Association between non-coding polymorphisms of *HOPX* gene and syncope in hypertrophic cardiomyopathy

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Abstract

Objective: Homeodomain Only Protein X (HOPX) is an unusual homeodomain protein which regulates Serum Response Factor (SRF) dependent gene expression. Due to the regulatory role of *HOPX* on SRF activity and the regulatory role of SRF on cardiac hypertrophy, we aimed to investigate the relationship between *HOPX* gene variations and hypertrophic cardiomyopathy (HCM).

Methods: In this study, designed as a case-control study, we analyzed coding and flanking non-coding regions of the HOPX gene through 67 patients with HCM and 31 healty subjects. Certain regions of the gene were investigated by Single Stranded Conformation Polymorphism (SSCP) and Restriction Fragment Length Polymorphism (RFLP). Statistical analyses of genotypes and their relationship with clinical parameters were performed by chi-square, Kruskal-Wallis and the Fisher's exact test.

Results: In 5' Untranslated Region (UTR) and intronic region of the *HOPX* gene, we found a C>T substitution and an 8-bp insertion/deletion (In/Del) polymorphism, respectively. These two polymorphisms seemed to constitute an haplotype. While the frequency of homozygous genotypes of In/Del and C/T polymorphisms were found significantly lower in the patients with syncope (p=0.014 and p=0.017, respectively), frequency of their heterozygous genotypes were found significantly higher in the patients with syncope (p=0.048 and p=0.030, respectively).

Conclusion: Though there was not found any mutation in coding sequence of *HOPX* gene, two non-coding polymorphisms were found related to syncope in HCM patients. While homozygous status of these polymorphisms was found to be protective against the syncope, their heterozygous status seemed to be a risk factor for syncope in HCM patients. Our results suggest that *HOPX* may contribute to pathogenesis or manifestation of HCM as a modifier gene. *(Anadolu Kardiyol Derg 2014; 14: 617-24)*

Key words: *HOPX*, hypertrophic cardiomyopathy, syncope, modifier gene, polymorphism

Introduction

Hypertrophic cardiomyopathy (HCM) is a familial disorder characterized by left ventricular hypertrophy in the absence of any cardiac or systemic disease which may cause cardiac hypertrophy (1). Most causal mutations in HCM have been identified in genes encoding sarcomeric proteins (2). More than 900 gene mutations have been found to be responsible for HCM, so far. Although most of those mutations were identified in genes which encode for proteins of the thick and thin filaments of the sarcomere, such as cardiac myosin binding protein C (MYBPC3), beta-myosin heavy chain (MYH7), troponin T (TNNT2) and troponin I (TNNI3) mutations in some non-sarcomeric genes were also found responsible for HCM (3). In addition to sarcomeric and non-sarcomeric gene mutations, many other metabolic gene mutations result in metabolic disorders which have similar phenotype with HCM (4).

The clinical course of HCM is characterized by a large inter- and intra-familial variability, ranging from severe symptomatic to asymptomatic individuals (5, 6). This clinical variability of the HCM is explained by environmental and genetic modifiers. Identification of these environmental and genetic modifiers is important for prognosis and treatment of the disease.

Histologically, HCM is characterized by left ventricular tickness which is consequent of cardiomyocyte hypertrophy. Since the cardiomyocytes loss their ability of division at first week after the birth, postnatal growth of the heart is performed by cardiomyocyte hypertrophy rather than cardiomyocyte proliferation (7, 8). In addition to its role on the post-natal heart growth, cardiomyocyte hypertrophy or cardiac hypertrophy, is also known to be a physiological response of myocardium to stress signals. This response of cardiomyocytes to stress signals is charecterizied by reactivation of fetal gene program (9).

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SRF, a member of the MADS (MCM1, Agamous, Deficiens, SRF) box family of transcription factors, is one of the developmental genes which are re-activated during cardiac hypertrophy (10). Binding to serum response element (SRE) which includes CArG box, SRF controls the transcription of genes including cellular immediate early genes, cytoskeletal and contractile proteins (11, 12). Activity of the SRF itself is also under the control of some co-activators like myocardin (13) and co-repressors like *HOPX* (14).

