The first report of c.4408T>C mutation on *FBN1* gene in a case with Marfan Syndrome in Iran

İran'da Marfan Sendrom'lu Bir Olguda *FBN1* Geninde c.4408T> C Mutasyonu ile ilgili İlk Rapor

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ABSTRACT

Marfan syndrome (MFS) is a rare autosomal dominant genetic disorder and the mutations on fibrillin-1 (*FBN1*) gene are the main cause of the disease. The MFS is associated with complications in cardiovascular, ocular, and skeletal systems. A 34-year-old man with clinical manifestation of the MFS disorder was referred to the Genetic diagnostic laboratory for genetic analysis. The NGS (Next-generation sequencing) panel for neuromuscular diseases that analyzes 14 different genes were applied for the detection of the related mutation and the c.4408T>C mutation (p.Cys1470Arg) on *FBN1* was detected in heterozygote state. The result was confirmed with Sanger sequencing method and the mutation was also detected in proband's mother and daughter. This is the first report of c.4408T>C mutation from Iran and Middle East. Genetic testing and specially NGS method is recommended to identify the mutations in individuals suspected of having MFS.

Key Words: Fibrillin-1, Marfan syndrome, Ghent Criteria, Next generation sequencing

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ÖZET

Marfan sendromu (MFS), nadir görülen otozomal dominant genetik bir hastalık olup, fibrillin-1 (*FBN1*) genindeki mutasyonlar hastalığın ana nedenidir. MFS, kardiyovasküler, oküler ve iskelet sistemlerindeki komplikasyonlarla ilişkilidir. MFS bozukluğunun klinik bulguları olan 34 yaşındaki bir erkek hasta genetik analiz için Genetik tanı laboratuvarına sevk edildi. NGS (Yeni Nesil Dizileme) paneli ile analiz eden nöromüsküler hastalıklar için ilgili 14 farklı gende mutasyon analizi yapılfı ve *FBN1* üzerindeki c.4408T> C (p.Cys1470Arg) mutasyonu heterozigot olarak tespit edildi. Bu sonuç, Sanger dizileme yöntemiyle doğrulandı ve mutasyon aynı zamanda probandın annesi ve kızında da tespit edildi. Bu, İran ve Orta Doğu'dan gelen c.4408T> C mutasyonunun ilk olgusudur. MFS'ye sahip olduğundan şüphelenilen bireylerde mutasyonları belirlemek için genetik testler ve özel olarak NGS yöntemi önerilir.

Anahtar Sözcükler: Fibrillin-1, Marfan sendromu, Ghent Kriterleri, Yeni nesil dizileme

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INTRODUCTION

Marfan syndrome is the most common variable heritable connective tissue disorder that was identified and introduced by Antoine Marfan in 1896. The disease is inherited in an autosomal dominant manner (1-5).

The most important gene responsible for the MFS, which is called fibrillin-1 (*FBN*1), is located on chromosome 15 (1, 4, 6, 7). Prevalence of the syndrome is about 2-3 per 10,000 individuals in a lifetime(3, 4) and it is reported that around 75% of the cases are individuals who take the disorder and related mutations from their affected parents and 25% have de novo mutations (1). The MFS is associated with complications in cardiovascular, ocular, and skeletal systems. Cardiovascular problems are known as the most common cause of morbidity and mortality in patients with MFS (2, 8). The defined clinical criteria named Ghent nosology was recommended in1996 as the main diagnostic criteria for MFS and it was revised in 2010(1, 9, 10). The Ghent nosology gives a list of possible clinical manifestations and symptoms of the disorder (11).

Since the introduction of DNA-sequencing method by Sanger in 1977 (12), the sequencing approaches have been evolved and these days the Next Generation Sequencing (NGS) is a completely new approach of sequencing technology. This technology leads to increased genetic diagnosis of rare and common heterogeneous disorders. NGS is a useful tool for detection of familial genetic disorders, de novo mutations and mosaicism. NGS is currently used in both clinical sets and research projects and research findings can significantly help diagnostic procedures (13).

In the present report, we introduced a patient with MFS and related mutation on *FBN1* gene, which was detected using NGS method. This mutation is reported from Iran and Middle East for the first time.

CASE REPORT

On November 2016, a 34-year-old man who was originally from Mazandaran (a northern province of Iran located on southern coastline of Caspian Sea) was referred to Fajr medical genetics and pathobiology laboratory (Sari, Iran) for genetic counseling. The case had Bile surgery due to gallstone at the age of 24. In this patient, mitral valve prolapse was observed and he was found to be taller than other members of the family.

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The patient had several clinical manifestations such as flat feet (pes planus), pectus excavatum, minor scoliosis in lower spine (lumbar), followed by lens dislocation in both eyes and teeth disarrangement. Moreover, hind foot deformity that existed in his father was also observed in the case and his daughter. Presenting these clinical symptoms, the case was suspected of having the Marfan syndrome. Based on the evidences, his father who died at the age of 44 was also suspected of having MFS. Although no history of the disease was reported in proband's child, his daughter was suspected of having MFS due to some symptoms such as arachnodactyly, pectus excavatum and some dislocations that had been treated by surgery. A possible pedigree chart was drawn for this family (Fig1).

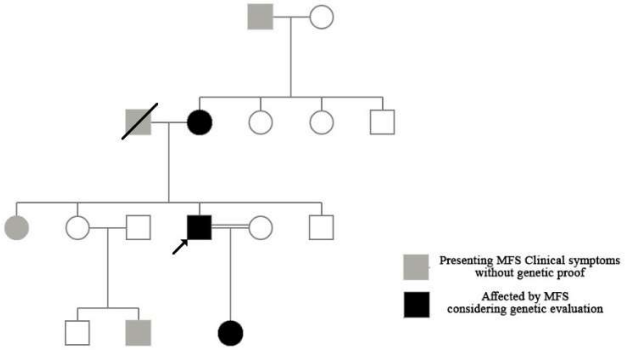

Figure 1. The family pedigree of the case with MFS

According to the above-mentioned clinical manifestations and possible diagnosis of the hereditary Marfan syndrome, NGS test was performed. DNA sample was sent to the Beijing Genomics Institute to confirm the diagnosis by detecting the likely mutation using NGS test.

