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Original Article

Fibrillin-1 gene intron 56 polymorphism in Turkish children with mitral valve prolapse*

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Abstract *Objective:* Mitral valvar prolapse is the most common anomaly of the mitral valve apparatus throughout childhood. Fibrillin is one of the structural components of the elastin-associated microfibrils found in the mitral valve. A case-controlled study has performed to investigate the relationship between *fibrillin 1* gene intron 56 polymorphism and risk of mitral valvar prolapse in Turkish children. *Patients and methods:* A total of 77 patients with mitral valvar prolapse diagnosed by clinical evaluation and echocardiography and 89 normal children of same age and sex were studied. The *fibrillin-1* gene intron 56 polymorphism was identified by the polymerase chain reaction-based restriction analysis. *Results:* There was a significant difference in the distribution of *fibrillin-1* gene intron 56 genotypes ($p = 0.0001$) and allelic frequency ($p = 0.0001$) between the cases and the controls. *Conclusions:* Patients with mitral valvar prolapse have higher frequencies of *fibrillin-1* gene intron 56 GC genotypes. Healthy children have higher frequencies of *fibrillin-1* gene intron 56 CC genotypes. We speculate that the higher frequency of *fibrillin-1* gene intron 56 G-allele increases the risk of mitral valvar prolapse.

Keywords: Fibrillinopathies; genetic disorders; cardiac valve diseases

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MITRAL VALVAR PROLAPSE IS ONE OF THE MOST common cardiac valvular diseases as well as the most common disorder of the mitral valve complex. It is characterised by the displacement of one or both mitral leaflets into the left atrium at the end of systole. Myxomatous degeneration of the valve leaflets is recognised as a characteristic histological change of mitral valvar prolapse.[1] According to recent community studies, it has an estimated prevalence of 2.4% in general population; thus, mitral valvar prolapse would be expected to be present in over 144 million worldwide.[2] But, it is unknown whether its results are applicable to other ethnic and racial groups. To date, this question remains still unanswered. Its aetiology and pathophysiology are unclear, with genetic markers and biomarkers for the disease still in the process of being elucidated. Although most cases with mitral valvar prolapse appear to be sporadic, it is shown that familial form is transmitted with autosomal dominant heritage.[3–5]

Mitral valvar prolapse with or without mitral regurgitation has been documented to be more prevalent in patients with Marfan syndrome, Ehlers Danlos syndrome, osteogenesis imperfecta, and other collagen-related disorders.[6] This association with inherited connective tissue disorders, plus the myxomatous degeneration observed in the mitral valves of patients showing mitral valvar prolapse, has suggested that the abnormality of some component of the connective tissue may be involved. The structural changes in the arrangement of both the

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amorphous elastin core and the microfibrils of elastic fibres associated with an increase in the number and a decrease in the size of the elastic fibres are examined in floppy areas of the mitral valvar prolapse. These alterations in the connective tissue of mitral valvar prolapse are involved in the pathological process.[7]

Fibrillin, which is a cysteine rich glycoprotein that weighs 350 kilograms Dalton and is composed of three similar forms, is the building stone of microfibrils in mitral valve. Coding of *fibrillin-1* gene, *fibrillin-2* and *fibrillin-3* genes are done by 15q21, 5q23, and 19p13 chromosomes, respectively. *Fibrillin-1* and *fibrillin-2* genes are structurally similar and both contain 65 exons.[8] Genetic variations of *fibrillin-1* gene are known to cause Marfan syndrome. Phenotypes of other disorders similar to Marfan are seen with *fibrillin-1* gene mutation. These disorders, including familial mitral valvar prolapse, Marfan syndrome, and neonatal Marfan syndrome, are called "type 1 fibrillinopathies".[8,9] Mutation of *fibrillin-2* gene is held responsible for congenital contractural arachnodactyly. No phenotype associated with mutations in *fibrillin-3* gene has been shown.[8] Although very limited data are currently available regarding the role of *fibrillin-1* gene polymorphisms in the pathogenesis of isolated mitral valvar prolapse, the current study was designed to determine whether the polymorphism of intron 56 of the *fibrillin-1* gene is associated with risk of mitral valvar prolapse in Turkish children.

