arrhythmic risk marker for HCMP.
Methods: This cross-sectional study consisted of 50 patients clinically diagnosed with HCMP and 60 healthy subjects. Exonic regions of BOP gene were amplified by polymerase chain reaction and amplified exonic regions were analyzed by Single-Strand Conformation Polymorphisms (SSCP). The samples with different migration patterns were sequenced through an automated sequencing system. Continuous variables were compared by unpaired t-test for independent samples or Mann-Whitney U test. Genotype-disease relationship was tested by Chi-square test.
Results: The nucleotide substitutions G275>A and C965>A in exon 2 and 7 were determined only in HCMP group. The G707>C, C710>T, T761>C, T1217>C SNPs in exon 6 and 9 are also found in the control group. Significant differences were found between two groups ($p=0.002$ and $p<0.001$). It was found that SNPs in exon 6 constitute a haplotype and that QT dispersion and corrected QT dispersion in the rare homozygote (707C/710T/761C) type carriers of HCMP patients for this haplotype were significantly lower than other genotypes ($p=0.032$ and $p=0.030$, respectively).
Conclusion: The human BOP gene was analyzed for the first time in HCMP to investigate possible association. The result that homozygosity of 707C/710T/761C haplotype is associated with lower QT dispersion and corrected QT dispersion supports the modifier role of BOP gene in HCMP. *(Anadolu Kardiyol Derg 2010; 10: 303-9)*
Key words: BOP, hypertrophic cardiomyopathy, clinical heterogeneity, modifying gene, genetic variation, QT dispersion

ÖZET

Amaç: Bop mutant farelerde, az gelişmiş sağ ventrikül ve aşırı büyümüş sol ventrikül varlığı, insan BOP geni ile hipertrofik kardiyomiyopati (HKMP) arasında olası bir ilişkiyi düşündürmektedir. Bu çalışmamızda, BOP genindeki varyasyonlar ile HKMP için noninvazif bir aritmik risk göstergesi olan QT dispersiyonu arasındaki olası ilişkiyi araştırdık.
Yöntemler: Enine-kesitli niteliğindeki çalışmaya 50 HKMP'li hasta ve 60 sağlıklı kontrol alındı. BOP geni ekson bölgeleri, polimeraz zincir reaksiyonu (PCR) ile çoğaltıldı ve çoğaltılan ekson bölgeleri, Tek İplikli Konformasyon Polimorfizm (SSCP) analizi ile incelendi. Farklı bantlara sahip örnekler otomatik dizileme yöntemi kullanılarak dizilendi. Sürekli değişkenler eşleştirilmemiş bağımsız örneklem t-testi ya da Mann-Whitney U testi ile karşılaştırıldı. Genotip-hastalık ilişkisi Ki-kare testi ile test edildi.
Bulgular: Ekson 2 ve 7'deki G275>A ve C965>A tek nükleotid değişimleri (TND) sadece HK grubunda belirlendi. Ekson 6 ve 9'daki G707>C, C710>T, T761>C, T1217>C TND'lerine kontrol grubunda da rastlandı. İki grup arasındaki fark ise anlamlı bulundu ($p=0.002$ ve $p<0.001$). Ekzon 6'daki TND'lerin haplotip oluşturduğu ve bu haplotipin nadir alleli (707C/710T/761C) için homozigot taşıyıcı olan HK hastalarında QT dispersiyonu ve düzeltilmiş QT dispersiyonu, diğer genotiplere göre anlamlı derecede düşük bulundu ($p=0.032$ ve $p=0.030$).
Sonuç: Bu çalışma, insanda BOP geni ile HKMP arasındaki ilişkiyi araştıran ilk çalışmadır. Üç farklı TND'yi içeren 707C/710T/761C haplotip takımını taşıyan HK'li olgularda QT dispersiyonu ve düzeltilmiş QT dispersiyonunun anlamlı derecede uzamış bulunması, BOP genindeki bu haplotipin ventiküler aritmilerle ve dolayısı ile ani ölümle ilişkili olabileceğini akla getirmektedir. *(Anadolu Kardiyol Derg 2010; 10: 303-9)*
Anahtar kelimeler: BOP, hipertrofik kardiyomiyopati, klinik farklılık, modifiye edici gen, genetik varyasyon, QT dispersiyonu

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Introduction

Hypertrophic cardiomyopathy (HCMP) is an inherited heart disease, which impairs myocardial function and leads heart failure or sudden death. HCMP is caused by mutations in genes which code for cardiac sarcomeric proteins. Although same mutation is generally responsible for the disease in a certain family affected by HCMP, clinical presentation of the disease shows diversity between patients in the same family. This diversity suggests the presence of modifier genes (1, 2).

