Informative STR Markers for Marfan Syndrome in Birjand, Iran

Ezzat Dadkhah¹, Masood Ziaee², Mohammad Hossein Davari³, Toba Kazemi⁴, Mohammad Reza Abbaszadegan*¹,⁵

Abstract

Objective(s)
Marfan syndrome (MFS) is a severe connective tissue disorder with an autosomal dominant inheritance pattern. Early diagnosis is critical in MFS. Because of the large size of fibrillin-1 gene (FBN1), the uniqueness of mutations, and the absence of genotype-to-phenotype correlations linkage analysis can be very helpful for early diagnosis of MFS. In this study, eight polymorphic markers were evaluated among families related to an affected pedigree.

Materials and Methods
An extended family in Birjand, Iran, with numerous cases of Marfan Syndrome in three consecutive generations, is being reported. From all consented members of these families, peripheral blood samples were collected in tubes containing EDTA. DNA extraction was performed by the conventional salting-out method. Eight STR markers were selected for linkage analysis, including four intragenic markers (MTS1, MTS2, MTS3, and MTS4) and another four flanking FBN1 markers (D15S119, D15S126, D15S1028, and D15S143). PCR-amplified fragments were evaluated on 15% polyacrylamide gel.

Results
MTS1, MTS2, and MTS3 were informative in the extended pedigree. D5S1028 was the only non-MTS marker which showed an informative diagnostic capability.

Conclusion
MTS markers were informative and useful in the molecular diagnosis of Marfan Syndrome in an extended pedigree. MTS1, MTS2, and MTS3 can be used as a prenatal or presymptomatic diagnosis for all members of the extended pedigree.

Keywords: Linkage analysis, Marfan syndrome, Microsatellite, MTS

¹Department of Human Genetics, Immunology Research Centre, Avicenna Research Institute, Mashhad University of Medical Science, Mashhad, Iran
*Corresponding author: Tel/fax: +98-511-7112343; email: abbaszadeganmr@mums.ac.ir
²Birjand Hepatitis Research Centre, Birjand University of Medical Sciences, Birjand, Iran
³Ophthalmology Department, Vali-e-Asr Hospital, Birjand University of Medical Sciences, Iran
⁴Birjand Atherosclerosis and Coronary Artery Research Centre, Birjand University of Medical Sciences, Birjand, Iran
⁵Medical Genetic Research Centre (MGRC), School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
Introduction
Marfan syndrome (MFS; MIM 154700) is an autosomal dominant disorder which affects connective tissues. MFS occurs in approximately 1 in 5000 to 1 in 10000 people (1). MFS can be diagnosed by certain physical examinations. Involvement of different organ systems, such as skeletal, cardiovascular, and ocular, and the degree of severity can be variable in MFS cases. The characteristics of the severe form of Marfan Syndrome are triad symptoms, consisting of skeletal changes (long thin extremities, loose joints, and arachnodactyly), ocular changes (such as dislocation of the lens), and cardiovascular problems (such as mitral valve prolapse and regurgitation, left ventricular dilatation, cardiac failure, and pulmonary artery dilatation). MFS predisposes patients to aortic complications. Aortic root dilatation is the main cause of morbidity and mortality in patients with MFS (2, 3). The syndrome has a complete penetrance, but it shows variability in the timing of onset, tissue distribution, and the severity of clinical manifestations, both between and within affected families. The molecular analysis of the phenotypic variation began in 1991, when the fibrillin-1 gene (FBN1) was introduced as the site of primary mutations (4). There are three human fibrillins: fibrillin-1, fibrillin-2, and fibrillin-3, which are encoded by different genes (5). It has been shown that mutations in the FBN1 gene (15q21.1), encoding fibrillin-1, can cause MFS (1). Fibrillin is a large glycoprotein which maintains the extracellular matrix and provides the elasticity and strength of connective tissues, including blood vessels, bones, and eyes. Mutations of the FBN1 gene are associated with 90% of Marfan cases (6). Of the remaining 10% of these cases, some have mutations in fibrillin-2 (5q23-31). There is some evidence showing that transforming growth factor β (TGF-β), TGF-β type I receptor (TGFBR1) and TGF-β type II receptor (TGFBR2) genes are also related to MFS (7). FBN1 is a 230 kb length gene, which encodes the Fibrillin-1 protein. This gene has 65 exons. Usually, major rearrangements are uncommon in the FBN-1 gene. Twelve percent of mutations are recurrent. Almost all of these mutations occur in CpG islands (8). In the Human Gene Mutation Database (HGMD), 782 different mutations have been documented for the FBN1 gene, of which 669 are related to Marfan Syndrome. FBN1 mutations include point mutations, gross and small deletions, gross and small insertions, and splicing mutations. According to HGMD, 66% of FBN1 mutations are missense/nonsense mutations. Approximately, 25-30% are new mutations. Missense, frameshift and splice site mutations are frequent mutations observed in MFS (9). Mutations in the FBN1 gene could be divided into two categories: mutations causing a truncated fibrillin-1 molecule (38.6%) and the missense mutations (60.3%) (8). Except for some particular cases, no clear genotype-phenotype correlation has been detected in the FBN1 gene (10). Since the FBN-1 gene is a large gene with 65 exons, screening and mutation detection for Marfan syndrome are not cost-effective methods. The FBN-1 mutation is not specific to Marfan syndrome, and the absence of mutation in this gene does not rule out the diagnosis of Marfan syndrome (8). Therefore, for prenatal diagnosis or presymptomatic detection of the disease in families with Marfan Syndrome linkage analysis can be useful for detecting the affected allele. In this study, eight polymorphic markers of the FBN1 gene were used to evaluate the informativity of these markers for an extended pedigree of MFS. The informative marker(s) could be used for prenatal or presymptomatic diagnosis of the family members.