HOPX gene codes for an unusual type of homeodomain protein and its expression is under the control of two promotors, one of which is regulated by cardiac specific transcription factor Nkx2-5 (15). *HOPX* protein is known to act as an antagonist of SRF in pre-natal cardiomyocyte proliferation and post-natal cardiomyocyte hypertrophy (14). This antagonistic action of *HOPX* on SRF-mediated transcription is mediated by recruiting histone deacetylase (HDAC) (16).

In some other tissues, however, *HOPX* is supposed to act as a tumor supressor gene, since it is expression was reported to be silenced or down-regulated in variable types of human carcinoma such as choriocarcinoma, lung cancer, head and neck squamous carcinoma and esophageal cancer (17-21).

In the heart muscle, *HOPX*, like myocardin, is known mainly as a regulator protein of SRF.

Due to its co-activator role on SRF activity, myocardin is thought to contribute to heart hypertrophy. This was shown in animal models (22). Similarly, due to its co-repressor role on SRF activity, we hypothesed that *HOPX* contributes to cardiac hypertrophy and HCM. Though *HOPX* gene expression was shown to be down-regulated in heart failure (23), there is not known any relation between *HOPX* gene variations and any heart disease, like heart failure or HCM. Since, the *HOPX* protein is not a component of the sarcomere, it is expected that *HOPX* plays role in HCM as a modifier gene rather than a disease-causing gene.

Although, the diversity of clinical parameters and manifestations like left ventricular wall diameter, ejection fraction, QRS duration, QT dispersion, appearance of syncope or sudden death through the HCM patients remain to be explained, the variations or the polymorphisms in modifier genes are supposed to be responsible for this diversity. In this study, to investigate the possible association between sequence variations of the *HOPX* gene and the diversity of clinical manifestations of the HCM, we analyzed coding regions of the *HOPX* gene in HCM patients with and without syncope, one of the clinical manifestations of HCM.

Methods

Study design

In this case-control study, we analyzed coding and flanking non-coding regions of the *HOPX* gene through patients with hypertrophic cardiomyopathy and healty subjects. To investigate the possible involvement of *HOPX* gene variations in HCM, we designed SSCP and RFLP analyses of certain regions of the gene, followed by statistical analyses of genotypes and clinical parameters.

Study population

This study included 67 patients (40 males, 27 females; mean age 47.94±15.62 years; range 17 to 74 years) who were diagnosed as hypertrophic cardiomyopathy at a tertiary referral hospital between 2002 and 2005.

All the patients were evaluated with a detailed history, physical examination, 12-lead electrocardiography and trans-thoracic echocardiography. The diagnosis of HCM was based on the demonstration of an unexplained left ventricular hypertrophy (wall thickness of at least 15 mm) associated with non-dilated ventricular chambers, in the absence of other cardiac or systemic diseases that might produce similar degree of hypertrophy (24, 25). Patients with co-morbid cardiovascular, pulmonary, or renal conditions were excluded. A detailed clinical evaluations and blood sampling for genetic analysis were obtained for each patient after echocardiographic examination.

Control group was composed of healthy volunteers without family history for any cardiac or inherited disease. Control group included 31 healthy subjects (20 females, 11 males; mean age 44.12±9.13; range 29 to 67 years) with normal 12-lead electrocardiography and trans-thoracic echocardiography.

This case-control study was approved by the local Ethic Committee in accordance with the Declaration of Helsinki, and each participant gave written informed consent after appropriate genetic counseling.

Study protocol

Electrocardiographic analysis (ECG)

The ECG recordings were obtained with a paper speed of 50 mm/sec at normal filtering. The QT interval was defined as the interval between the beginning of the QRS complex and the end of the T wave. Three consecutive cycles were manually measured in each of the standard 12 leads, and a mean value was calculated from these three measurements.

The QT interval was then corrected (QTc) using Bazett's formula. The corrected dispersion of QT intervals was defined as the difference between the maximum and minimum of the corrected QT interval which could be measured in any of the 12 ECG leads.