The Genetic Testing for 1445 Monogenic Diseases (10-99 genes), Marfan, Aneurism and related disorders was tested on 14 genes including *ACTA2, CBS, FBN1, FBN2, MYH11, COL3A1, SMAD3, TGFBR1, TGFBR2, MYLK, MSTN, COL5A2, TGFB2* and *SLC2A10* by BGI Clinical Laboratories in 2016-10-27.

Target regions of interested genes were captured with NimbleGen chip, followed by Next-generation sequencing. In general, the test platform (Illumina) examined>95% of the target gene with coverage>99%. The average depth of target region was 268.78X and length of target region was 49383 base pair. Point mutation, micro-insertion, deletion and duplication (<20bp) can be simultaneously detected. *FBN1* sequences were compared to Human Genome database (GenBank NM_000138).

Genetic evaluation of the proband showed a heterozygote mutation in exon 36 (CDS35) with a variation of c.4408T>C (p.Cys1470Arg) on *FBN1* which is located on chr15:48762882 (Table1). The detected mutation was confirmed in the proband using Sanger sequencing method. The proband's mother and daughter were also found to be heterozygote for the c.4408T>C mutation (Fig2).

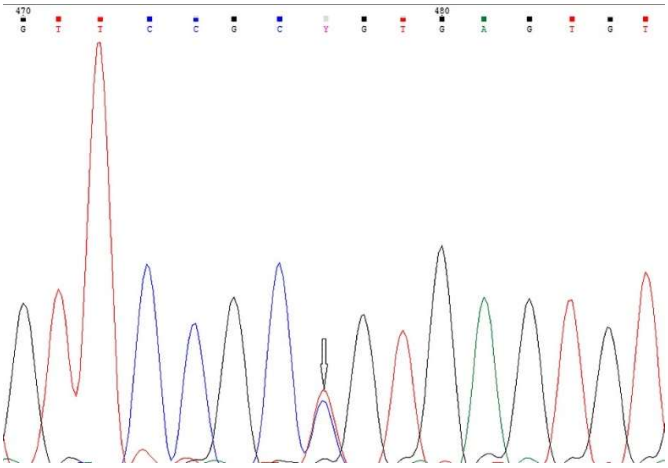

Figure 2. The Sanger sequencing test result of the *FBN1* gene in the patient. The arrow shows the location of the c.4408T>C mutation.

The c.4408T>C Mutation affects a disulfide-bonding cysteine (p.Cys1470Arg) and this variant has been described as a disease-causing mutation (14). The translated protein is predicted to be damaged using sorting intolerant from tolerant (SIFT) or polymorphism phenotyping (PolyPhen) soft wares (15).

Table 1. Results of NGS test for the case

Genes	Reference Sequence	Nucleic Acid Alteration	Amino Acid Alternation	Mutation location	Zygosity	Chromosome location	Mutation function
FBN1	NM_000138	c.4408T>C	p.Cys1470Arg	EX36/CDS35	Het	chr15:48762882	VUS

VUS: variant of uncertain significance

DISCUSSION

The mutations on several genes have been reported to be responsible for MFS including *FBN1, TGFBR1, TGFBR2, SMAD3, TGFB2, TGFB3* etc. (16, 17). However, identification of a mutation on *FBN1* is the main criteria for the diagnosis of the disorder (4). Previous studies have reported that there are around 3000 mutations on *FBN1* gene that most of the mutations are unique to the individuals or families (4, 18). Depending on the type and location of the mutation (missense, deletion, nonsense or frameshift mutations), clinical manifestations of the MFS are varied (19, 20).

According to Ghent nosology, an individual with a combination of aortic dilation, ocular systems problems, systemic symptoms, *FBN1* mutation and family history can be categorized as a patient with MFS (10). Since the presented case met the criteria of this nosology, he was diagnosed as a patient with MFS.

According to UMD-FBN1 mutations database (18), in 1994 Kainulainen et al reported (21) a Finnish female case with c.4537T>C mutation that resulted in p.Cys1513Arg due to familiar transition with clinical symptoms such as aortic dilatation, Mitral valve prolapse, Ectopia lentis, Myopia, Arachnodactyly, Chest deformity and increased body length. Our study showed some similarity in terms of mutation event and symptoms with this report.

Like the present study, two other cases with MFS have also been reported in UMD-FBN1 mutations database with c.4408T>C (p.Cys1470Arg) mutation: A female case from France and an unknown patient from United Kingdom while the present case is the first report of c.4408T>C (p.Cys1470Arg) mutation in Iran and Middle East (18, 22).

CONCLUSION

The present report showed that c.4408T>C mutation might be responsible for some changes in function and stability of *FBN1* gene that can lead to various clinical manifestations of patients with MFS. Due to unclear clinical symptoms in these patients such as different manifestations and overlaps with the other disorders such as Loeys-Dietz syndrome, genetic screening tests like NGS can be helpful for confirm the diagnosis of MFS. Considering the modernity of this approach, its high cost, lack of awareness for using this method, NGS is not a common test in Iran. Therefore, it is highly recommended to put more effort into introducing the NGS as an efficient approach in diagnosis of complicated cases to the physicians in order to avoid confusion and access to a rapid diagnosis of the genetic disorders.

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Conflict of interest
No conflict of interest was declared by the authors.

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