Materials and methods

Study population

This study was carried out the Department of Paediatric Cardiology in Gazi University, School of Medicine. A total of 77 patients (44 females and 33 males) with an echocardiographically diagnosed mitral valvar prolapse were enroled in this study. Diagnosis of mitral valvar prolapse was made by echocardiography. Patients with familial mitral valvar prolapse, Marfan syndrome, Ehlers Danlos syndrome, rheumatic cardiac disease, and congenital cardiac disease were excluded. The control group comprised 89 age- and sex-matched healthy children (49 females and 40 males) with a normal echocardiography verified before inclusion. All subjects were of Turkish ethnicity and lived in central Turkey.

All subjects signed an informed consent; the study complies with the Declaration of Helsinki and was approved by the local ethics committee.

Echocardiographic method

A ViVid 3 Expert echocardiography device of General Electric Medical Systems Company (Milwaukee, Wisconsin, United States of America) with 7 and 3 MegaHertz probes was used for echocardiographic examination of patients. Structure and movement of mitral valve in more than one position; parasternal long axis, and apical 4 cavity positions were evaluated. Displacement of anterior and/or posterior leaflet of mitral valve more than 2 millimetres towards left atrium and measurement of this leaflet in diastole over 5 millimetres is accepted as classic mitral valvar prolapse.[10,11]

Genetic analysis

Blood samples were obtained from the patients and put in tubes with ethylene diamine tetra-acetic acid. The tubes were protected to minus 20 degree Celsius. The genomic deoxyribonucleic acid was prepared from peripheral blood leucocytes using a genomic deoxyribonucleic acid isolation kit (Dr Zeydanli, Istanbul, Turkey). Analyses of the blood samples were blinded to our genetic laboratory.

The polymerase chain reaction technique was used to amplify specific regions of a deoxyribonucleic acid strand. The primers were designed according to the sequences of human *fibrillin-1* gene, which were available on the web site (http://www.ncbi.nlm.nih.gov/). Primers for intron 56 polymorphism were (forward) 5′ CGAGTGCCTT GGTGAGTACA 3′ and (reverse) 5′ AATTTAGCT GCAGGGTGGTG 3′.

The polymerase chain reaction mixture contained 0.8 microlitre (0.3 micromole) of each primer, 0.8 microlitre (100 micromole) $MgCl_2$, 1 microlitre (10 micromole) dNTPs, 5 microlitre (10×) polymerase chain reaction buffer, 0.3 microlitre (5 unit per 50 microlitre) Taq deoxyribonucleic acid polymerase, 4 microlitre (40 micromole) genomic deoxyribonucleic acid, and 37.3 microlitre deoxyribonuclease, and ribonuclease free water. The final reaction volume was 50 microlitre. Deoxyribonucleic acid was denatured at 94 degree Celsius for 5 minutes followed by 35 cycles of amplification beginning with a denaturation step at 94 degree Celsius, a primer annealing step at 60 degree Celsius, and an extension step at 72 degree Celsius, each step took 75 seconds. A 5-minutes final extension step was performed at 72 degree Celsius. After polymerase chain reaction, the fragment was digested by the restriction enzyme Aci I for the intron 56 polymorphism. So 15 microlitre polymerase chain reaction product, 1.6 microlitre buffer, and Aci I restriction enzymes were added in the ependorf tubes (0.5 millilitres) for each product. The polymorphisms on the *fibrillin-1* gene were confirmed by electrophoresis of 8 microlitre reaction aliquot in 2% agarose gels, followed by visualisation of bands under ultraviolet light and photographed by Canon powershot G5 camera (Japan) with Syngene system.

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Statistical method

The differences between ages were compared using "Student's *t*-test" in the mitral valvar prolapse and control groups. The gender distributions were tested using a "χ^2 test" in the groups. Genotypic differences and allelic frequencies of intron 56 between patients with mitral valvar prolapse and control subjects were compared using the "χ^2 test". The polymorphism of intron 56 of *fibrillin-1* gene with "Hardy–Weinberg principle" was controlled using the "χ^2 test" in the study. The associations between the intron 56 GC genotypes and mitral valvar prolapse risk were estimated by computing the odds ratios and their 95% confidence intervals. A p-value, less than 0.05, was considered statistically significant. All statistical analyses were performed using "SPSS for Windows".