Echocardiographic analysis

A complete echocardiographic examination was performed with a Vivid Five System (GE, Vingmed Ultrasound, Horten, Norway) in each patient at rest by a single-blinded observer. LV hypertrophy was assessed with two-dimensional echocardiography according to published criteria (24). The greatest thickness measured at any site in the LV wall was considered to represent LV maximal wall thickness (26). Peak instantaneous LV outflow gradient was estimated under basal conditions with continuous

Table 1. Sequences of the primers used for three regions of the *HOPX* gene

Region	Exon	Primer	Sequence (5'-3')	Expected product lenghth, bp
I	1	HOPXe1f	AACGTGCTATCAGCAGCCTG	177
		HOPXe1r	GCATTTTGGTCTAGTTCCTGCAC	
II	4	HOPXe2f	CGACCGCCTTCCTTCGCTGC	308
		HOPXe2r	GACGAACAGGACCGCCCAGC	
III	5	HOPXe3f	CTTGTGCCACAGAGGCTACC	206
		HOPXe3r	CCTTCATGGAGTGAAGCTGTC	

wave Doppler (27). Two-dimensional measurements included LV end-diastolic and end-systolic diameters, posterior wall thickness, interventricular septal thickness, and LV ejection fraction (28). Mitral inflow Doppler was measured in standard fashion to determine peak E- and A-wave velocities, deceleration time of the transmitral E wave, and isovolumic relaxation time (29).

Description of syncope

Syncope was defined as loss of consciousness with interruption of awareness of oneself and ones surroundings with spontaneous recovery.

DNA isolation

DNA was yielded from peripheral blood of both patients and healthy subjects by using standard ammonium acetate method. After informed, each participant was subjected to blood sampling for DNA isolation. Briefly, 10 mL peripheral blood sample in EDTA tube was treated with red blood lysis buffer for three times to yield white blood cells. After treatment with white blood cell lysis buffer in the presence of proteinase K and Sodium dodecyl sulphate (SDS), cellular debris was removed by ammonium acetate treatment followed by centrifugation. DNA was precipitated by ethanol addition.

To yield specific DNA material for further genetic analyses, we used standard PCR technique. Three exonic regions of *HOPX* gene from DNA samples of the patients and the controls were amplified using primers listed in Table 1. PCR reactions was carried out in 25 mL volume containing 10x PCR buffer (50 mM KCl; 10 mM Tris-HCl; 1.5 mM $MgCl_2$), 2 mM $MgCl_2$, 0.8 μM each of primer, 200 μM dNTP mix, 1% DMSO, 0.5 U Taq DNA polymerase and 50 ng genomic DNA. Taq DNA polymerase was obtained from Roche (MBI Fermentas, Hanover, MD, USA). PCR amplification was carried out in a DNA Thermal Cycler (MJ Research Techne, Berlin, Germany).

Amplification conditions were as follows:

Initial denaturation at 95°C for 5 min followed by 30 cycles of denaturation at 95°C for 60 s, annealing at 59°C (for primers of regions 1 and 3) or 65°C (for primers of region 2) for 60 s, extension at 72°C for 60 s with a final extension at 72°C for 10 min.

Restriction fragment length polymorphism (RFLP)

For the genotyping of the identified known polymorphism (rs4626270) in the *HOPX* gene, PCR products were digested with Mbil.

Single strand conformation polymorphism (SSCP)

For the determination of unknown mutations or Single Nucleotid Polymorphisms (SNPs) in *HOPX* gene, we used SSCP analysis. SSCP analysis was performed using non-denaturing polyacrylamide gels on the Owl Separation Systems (Thermo Scientific, Rochester, NY, USA).

For SSCP detection, a volume of 2 μL PCR product was transferred into an Eppendorf tube, mixed with 5 μL gel loading solution containing 98% formamide, 0.025% bromophenol blue, 0.025% xylene cyanol, 20 mmol/l EDTA (pH 8.0) and 10% glycerol. The mixture was centrifuged and denatured at 98°C for 10 min, then chilled on ice for 5 min and loaded on 12% polyacrylamide gels (acrylamide:bisacrylamide=99:1). Electrophoresis was performed in Tris borate (pH 8.3)-EDTA buffer at 600 V/cm at 14°C. After electrophoresis, the DNA fragments in the gels were visualized by silver-staining method using standard protocols. All chemicals used in gel electrophoresis and SSCP analysis were obtained from Sigma-Aldrich (Stockholm, Sweden), Merck (Darmstadt, Germany), and AppliChem GmbH (Darmstadt, Germany). Samples with different SSCP patterns were sequenced by commercial sequencing service, Iontek.