Mice Bop gene is located in close proximity to CD8b gene. Because of it's head-to-head arrangement with CD8b gene, this gene is called as Bop (CD8b opposite) (3). Human BOP gene (also known as SET and MYND domain-containing protein 1 - SMYD1) is localized to chromosome 2p11.2 and codes for a 490 amino acid protein. Two transcript forms are known to be expressed from this gene in different cell types. The form expressed in cytotoxic T cells is called as t-Bop and the form expressed in skeletal and heart muscle is called as skm-Bop (M-Bop) (4). There are two isoforms of skm-Bop in mice and chicks, which are called as skm-Bop 1 and skm-Bop 2 that differ by an insertion of 13 amino acids in skm-Bop 2. The Bop protein contains SET and MYND domains, which have been shown to regulate transcription by mediating distinct chromatin modification. Bop gene is expressed in cardiac precursor cells beginning at E8.0 embryonic stage in mice. Expression of Bop gene is maintained throughout the linear and looping heart tube, as well as in the atrial and ventricular chambers of the mouse heart. It has been demonstrated that Bop null allele mouse embryos showed structural retardation during E 9.5 embryonic stage and could live up to E 10.5 embryonic stage. In $Bop^{-/-}$ embryos, right ventricle has not been developed and left ventricle showed excessive development (5). These observations suggest that Bop expression is important for cardiomyocyte differentiation and cardiac morphogenesis. Targeted deletion of Bop in mice disrupted maturation of ventricular cardiomyocytes and interfered with formation of the right ventricle (5). Mef2C, the transcription factor which regulates Bop gene in developing heart, is also known to be involved in hypertrophic response in the adult heart (6).

Bop seems to take a part not only in structure, but also in function of the heart. Rhythmic cardiac contraction is mediated by coordination between depolarization and repolarization of myocardium. This harmony depends on unequal distribution of ion channels through myocardium (7- 9). One of these ion channels is potassium channel protein, Kv4.2. Kv4.2 concentration decreases through myocardium, from epicardium with high Kv4.2 to endocardium with low Kv4.2 (10- 12). Costantini et al. (13) showed that myocardial gradient of Kv4.2 is mediated by Irx5 dependent transcriptional repression. Irx5 dependent Kv4.2 repression, which results on inverse gradients between Irx5 and Kv4.2 across the ventricular myocardium, is main determinant of the rhythmic cardiac contraction. In the same study, it was shown that this transcriptional repression depends on mBop mediated interaction between Irx5 and HDAC (histon deacetilase) (13).

Coordination between depolarization and repolarization of the myocardium could be affected by certain pathological conditions. These pathological conditions might cause changes in waves of surface electrocardiography (ECG). One of these changes of ECG waves is QT dispersion. QT dispersion (QTd) (14-17) have been used previously as noninvasive prognostic tools in the evaluation and risk stratification of HCMP patients. Currently available studies report controversial results regarding QTd and prognosis in HCMP. QTd (14) and QT prolongation (15) have been reported in HCMP previously. Maron et al. (16) found that QT dispersion has no prognostic value in term of sudden death in patients with HCMP. Göktekin et al. (17) have shown no prognostic information from QTd regarding risk factors for sudden cardiac death in patients with HCMP.

Considering all data, human homologous of Bop gene (BOP) seems to be candidate modifier gene for HCMP in which both cardiac hypertrophy and cardiac contraction have central role. We supposed that, due to relationship of Bop gene with cardiac hypertrophy and arrhythmia in mice, BOP gene could play role in clinical presentation of HCMP in humans.

In this study, we aimed to investigate whether there is a relationship between sequence variations in BOP gene and QTd as an noninvasive risk marker for HCMP.