Materials and Methods

Patients
The proband, the twenty-eight-year-old male in the pedigree, was referred to an ophthalmologist because of a vision problem. The medical examination revealed a lens displacement, iridodenosis and high intraocular pressure. The patient was of tall stature and exhibited arachnodactyly. He was referred to a cardiologist for further observation. The echocardiography supported the diagnosis of MFS by demonstrating aortic
root dilation and mitral valve prolapse. Other members of the family were also examined for ophthalmologic and cardiologic symptoms. Diagnosis parameters were consistent with Ghent nosology (1996) (11). An extended pedigree of these relatives was recruited in this study (Figure 1). Peripheral blood samples of available members of the pedigree were collected in EDTA containing tubes, after consent forms were completed.

Linkage analysis
DNA extraction was performed using the conventional salting-out method. The PCR reaction mixture consisted of 1X GENETBIO PCR buffer (GENETBIO, Korea), 500 nmol/l of each PCR primer, 1.5 mmol/l MgCl₂, 200 μmol/l dNTPs and 1U of Taq DNA Polymerase (GENETBIO, Korea). A total of 50 ng DNA was used in a reaction volume of 20 μl. PCR conditions for all markers were as follows: 5 min at 95 °C followed by 35 cycles of 20 sec at 95 °C, 20 sec at an annealing temperature and 20 sec at 72 °C followed by 5 min at 72 °C as final extension, with maximum heating and cooling settings in the Techne Thermal Cycler (Techgene, Techne, UK). Eight primer sets were selected for linkage analysis, D15S119, D15S126, D15S1028, D15S143, MTS-1, MTS-2, MTS-3 and MTS-4 (Table 1) (12). Single tandem repeat (STR) segments were amplified. PCR products were electrophoresed on 2% agarose gel and stained with ethidium bromide to analyze the strength of the amplified band and the possible existence of any nonspecific bands. The PCR products were electrophoresed on 15% acrylamide gel. The allelic pattern was analyzed on the acrylamide gel and informative alleles were reported. When the affected allele could be followed in a family, it was considered an informative marker.

Results
Patients exhibited complex symptoms, such as cardiovascular disorders and ocular and skeletal problems, that confirmed MFS. All patients were examined by an ophthalmologist and a cardiologist. The most common cardiac involvement in these patients was mitral valve prolapse and mitral regurgitation followed by the dilatation of the aortic root and aortic insufficiency. It was clarified that two sisters, two brothers, and the father of the proband's family were also afflicted with MFS. The mother and one of the brothers were healthy. There were thirteen affected MFS individuals in this pedigree of three generations (Figure 1). MTS-1, MTS-2, and MTS-4 are (CA)ₙ repeat markers, and MTS-3 contains the (TAAAA)ₙ repeat (13). The informativity status of the markers is shown in Table 1. Informative markers could be found in all accessible families of this pedigree. D15S1028, MTS1, MTS2, and MTS3 were informative in this pedigree (Table 2). The mutant allele was detected using these intragenic and extragenic short tandem repeat markers.
Linkage Analysis of Marfan Syndrome