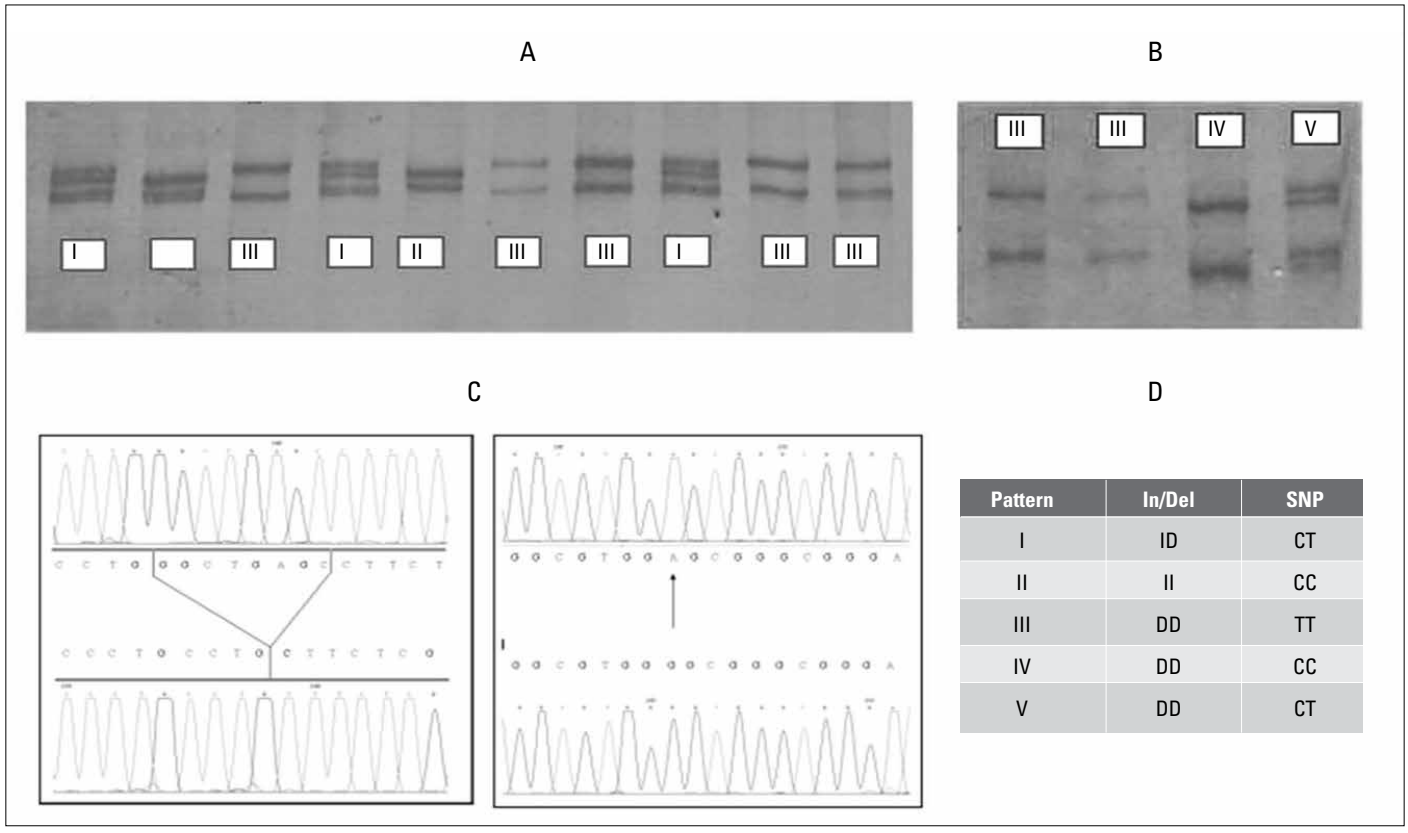
Statistical analysis

The frequencies of the alleles and genotypes were compared among patient and control groups using the chi-square. Comparation of genotype and allele distributions through the patients with and without syncope were performed by Kruskal-Wallis and the Fisher's exact test, respectively. SPSS 10.0 (SPSS, Inc., Chicago, IL, USA) for Windows and the Microsoft Excel were used for statistical analysis. Statistical significance was taken as $p<0.05$.