Methods

Study population

This cross-sectional study was approved by the local Ethics committee and each participant gave written informed consent after appropriate genetic counseling. The study consisted of 50 patients (30 males, 20 females; mean age 47±17 years; range 17 to 74 years) with clinically diagnosed HCMP. All the patients were evaluated with a detailed history, physical examination, 12-lead electrocardiography and transthoracic echocardiography. Gender matched 60 healthy subjects (30 males, 30 females) with normal transthoracic echocardiograms were also included as controls. The diagnosis of HCMP was based on the demonstration of a hypertrophied, non-dilated left ventricle (wall thickness of at least 15 mm) by two-dimensional echocardiography, in the absence of other cardiac or systemic diseases that might produce hypertrophy of similar degree (19).

Genetic analysis

Taq DNA polymerase was obtained from Roche (MBI Fermentas, Hanover, MD). All chemicals used in polymerase chain reaction (PCR), gel electrophoresis and SSCP analysis were obtained from Sigma-Aldrich (Stockholm, Sweden), Merck (Darmstadt, Germany) and AppliChem GmbH (Darmstadt, Germany).

DNA samples were isolated from the peripheral blood using standard ammonium acetate method. Primers (Table 1) from flanking intronic sequences for all exons of the human BOP

Table 1. Primers used for the amplification of BOP gene

Exons	Oligonucleotids (5'->3')	Length,	Annealing
Exon 1	F: TTA AAT AAC TGC CGC GCT G R: TAA CAG GAG AGA AGG CAA C	208	55°C
Exon 2	F: GTC TTC TTT CTC CAT TTC CA R: GAA ATC TGA AGC ACC ACC A	234	54°C
Exon 3	F: CCT CCT GAC GCT GCC CTT C R: AGG AGA CAG ACA GGG AGG AT	284	60°C
Exon 4	F: ACT CTT TCA TCT TTT CCC CT R: CAT CCC CTT CAC ACA CAC	196	54°C
Exon 5	F: GGG TGT CTG TTT TGT CTT TC R: CCA GGT AAG TGA GGG AGA T	118	55°C
Exon 6	F: ATT CAC CTT TTC CTT TCA CCC T R: CGG AGA AGA CAG ACA CAA CAC C	263	56°C
Exon 7	F: GCT CAA TGT GTC TCT CTT TCC R: TCC TTT CTA CCC CCA TCC TC	157	58°C
Exon 8	F: GCA ATG GTA ATG GGC AGG R: ATA GCC GTC CAC CAT CCT	197	57°C
Exon 9	F: GGG TGA TTG GTA TGA TGT AT R: GAA CAC CAG CCT CAA GAC G	238	59°C
Exon 10	F: GTG TGT GTG TAT TCT GAG G R: CCC GAG AGA CCC ACC AC	291	58°C

gene were designed using Primer 3 software. Specificity of the primers was checked by using the 'BLAST'' program at http://www.ncbi.nlm.nih.gov/blast.

PCR amplification was carried out in a DNA Thermal Cycler (MJ Research Techne). SSCP analysis was performed using non-denaturing polyacrylamide gels on the Owl Separation Systems (Thermo Scientific, Rochester, NY). Bands were visualized by silver-staining method using standard protocols.

Electrocardiographic analysis

The ECG recordings were taken with a paper speed of 50 mm/sec at normal filtering. Several ECG parameters were measured manually. QRS duration was defined as the maximum QRS duration in any lead from the first to the final sharp vector crossing the isoelectric line. QT interval was measured from the lead II using calipers. QT interval was defined as the interval between the beginning of QRS complex and the end of T wave. The onset and offset of T wave were defined as the intersections of the isoelectric line and the tangent of the maximal slope on the up and down limbs of T wave, respectively. Care was taken to avoid U waves in any measurement, and when U waves were present, the end of T wave was taken as the nadir between T and U waves. Three consecutive cycles were measured in each of the standard 12 leads and a mean value was calculated from the three values. The JT interval was then calculated by subtracting QRS from QT in individual leads. Bazett's formula was used to obtain corrected QT and these were represented as QTc. The dispersion of QT intervals was defined as the difference between the maximum and minimum of QT interval, which could be measured in any of the 12 ECG leads and was represented as QTd. At the time of QT evaluation, all patients were hemodynamically stable and none had electrolytic disturbances, atrial fibrillation or significant intraventricular conduction defects.