Table 1. Primer sequences of eight STR markers of the FBN1 gene

| Primer | Sequence | Size (bp) | Annealing temperature |
|--------|----------|-----------|-----------------------|
| MTS-1F | 5’-CAACAAAAGAGGAGAAACAG-3’ | 128-146 | 57 |
| MTS-1R | 5’-GACAATGTATTCAGAGGC-3’ | 137-165 | 59 |
| MTS-2F | 5’-GTAAGTGTATCTTCAGA-3’ | 112-128 | 61 |
| MTS-2R | 5’-CTGCCCTCTAGACTCTAG-3’ | 182-197 | 57 |
| MTS-3F | 5’-GAGTTCAAGTGGTTTATGGG-3’ | 185-197 | 56 |
| MTS-3R | 5’-CCTGGCTATCTACAATCCC-3’ | 171-187 | 50 |
| MTS-4F | 5’-GATGTCCTCTATGCCATACAC-3’ | 189-199 | 59 |
| MTS-4R | 5’-CCTGGCTACCATTCAACTCCC-3’ | | |
| D15S119F | 5’-ACTTCTTGTGCAATTAGAGTT-3’ | | |
| D15S119R | 5’-AACAGAAATCCGTAACATAACATA-3’ | | |
| D15S126F | 5’-GTGAGCCAAGATGCGACACT-3’ | 188-218 | 56 |
| D15S126R | 5’-GCCAGCAATAATGGGAAGTT-3’ | | |
| D15S1028F | 5’-GAACTGTGCTCTGTGC-3’ | 182-197 | 57 |
| D15S1028R | 5’-GTGAGCCAAGATGCGACACT-3’ | | |
| D15S143F | 5’-CAACAAAAGAGGAGAAACAG-3’ | 128-146 | 57 |
| D15S143R | 5’-ATGTAAGAGCAGCTGATCT-3’ | 182-197 | 57 |

Table 2. The informativity of eight STR markers in the pedigree with Marfan syndrome

| STR      | Informativity |
|----------|---------------|
| D15S119F | Noninformative |
| D15S126F | Noninformative |
| D15S143F | Noninformative |
| D15S1028F | Noninformative |
| MTS1     | Noninformative |
| MTS2     | Noninformative |
| MTS3     | Noninformative |
| MTS4     | Noninformative |

Discussion

Marfan Syndrome is a clinically diagnosed disease. It has no treatment and, without any intervention, the patients may survive approximately 37 years (14). However, if intervention and attentive follow-up are provided, a normal life span is possible (6). The prognosis of MFS patients is determined by the extent and severity of the cardiovascular disorders (15). Besides the physical frustrations, patients with MFS have many psychological challenges. Living with a genetic disorder like Marfan Syndrome can exert social and emotional stress along with financial costs. Studies showed that the quality of life of the MFS patients was represented as poor. It was also clarified that 20% of patients with MFS suffer from depression (16). Mutational analysis using techniques such as single-strand conformation polymorphism (SSCP), denaturing high performance liquid chromatography, and direct sequencing can be employed for the genetic diagnosis of MFS. However, these techniques need extra money and time. In addition, only 8-30% of mutations can be diagnosed (17, 18). Most families with MFS have private mutations (19).

Using intragenic markers for linkage analysis can be a supplementary method for the MFS diagnosis of families, who have young members or show diverse or incomplete clinical symptoms (13). Moreover, molecular methods can be beneficial for prenatal diagnosis or presymptomatic individuals (12). A study in 1994 introduced four intragenic microsatellite markers for the linkage analysis of MFS: MTS1, MTS2, MTS3, and MTS-4 (20). They are located in introns 1, 5, 28, and 43, respectively. As these markers do not cover the entire gene, another group of researchers developed four other microsatellite markers spanning from the 5’ end of the gene to the intron 1 and from the intron 43 to the 3’ end of the gene. A restriction dimorphism in 3’-UTR was also employed. Their research showed that the combination of these eight microsatellite markers and the restriction dimorphism of 3’-UTR could cover complete haplotype heterozygosity in a population of 50 unrelated individuals. This panel offered a complete informativity for prenatal and presymptomatic diagnosis (12). The arrangement of these markers is shown in Figure 3. Lee et al., 2005 used four intragenic markers, MTS-1, MTS-2, MTS-3, and MTS-4,
for linkage analysis in six families with 18 MFS patients. It was shown that MTS-2 was fully informative in four families, MTS-4 in two families and MTS-3 in just one family. In the Lee et al study, the MTS-1 was not informative in any family (13). Mottes M et al (2000) evaluated five polymorphic markers, MTS-1 to MTS-4 and 3’-UTR RsaI polymorphic site, on 50 unrelated Italian subjects. It was indicated that the MTS-1 marker did not have any significant linkage disequilibrium with the three others, MTS2, MTS3, and MTS4, even though they appeared closely linked to each other. Mottes M et al reported that these polymorphic markers were very effective for the identification of the disease haplotype. The informative markers were found in all 12 families, except for one (21).