Results

***HOPX* gene did not harbor any disease-causing mutation in the HCM patients**

In SSCP analysis of the 1st and 5th exons of *HOPX* gene, there was not found any variation. In SSCP of fourth exon of the gene, however, we found 5 different patterns (I-V in Fig. 1). After sequencing of the samples from each pattern, it was shown that these 5 patterns in SSCP gel were due to genotypic combinations of two sequence variations. One of these two sequence



Pattern	In/Del	SNP
I	ID	CT
II	II	CC
III	DD	TT
IV	DD	CC
V	DD	CT

Figure 1. A-D. Five different patterns (I, II, III, IV and IV) observed in SSCP (A, B), sequencing results that show 8-bp-In/Del polymorphism and C-T substitution (C), and corresponding genotypes of each pattern observed in SSCP (D). While pattern I, II and III had "C-In" and/or "T-Del" haplotype, pattern IV and V had "C-Del" haplotype as homozygous and heterozygous, respectively

variations was 8-basepair (8-bp) insertion-deletion (In/Del) polymorphism (-/GCTCAGCC, rs11279383) and the other was single nucleotide polymorphism (SNP) (C>T, rs4626270).

For genotyping of 8-bp In/Del polymorphism and SNP, we used polyacrylamide gel electrophoresis and restriction enzyme digestion, respectively.

PCR products were loaded and electrophoresed in polyacrylamide gel for genotyping the patients and the controls for In/Del polymorphism. Samples were genotyped considering to Del specific and In specific patterns (pattern I, II and III at Fig. 1a), which were confirmed by sequencing.

To genotype the samples for SNP, PCR products were digested with restriction enzyme MbiI (BsrB1), followed by polyacrilamide gel electrophoresis.

In the case of T allele, MbiI enzyme digested PCR product giving two fragments 30 bp and, 270/278 bp (depending on the presence of 8-bp insertion) in length. In the case of C allele, PCR product remained undigested as 300/308 bp (depending on the presence of 8-bp insertion) fragment. Heterozygouses had 5 fragments (30 bp, 270/278 bp, 300/308 bp and two heterodublex bands) (Fig. 2).

Pattern I, II and III were consideres as In/Del (ID), In/In (II) and Del/Del (DD), respectively. While most samples showed one of those three patterns (pattern I, II and III), two other different patterns (pattern IV and V in Fig. 1a) were shown through four samples. Those two different patterns were revealed to be caused by the presence of a different haplotype. While all samples with pattern I, II or III had homozygosity or heterozygosity of "C-In" and "T-Del" haplotypes, samples with pattern IV and V had "C-Del" haplotype as homozygous and heterozygous, respectively.

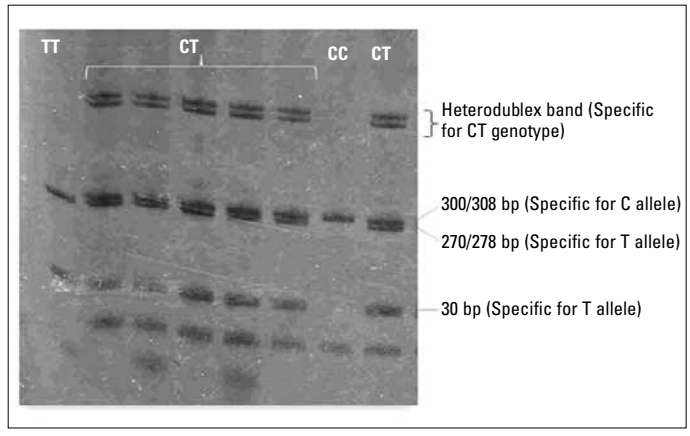


Figure 2. Polyacrilamide gel electrophoresis image of PCR products after MbiI digestion. In the presence of C allele, MbiI does not digest the PCR product. Depending on the 8-bp insertion/deletion polymorphism, C allele gives a single band in length of 300 or 308 bp, which can not distinguished in the gel. In the presence of T allele, MbiI digestion gives two bands, one of which is 30 bp and the other is 270 or 278 bp (depending on the In/Del polymorphism). In the presence of both alleles, two heterodublex bands appear as well