Results

Patient characteristics

The gender and age distributions of the patients and healthy control subjects were found similar and listed in Table 1.

Fibrillin-1 *gene intron 56 polymorphism*

A 170 plus 28 base pair band was seen in individuals with wild-type genotype when bands formed after electrophoresis was examined. A non-digested fragment was determined in individuals with mutant genotype (198 base pair; Fig 1).

As Table 2 indicates, homozygote genotype was determined in 32.5% of patients in mitral valvar prolapse group, and 70.8% of controls. Consequently, a statistical difference between groups in terms of *fibrillin 1* gene intron 56 polymorphism was detected ($\chi^2 = 24.335$, $p = 0.0001$, odds ratio = 5.040, 95% confidence intervals = 2.604–9.756). Using Hardy–Weinberg method, allelic frequencies in terms of *fibrillin-1* gene intron 56 polymorphism were found as 34% G-allele, 66% C-allele in mitral valvar prolapse group; and 15% G-allele, 85% C-allele in control group and a statistical difference ($\chi^2 = 16.862$, $p = 0.0001$, odds ratio = 2.980, 95% confidence intervals = 1.748–5.081) was determined between groups.

Discussion

Mitral valvar prolapse is a complex clinical entity that has been well known for more than 45 years. It is generally understood to be the displacement of abnormally thickened, redundant mitral leaflet(s) into the left atrium during systole.[1,2] The main histological features of mitral valvar prolapse are marked proliferation of the spongiosa of the mitral valve leaflets and mucopolysaccharide acid replacement of leaflet collagen, causing myxomatous leaflet thickening and mitral leaflet redundancy.[12] The technique of

Table 1. Gender and age distributions of patients with mitral valve prolapse and control groups.

Parameter	MVP group (n = 77)	Control group (n = 89)	p
Gender (female/male)	44/33	49/40	0.787*
Age (years)	3–18	3.5–17	0.983**
Median (years)	11.29	11.34	

MVP, Mitral valve prolapse
*χ^2 test
**Student's *t*-test

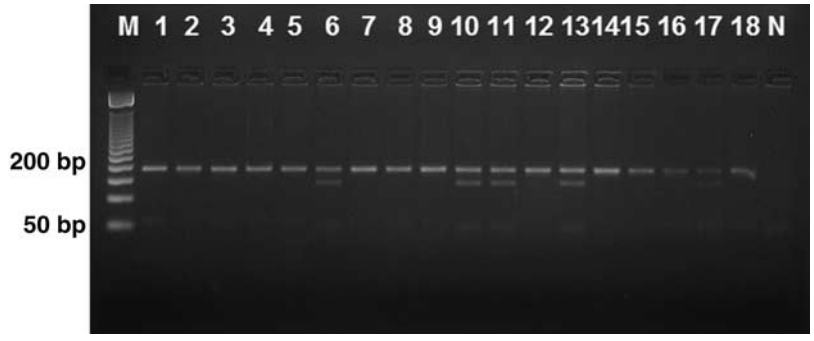

Figure 1.
Photograph of intron 56 taken with the camera including Syngene system.

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Table 2. Distribution of *fibrillin-1* gene intron 56 polymorphism in patients with mitral valve prolapse and healthy control subjects.

	MVP group		Control group					
Genotype	n	%	n	%	OR	95% CI	χ^2	p*
GG	–	–	–	–	5.040	2.604–9.756	24.335	0.0001
GC	52	67.5	26	29.2				
CC	25	32.5	63	70.8				
G/C	0.34/0.66		0.15/0.85		2.980	1.748–5.081	16.862	0.0001

CI, confidence interval; MVP, mitral valve prolapse; OR, odds ratio
*χ^2 test

echocardiography has evolved from transthoracic to transoesophageal and from one dimensional to three dimensional modes. Thus, the diagnosis of mitral valvar prolapse has become easier and more accurate.[11] Primary mitral valvar prolapse can be familial or sporadic. Identification of the locus for autosomal dominant familial mitral valvar prolapse to chromosome 16p11.2-p12.1, 11p15.4, and 13q31.3-q32.1 have been reported.[3–5] In addition, X-linked inheritance has been reported in a special form of familial mitral valvar prolapse, called myxomatous valvular dystrophy, which has been mapped to Xq28 in a large French pedigree.[13]