Statistical analysis

SPSS for Windows 10.0 (SPSS, Inc., Chicago, IL, USA) and the Microsoft Excel were used for statistical analysis. The frequencies of the alleles and genotypes were compared among patients and control groups using the Chi-square and the Fisher tests when appropriate. Prevalence odds ratios (OR) considered as prevalence of existing disease and 95% confidence intervals (CIs) were calculated using the normal approximation. Continuous variables were expressed as mean±standard deviation (SD) and were compared with a two-tailed t-test for independent samples or Mann-Whitney U test. Mann-Whitney U test was used to compare continuous variables with skewed distributions such as cQT dispersion and QT dispersion. Statistical significance was considered when $p<0.05$.

Results

The evaluation of clinical data of patients is shown in Table 2. SSCP analysis of each one of the exons in BOP gene demonstrated the samples with possible mutation or polymorphism, and those

samples were sequenced. In addition to known substitutions, which have been informed to database before, novel nucleotide substitutions were identified as well. Two undefined substitutions, namely, G275>A, and C965>A were identified in the BOP gene and other identified four substitutions were confirmed as known substitutions, namely G707>C, C710>T, T761>C and T1217>C. The location of these nucleotide substitutions in relation to genomic structure of the BOP gene is shown in Figure 1. These polymorphisms do not cause any change at amino acid sequence of BOP.

The comparison of genotypic frequencies of each polymorphism between patients and controls is shown in Table 3. G707>C, C710>T, T761>C known substitutions in exon 6 were defined in both heterozygote and homozygote types. In this study, the three SNPs in exon 6 of BOP gene at nucleotides 707 (G or C), 710 (C or T) and 761 (T or C) were found to be linked with each others in genotyped every subjects. The genotype distributions of the SNPs in exon 6 and exon 9 were statistically significant between patients and controls ($p<0.005$, Table 3). In the exon 6, the frequency of homozygote wild type (NN=707GG/710GG/761CC) was 68% (34/50) in the patient group, whereas it was 91.7% (55/60) in the control group; heterozygote type (NM= 707GC/710GC/761 CT) frequency was 22 % (11/50) in patients and 5% (3/60) in the controls and homozygote mutant type (MM= 707CC/710CC/761TT) frequency was 10% (5/50) in the patients and 3.33% (2/60) in the controls ($p=0.002$) (Table 3).

QT dispersion and corrected QT dispersion of HCMP patients with 707CC/710TT/761CC genotype of linked 707G>C, 710C>T, and 761T>C polymorphisms in exon 6 were found to be significantly lower ($p=0.03$ and $p=0.032$) than in the others genotypes (GG/CC/TT and GC/CT/TC) (Table 4).

For the polymorphism identified in the exon 9, the frequency of homozygote wild type (TT) was 61.23% (30/49) in the patient group, whereas it was 91.07% (51/56) in the controls. Heterozygote type (TC) of the same polymorphism was 24.49% (12/49) in patients and 8.93% (5/56) in the controls and homozygote type for rare allele (CC) was 14.28% (7/49) in the patients ($p= 0.002$).

G275>A nucleotide substitution in exon 2 was found only in one HCMP patient (1/50) and C965>A nucleotide substitution in exon 7 was found in three HCMP patients (3/49) (Fig. 1). Both of the single nucleotide changes were found as heterozygous. These substitutions were not detected in the control group. The heterozygote patient for the G275>A substitution had a clinical feature with asymmetric and non-obstructive type of hypertrophy without family history. Clinical features of three heterozygote patients carrying the 965C>A substitution include non-obstructive asymmetric septal type, obstructive asymmetric septal type and obstructive concentric type hypertrophy. Only two patients of these had family history for HCMP, but did not have sudden-death history in their family.

Discussion

The main observation of this study was that sequence variations in human BOP/SMYD1 gene are related to QT dispersion and cQT dispersion in HCMP patients. This observation supports the hypothesis that BOP/SMYD1 gene might be a modifier gene for HCMP. However, due to controversial results on prognostic value of QT dispersion in HCMP patients, clinical importance of this observation is open the question.