***HOPX* gene polymorphisms were associated with syncope in the patients with HCM**

Between patient and control group, there was not any significant difference in allele or genotype frequencies (Table 2). Allele frequency of C allele of C>T polymorphism was found 61.65% and 59.79% in the patient and control group, respectively. Similarly, allele frequency for In allele of In>Del polymorphism was found 60.25% and 56.50% in the patient and control group, respectively. For both patient and control group, highest genotype frequency belonged to the heterozygous genotypes (Table 2). Clinical properties of the patients are presented in Table 3. After comparing the genotypes and alleles of 8-basepair deletion-insertion polymorphism (-/GCTCAGCC, rs11279383) and single nucleotide polymorphism (SNP) (C>T, rs4626270) with clinical parameters in detail, we have found that both polymorphisms were significantly associated with syncope in HCM patients. Distribution of genotypes through the patients demonstrated an increased risk of syncope in heterozygous status of In/Del and C/T polymorphisms, with p values of 0.048 and 0.030, respectively. Homozygous status of both polymorphisms, however, had lower frequency in patients with syncope (Table 4). Lower frequency of homozygous status through the patients with syncope suggested the possible protective role of homozygosis of both alleles of both polymorphisms. After considering the zigosity status of the patients, frequency of syncope was found significantly lower in homozygous than heterozygous patients for both In/Del (p=0.014) and C/T (p=0.017) polymorphism (Table 5). Increased significancy after considering the patients' zigosity status, supported the protective role of homozygosis of both polymorphisms.

C/T and In/Del are linked polymorphisms

Genotyping of the samples have also shown that the C allele was linked to In (Insertion) allele with 240bp-interval as an haplotype ("C-Ins" haplotype), and the T allele was linked to Del (Deletion) allele ("T-Del" haplotype). Only two patients (2.73%) and two controls (6.45%) were found to have "C-Del" haplotype. While one control was homozygous for "C-Del" haplotype (C-Del/C-Del), other control and two patients were heterozygous (C-Del/T-Del).

Discussion

In this study, we dealed *HOPX* as a potential modifier gene for HCM. In this purpose, we analyzed certain regions including coding exons and flanking non-coding sequences of the *HOPX* gene among HCM patients and healthy subjects. In non-coding region, 5'UTR and 42-bp downstream of the common coding exon, we defined two sequence variations. One of these variations was single nucleotide polymorphism (C>T substitution) and the other was a 8-bp In/Del (insertion/deletion) polymorphism. While heterozygosity of these polymorphisms seemed like a risk factor for syncope in HCM, their homozygosis was found significantly associated with decreased frequency of syncope.

Table 2. Genotype distribution and allele frequencies of two polymorphisms

Genotype distribution			
Polymorphism	**Genotype**	**Patient n (%)**	**Control n (%)**
C/T	CC	26 (35.6)	9 (29.1)
	CT	38 (52.1)	19 (61.3)
	TT	9 (12.3)	3 (9.6)
In/Del	II	26 (35.6)	9 (29.1)
	ID	36 (49.3)	17 (54.8)
	DD	11 (16.1)	5 (16.1)
Allele frequencies			
Polymorphism	**Allele**	**Patient %**	**Control %**
C/T	C	61.65	59.75
	T	38.35	40.25
In/Del	I	60.25	56.5
	D	39.75	43.5