During intrauterine life, differentiation of mitral valve and formation of chest cavity and bone structures in vertebrae occur in the fifth and the sixth weeks of pregnancy.[12] Therefore, disorders in mitral valve and connective tissue are accepted to be closely associated. As mitral valvar prolapse has been discovered, similarities with Marfan syndrome have been described. More recently, it has been estimated that only 0.25% of patients with mitral valvar prolapse have Marfan syndrome. This percentage might be higher if the more recent and stringent diagnostic criteria are used, but it is unlikely that more than 1 and 2% of patients with mitral valvar prolapse have an associated connective tissue disorder.[14] Marfan syndrome is associated with mutations in *fibrillin-1* gene on chromosome 15q21.1.[8] Because mitral valvar prolapse is found in many, but certainly not all patients with Marfan syndrome, it was suggested that isolated mitral valvar prolapse may also be because of a mutation of *fibrillin-1* gene. However, despite the availability of millions of patients for study no convincing association has been found to date.[14] Since familial mitral valvar prolapse and Marfan syndrome are transmitted to future generations by autosomal dominant heritage, genetic inspections of mitral valvar prolapse, "genetic fibrillinopathies" or "type 1 fibrillinopathies", are recently done especially over Marfan syndrome.[9]

In this study, a method of polymerase chain reaction- based restriction analysis was used to approach the single nucleotide polymorphism. In this case-controlled study, an association was found between *fibrillin-1* gene intron 56 polymorphism and mitral valvar prolapse. The pathogenesis of elastic fibre abnormality in mitral valvar prolapse might be partially explained by this finding. *Fibrillin-1* gene was evaluated in terms of intron 56 polymorphism and it was found that individuals with intron 56 heterozygote genotype are under higher risk of mitral valvar prolapse. Distribution of intron 56 homozygote and heterozygote genotypes in mitral valvar prolapse, and control groups is given in Table 2. The role of *fibrillin-1* gene was hypothesised to be involved in the pathogenesis of isolated mitral valvar prolapse. A statistically significant difference in terms of intron 56 polymorphism was found between groups ($p = 0.0001$). In mitral valvar prolapse group, intron 56 heterozygote genotype was found to be statistically higher than that in controls. As a result, it was found that mitral valvar prolapse is more frequent in individuals with intron 56 heterozygote genotype and individuals with intron 56 homozygote genotype are under lower risk of mitral valvar prolapse. Allelic frequencies in terms of intron 56 polymorphism using Hardy–Weinberg method are also given in Table 2 and a statistical difference was determined between groups ($p = 0.0001$). Risk of mitral valvar prolapse was seen to increase with increasing G-allele frequency in intron 56 polymorphism.

A recent study reported that *fibrillin-1* gene exons 15 and 27 polymorphisms were associated with an increase risk of mitral valvar prolapse in Chinese adults.[15] In this study, it was reported that association between *fibrillin-1* gene intron 56 polymorphism and mitral valvar prolapse in Turkish children. The current sample of 166 individuals is quite small in terms of genetic epidemiological studies. Although all people were of Turkish ethnicity and lived in the central Turkey, they could still be a certain ancestry prone to develop mitral valvar prolapse and the *fibrillin-1* genotype is a mere marker for such a different population.

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Mitral valvar prolapse is a common clinical phenotype. Its aetiology has not been elucidated yet. The scientific community has made significant efforts in identifying genetic profiles related to mitral valvar prolapse, especially through the use of linkage analysis. This resulted in the discovery of four different loci linked to mitral valvar prolapse on chromosomes 11, 13, 16, and X.[3–5,13] Currently, no set of specific genes has been identified. Elucidating the genetic basis for mitral valvar prolapse using well-designed case control or family-based studies may have substantial implications in clinical practice. This study shows that patients with mitral valvar prolapse have a higher frequency of *fibrillin-1* gene intron 56 GC genotype and G-*allele* that supports a role of *fibrillin-1* gene intron 56 polymorphism in determining the risk of mitral valvar prolapse among the Turkish children. Therefore, the study indicates the association between *fibrillin-1* gene intron 56 polymorphism and mitral valvar prolapse and further investigation is required on this issue.

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