In this study, we have demonstrated that electrocardiographic presentation of HCMP is influenced by the allelic vari-

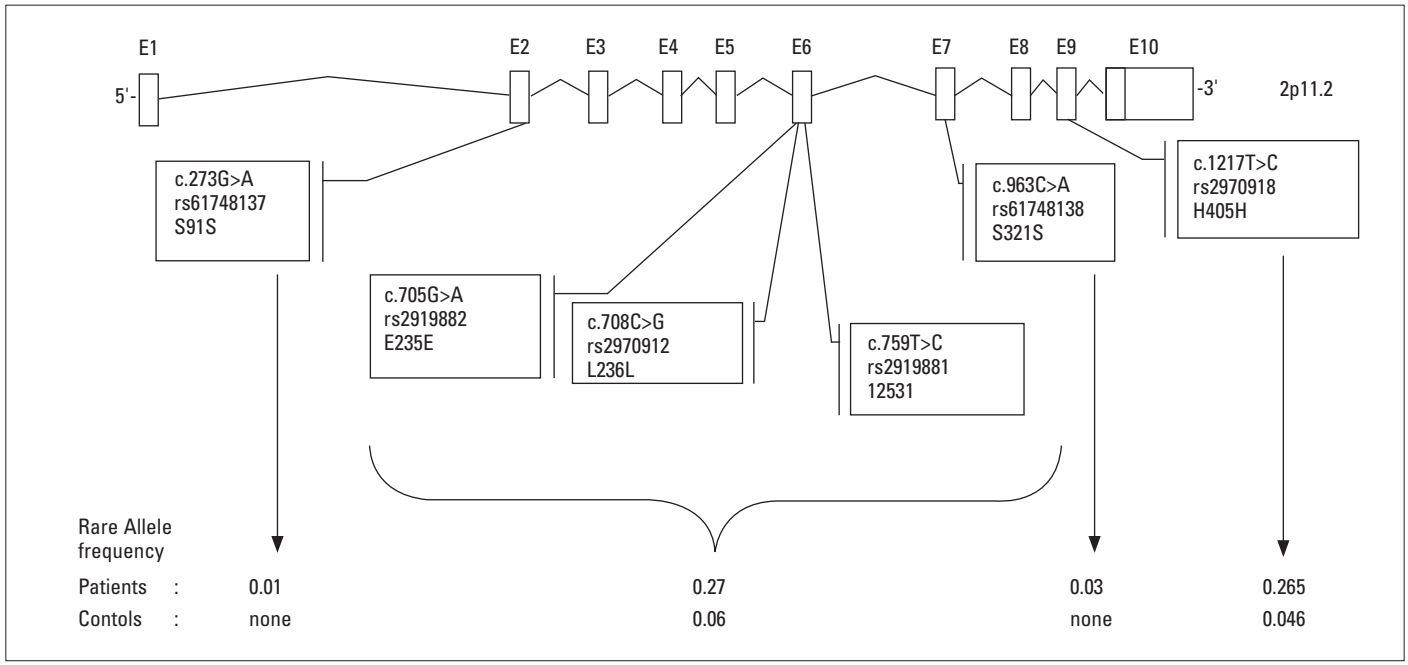

Figure 1. Schematic structure of the human BOP gene showing the localization of six nucleotide substitutions identified in this study and the rare allele frequencies of these substitutions in HC MP patients and controls.

E - exon, HCMP - hypertrophic cardiomyopathy

Table 2. Clinical and echocardiographic characteristics of patients with hypertrophic cardiomyopathy

Variables	n	%	Mean±SD	Range
Age, years			47±17	17-74
Male gender	30	60		
Family history				
Hypertrophic cardiomyopathy	15	30		
Sudden death	11	22		
Clinic status				
Symptomatic	42	84		
Asymptomatic	8	16		
Left ventricle				
End-systolic diameter, cm			2.46±0.54	
End-diastolic diameter, cm			4.36±0.67	
Maximal wall thickness, cm			2.5±0.54	
Ejection fraction, %			75.42±9	51-93
Left atrium size, cm			4.6±0.7	2.9-6.5
QRS duration, msec			121.49±27.25	
QT dispersion , msec			75.41±25.53	
Corrected QT dispersion, msec			82.97±26.52	

Continuous variables are presented as mean±SD, dichotomous variables as percentages
SD - standard deviation

Table 3. The genotypes distributions of the SNPs in exon 6 and 9 in patients and controls