Table 3. Clinical characteristics of patients with hypertropic cardiomyopathy

Variables	**n**	**%**	**Mean±SD**	**Range**
Age, years			47.94±15.62	17-74
Male gender	40	59.7		
Family history				
Hypertrophic cardiomyopathy	20	29.9		
Sudden death	14	20.9		
Clinical status				
Symptomatic	38	56.7		
Asymptomatic	29	43.3		
Left ventricle				
End-systolic diameter, cm			2.48±0.49	
End-diasystolic diameter, cm			4.40±0.59	
Maximal wall thickness, cm			2.43±0.53	
Ejection fraction, %			75.19±8.79	72-93
Left atrium size, cm			4.55±0.77	2.9-6.49
QRS duration			120.70±27.95	
QT dispersion, msec			69.61±27.65	
Corrected QT dispersion, msec			77.02±29.87	

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

As a genetic disease of cardiac-muscle sarcomere, HCM helped scientists to understand the molecular mechanism of cardiac function. In medical practice, however, HCM is one of the main problem concerning the people which have history of sudden death (2, 30). Due to its clinical importance, HCM needs to be predicted and prevented before the apperaence of life-

Table 4. Syncope episode and genotype distribution of In/Del (I/D) and C>T (C/T) polymorphisms

Genotype	II n (%)	ID n (%)	DD n (%)	*P* value*	CC n (%)	CT n (%)	TT n (%)	*P* value*
Syncope								
No	21 (39.6)	22 (41.5)	10 (18.9)	0.048	19 (35.9)	25 (47.1)	9 (17)	0.030
Yes	1 (7.1)	11 (78.6)	2 (14.3)		2 (14.3)	12 (85.7)	0 (0)	
*Performed with Kruskal-Wallis								

threating complications. Since the HCM is inherited as an autosomal dominant trait, it seems easy to follow the people at risk. However, monitoring of the people at risk is not so smooth, because the clinical course and prognosis of the HCM vary among the patients, even among them which have same mutation within a family. This heterogenity is explained by environmental and genetic modifiers.

Searching the modifier genes for HCM is an ongoing field of the cardiovascular genetics, because the clinical heterogeneity of HCM needs to be explained for correct and personalised approach to the patients.

So far, some genes were shown to play modifier role in HCM, including ACE (31) and sex hormone receptor gene (32). In/Del polymorphism of ACE was shown to be involved in the phenotypic expression of left ventricular hypertrophy in HCM patients (31). Same polymorphism was shown to be related to QT-dispersion in hypertrophic cardiomyopathy as well (33). QT-dispersion in patients with hypertrophic cardiomyopathy was also shown to be associated significantly with an haplotype in another modifier gene, SMYD1/BOP (34). In addition, the genome-wide mapping analysis of 100 HCM patients with *MYBPC3* mutation offered the 10p13 region as a candidate modifier locus for the left ventricular weight in HCM (35). All these studies support the importance of modifier genes in heterogenity of the HCM. In the future, considering the polymorphisms in proved modifier genes will help the phisicians to predict clinical progress of the patients with HCM more accurately.

HOPX gene has been subjected to many studies, mostly as a tumor supressor gene. Although, those studies have demonstrated significant change of *HOPX* level in certain tumor materials (19, 36) or failed heart muscle (23), polymorphisms in this gene have not been found related to any pathological condition, yet. Considering the fact that *HOPX* plays role in hypertrophic response of myocardium, and its expression was significantly changed in failed heart, *HOPX* gene seems like worthwhile to be investigated as a modifier gene for HCM.

Since the *HOPX* gene codes for a small protein, we dealed whether any change in protein coding sequence of *HOPX* gene may be responsible for HCM pathogenesis. *HOPX* gene codes for 13 transcripts and 3 proteins (112, 91 and 73 aa in length) with alternative splicing and/or using different transcription start sites. We analyzed three regions of the gene, two of which are common to all transcripts and include coding exons. In the first (1st exon) and the third region (5th exon) of *HOPX* gene, we did not find any different pattern in SSCP analysis.