Figure 3. Position of polymorphic markers within and flanking the FBN1 gene (12)
Conclusion

In the current study of a Birjand extended pedigree, MTS markers were more informative, compared to other markers. MTS-1, MTS-2, and MTS-3 were the common informative markers for the families, and they could be used for the prenatal or presymptomatic diagnosis in this pedigree. Selected extragenic markers did not exhibit any great informative advantage for the pedigree in this study.

Acknowledgment

This study was supported by a grant from Birjand University of Medical Sciences, Birjand, Iran. The authors are thankful to Ms Samira Hosseini for her assistance with the molecular experiments.

References

1. Dietz HC, Loeys B, Carta L, Ramirez F. Recent progress towards a molecular understanding of Marfan syndrome. Am J Med Genet C Semin Med Genet 2005; 139C:4-9.
2. Judge DP, Dietz HC. Marfan's syndrome. Lancet 2005; 366:1965-1976.
3. Gao LG, Luo F, Hui RT, Zhou XL. Recent molecular biological progress in Marfan syndrome and Marfan-associated disorders. Ageing Res Rev 2009; 9:363-368.
4. Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, et al. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature 1991; 352:337-339.
5. Sakai LY, Keene DR, Engvall E. Fibrillin, a new 350-kD glycoprotein, is a component of extracellular microfibrils. J Cell Biol 1986; 103:2499-2509.
6. Judge DP, Dietz HC. Therapy of Marfan syndrome. Annu Rev Med 2008; 59:43-59.
7. Mizuguchi T, Matsumoto N. Recent progress in genetics of Marfan syndrome and Marfan-associated disorders. J Hum Genet 2007; 52:1-12.
8. Boileau C, Jondeau G, Mizuguchi T, Matsumoto N. Molecular genetics of Marfan syndrome. Curr Opin Cardiol 2005; 20:194-200.
9. Nollen GJ, Mulder BJ. What is new in the Marfan syndrome? Int J Cardiol. 2004 Dec; 97 Suppl 1:103-8.
10. Revencu N, Quenum G, Detaille T, Verellen G, De Paepe A, Verellen-Dumoulin C. Congenital diaphragmatic eventration and bilateral uretero-hydronephrosis in a patient with neonatal Marfan syndrome caused by a mutation in exon 25 of the FBN1 gene and review of the literature. Eur J Pediatr 2004; 163:33-37.
11. Davari M, Kazemi T, Alimirzaei H, Rezvani M. Cardiovascular Manifestation of a Family with Marfan’s Syndrome. J Teh Univ Heart Ctr? 2011; 6:37-40.
12. Judge DP, Biery NJ, Dietz HC. Characterization of microsatellite markers flanking FBN1: utility in the diagnostic evaluation for Marfan syndrome. Am J Med Genet 2001; 99:39-47.
13. Lee NC, Hwang B, Chen CH, Niu DM. Intrafamilial phenotype variation in Marfan syndrome ascertained by intragenic linkage analysis. J Formos Med Assoc 2005; 104:964-967.
14. Van Karnebeek CD, Naef MS, Mulder BJ, Hennekam RC, Offringa M. Natural history of cardiovascular manifestations in Marfan syndrome. Arch Dis Child 2001; 84:129-137.
15. Gonzales EA. Marfan syndrome. J Am Acad Nurse Pract 2009; 21:663-670.
16. Hasan A, Poloniecki J, Child A. Ageing in Marfan syndrome. Int J Clin Pract 2007; 61:1308-1320.
17. Loeys B, De Backer J, Van Acker P, Wettinck K, Dae Peape A, Verellen-Dumoulin C. Congenital diaphragmatic eventration and bilateral uretero-hydronephrosis in a patient with neonatal Marfan syndrome caused by a mutation in exon 25 of the FBN1 gene and review of the literature. Eur J Pediatr 2004; 163:33-37.
18. Loeys B, Nuytinck L, Delvaux I, De Bie S, De Paepe A. Genotype and phenotype analysis of 171 patients referred for molecular study of the fibrillin-1 gene FBN1 because of suspected Marfan syndrome. Arch Intern Med 2001; 161:2447-2454.
19. Colloid-Beroud G, Le Bourdelles S, Ades L, Ala-Kokko L, Booms P, Boxer M, et al. Update of the UMD-FBN1 mutation database and creation of an FBN1 polymorphism database. Hum Mutat 2003; 22:199-208.
20. Pereira L, Levrn O, Ramirez F, Lynch JR, Sykes B, Pyeritz RE, et al. A molecular approach to the stratification of cardiovascular risk in families with Marfan's syndrome. N Engl J Med 1994; 331:148-153.
21. Motte M, Mirandola S, Rigatelli F, Zolezzi F, Lisi V, Gordon D, et al. Allelic frequencies of FBN1 gene polymorphisms and genetic analysis of Italian families with Marfan syndrome. Hum Hered 2000; 50:175-179.