Genotype	Patients (n=50)	%	Exon 6 Controls (n=60)	%	p	Genotype	Exon 9 Patients (n=49)	%	Controls (n=56)	%	p
NN*	34	68	55	91.7	0.002	TT	30	61.2	51	91.1	0.002
NM**	11	22	3	5		TC	12	24.5	5	8.9	
MM***	5	10	2	3.3		CC	7	14.3	0	0	
NN	34		55		0.002	TT		30		51	<0.001
NM and MM	16		5			TC and CC		19		5	
OR, (95%CI)				5.18 (1.738-15.418)				6.46 (2.186-19.088)			

Data are presented as proprotionspercentages
Chi-square-test
* 707GG/ 710 GG/ 761CC homozygotes (wild type) for three SNPs in exon 6
** 707GC/710 GC/761 CT heterozygotes for three SNPs in exon 6
*** 707CC/710 CC/761 TT homozygotes for three SNPs in exon 6

Table 4. The distribution of cQT dispersion and QT dispersion in exon 6 haplotypes

Genotype	n=50	cQT Dispersion, ms	p	QT Dispersion, ms	p
MM*	5	54.00±16.73	0.030	60.25±21.33	0.032
NM and NN**	45	77.84±25.34		85.55±26.00	

Continuous variables are presented as mean ± SD
Mann-Whitney U test
* 707CC/710 CC/761 TT homozygotes for three SNPs in exon 6
**707GG/710 GG/761CC homozygotes (wild type) and 707GC/710 GC/761 CT heterozygotes for three SNPs in exon 6

ants of the BOP/SMYD1 gene. Furthermore, we observed that BOP gene 705A/708G/759C haplotype is associated with QT dispersion and corrected QT dispersion in HCMP. Moreover, it is the first report to describe the novel BOP variants (c275G>A, and c.965C>A) in HCMP.

Since HCMP is mostly hereditary disease, it is important to give medical and genetic advice to other members of the patient's family. However, it is difficult to predict clinical outcomes of the disease, because the HCMP has clinical heterogeneity. Therefore, environmental and genetic factors, which could

contribute to the heterogeneity of the HCMP have importance for physicians.

Although HCMP is a genetic disease, the exact molecular mechanism of the hypertrophy is not known. Most HCMP patients are supposed to carry mutations in their genes coding for contractile proteins like beta myosin heavy chain or tropomyosin. But, mutations in all these defined genes could not explain the clinical diversity of the disease alone. Therefore, polymorphisms in other genes, which play role in cardiac hypertrophy, cardiomyocyte differentiation or cardiac function, are believed to be modifier genes for the HCMP. Although, in general, polymorphisms at modifier genes do not cause disease directly, combinations of these polymorphisms are believed to ensure predisposition or protection for the disease, thus diversification of clinical presentation of a disease (1, 2).

Defective development of right ventricle and excessive development of the left ventricle in the absence of Bop gene expression in mice, might support the idea that human BOP gene might play role in HCMP pathogenesis (1, 2). Therefore, we attempted to understand whether human BOP gene might be a modifier gene for HCMP. In this purpose, we analyzed all coding regions of human BOP gene both in the patients diagnosed with HCMP and in the healthy controls.

For the first time we examined all exons of the human BOP gene and investigated unknown and known single nucleotide changes G275>A, G707>C, C710>T, T761>C, C965>A and T1217>C. The fact that no nucleotide substitutions we identified in coding regions of the gene, caused any change at amino acid sequence, shows the protein-level conservation of the BOP.

In our result, the known substitutions in exon 6 and exon 9 were significantly different between HCMP patients and controls. Especially rare alleles of both exons had higher incidence in patient group. However, comparison between genotypes and type or localization of hypertrophy did not show any significance. These results suggest the possible relationship between genetic variations at the BOP gene and clinical manifestation of the HCMP.

In this study, we first showed that human BOP gene might be modifier gene for HCMP. The linked rare allele of three SNPs at exon 6 of human BOP gene had significantly relationship with QT dispersion values of HCMP patients. QT dispersion and corrected QT dispersion in the homozygote for rare type of HCMP patients (MM) were significantly lower than heterozygote and wild type homozygote (p=0.03 and p=0.032, respectively). It seems likely that existence of haplotype with homozygote for rare allele at exon 6 have protective role against QT dispersion in HCMP patients. This effect of BOP gene might be through regulating Irx5 dependent Kv4.2 repression at cardiac myocardium (13). However, the molecular mechanism of BOP dependent shortening of QT dispersion needs to be elucidated.