Table 5. Genotypic distribution of two polymorphisms between patients with and without syncope

	C>T substitution		In/Del polymorphism	
	Homozygous (CC+TT)	Heterozygous (CT)	Homozygous (II+DD)	Heterozygous (ID)
With syncope	2	12	3	11
Without syncope	28	25	31	22
		p=0.014*		p=0.017*
*Performed with Fisher's exact test				

Within second region, however, we found one SNP (C>T substitution) at 5'UTR (untranslated region) and one InDel polymorphism at intronic region (42-bp downstream to exon-intron boundary). In addition, we observed that "C" allele at the 5'UTR was linked to "In" allele at the intronic region. Consistent rates of the polymorphic alleles in both patient and control group, suggests that frequency of "C-In" and "T-Del" haplotypes is nearly 60% and 40% for Turkish population, respectively. While there was not found "T-In" haplotype, "C-Del" haplotype was found as heterozygous in two patients and one control, and as homozygous in one control subject. Frequency of "C-Del" haplotype seemed to have little difference between two groups with 1.36% in the patient group and 9.67% in the control group.

Although both C>T substitution and In/Del polymorphism did not lead change in protein sequence, they showed significant association with syncope, one of the risk factors for death in HCM (37). Association of syncope with heterozygosity, but not with a certain allele of these polymorphisms, suggests that the co-existance of different alleles at different transcripts may interfere the transcriptional or translational efficiency of the gene at RNA level.

Mutation screening results of the coding region by SSCP, suggest the conservation at protein sequence. Even though *HOPX* protein seems to be conserved structuraly, its level shows alterations in some pathological tissues. Morover, *HOPX* was shown to be highly expressed in the adult murine cardiac conduction system, and its disruption was shown to lead infranodal conduction defects with downregulation of connexin40

expression (38). This leads us to conclude that polymorphisms which cause quantitative rather than qualitative variations in HOPX protein may be involved in HCM as modifier factor. Therefore, it is considerable that association between non-coding polymorphisms of *HOPX* gene and syncope in HCM is consequence of quantitative alteration of *HOPX* in the heart.

It was noteworthy that the expression of many SRF-dependent genes were decreased in the heart muscle of the *HOPX* mutant mice (39). Of these genes, beta myosin heavy chain (βMyHC) and myosin binding protein C (MyBPC) showed 15 and 10 fold decreased expression level, respectively (40). Considering the fact that the mutations in human homologous of these two genes are responsible for most of the HCM patients (40, 41), it could be concluded that any change in *HOPX* expression level, due to a promotor or a non-coding polymorphism for instance, may effect the expression level of the mutant gene at great degree. This effect is expected to result in clinical heterogenity within the patients carying the same mutation. Therefore, sequences variations in regulatory region rather than coding region of the *HOPX* gene may have greater importance for its possible modifier role in HCM.

Relationship between *HOPX* and syncope in HCM may be SRF-dependent or independent. If *HOPX* gene plays role in HCM pathogenesis through SRF-dependent genes, this modifier effect may be more obvious among the patients which have mutations in their SRF-target genes, like βMyHC and MyBPC. This question remains to be solved by further studies with study group composed of the patients which are diagnosed by mutation analysis, like that performed by Daw EW (35). It also remains to investigate the expression levels of *HOPX* and *Connexin 40* in cardiac conduction system of the patients which suffer syncope.

In addition to genetic variations, epigenetic status of *HOPX* gene also may have importance for its possible modifier role in HCM. Considering that epigenetic alteration in *HOPX* gene promoter was found related to some cancers (42, 43), and that methylation level in exonic CpG sites of cardiac *MYBPC3* gene, a common causal gene for HCM, may result in increased genetic mutability (44), epigenetic evaluation of *HOPX* gene promoter is wothy of investigation in HCM patients.

Study limitations

Main limitation of the recent study was that it included only certain regions, especially coding exons of the gene, and that the regulatory regions were excluded.

Lack of our knowledge on mutant gene profile of our patients is the other limitation for the study to investigate whether the modifier effect of *HOPX* depends on SRF.

Conclusion

In conclusion, we found a correlation between non-coding polymorphisms of *HOPX* gene and syncope episode in HCM patients. This correlation supports the idea that *HOPX* gene may play modifier role in HCM, and emphasizes the clinical importance of modifier genes. Since the syncope is supposed to be a risk factor for HCM, screening of *HOPX* polymorphisms may help to estimate the patients which have syncope risk, in the future.

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