Although, there are many controversial studies on it's prognostic value (14-18), QT dispersion is supposed to be clinically important measure for heart diseases. Both QT dispersion and corrected QT dispersion are accepted as an indicator of arrhythmic events and general abnormality of repolarization (20-23). Therefore, the factors, which change QT dispersion might effect clinical presentation of the disease like HCMP and arrhythmic heart diseases. Individual response to arrhythmic events of HCMP could be explained by SNPs at modifier genes. Significant difference of QT dispersion within HCMP patients, who were separated according to haplotype at exon 6 of BOP gene, indicates that BOP gene might be one of these modifier genes.

Costantini et al. (13) showed that the production of the atrial natriuretic factor (ANF) which was the most important feature of HCMP, increased when the expression of the BOP in HCMP patients increased. Possible role of the BOP gene in cardiac hypertrophy was shown to be increased with elevated production of ANF, one of important markers of HCMP. The appearance of arrhythmia in Irx5 mutant mice and the fact that Kv4.2, which is repressed by Bop through Irx5, regulates heart rhythm, suggest that Bop might have a role not only in HCMP, but also in arrhythmic heart diseases (13).

Three SNPs in exon 6 (G707>C, C710>T and T761>C) seem like to be inherited as a haplotype block. As this haplotype block shows a statistically significant relationship with HCMP, it could be claimed that one of those substitutions or other undefined substitution within this haplotype block might affect gene function. Because there was not found any amino acid change in exon 6, this possible affect should be through quantitative trait like mRNA expression level or translation ratio.

To elucidate the possible modifier role of BOP gene in the HCMP, we analyzed all coding regions of the human Bop gene at 50 HCMP patient and 60 control. Although we did not found mutation, which could change protein structure, we identified known and undefined SNPs in both coding and non-coding regions of the gene. Our results indicate the possible modifier role of BOP gene in HCMP.

Study limitations

Study group: As the diagnosis of HCMP requires advanced cardiological techniques and exclusion of other possible causes of cardiac hypertrophy, patient number of recent study was limited.

Technical: Because primers were designed from intronic regions near to exon-intron boundaries of BOP gene to analyze coding regions, introns and regulatory elements were not included to this study. Therefore, polymorphisms in intronic and regulatory regions, which have importance for gene expression level, had to be excluded. However, many studies have shown that polymorphisms at noncoding regions might effect gene function through gene expression level, RNA splicing, RNA conformation or mRNA decay. Substitutions in the noncoding regions have also been found to be related to some disease such as beta thalassemia, in which many intronic mutations of beta-globin gene are known to cause the disease by RNA splicing and exon skipping (24).

Another limitation of this study was that other modifier genes were excluded. Our study is a preliminary one to pioneer next studies. Modifier role of BOP gene in the HCMP will be confirmed by future studies with higher number of patients.

In addition to HCMP, BOP might have modifier role in other cardiac disease, especially that which are related to cardiac rhythm and QT dispersion. Therefore, studies with other cardiac disorders are needed to confirm modifier role of BOP gene in other cardiac diseases.

Conclusion

In conclusion, though we have not found any mutation in BOP gene, it seems like that human BOP gene plays role in HCMP as a modifier gene. To elucidate the modifying role of BOP gene in HCMP, noncoding regions like regulatory sequences and intronic sequences of human BOP gene and expression level in cardiac tissue material from HCMP patients need to be analyzed in further studies. In addition to HCMP patients, other patients with heart disease related to depolarization abnormality might also be subjected to studies on BOP gene.

This study showed that BOP is a conserved gene at the level of protein structure. No mutation in human BOP gene, has been not found yet. On the other hand, it's function in the cardiomyocyte development and in the heart contractility suggest that BOP gene might be a modifier gene for heart diseases like HCMP or arrhythmic heart diseases. For these reason it might be important to analyze polymorphisms in promotor region and splicing sites of this gene.

The human BOP gene was analyzed for the first time in HCMP to investigate possible modifier role. QT dispersion and corrected QT dispersion in homozygote mutant carrier for 707C/710T/761C allele were significantly lower than in homozygote wild and heterozygote carriers. This haplotype may be is related to ventricular arrhythmia and sudden death.

Conflict of interest: None declared.

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