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Updated genetic studies of Marfan syndrome in China

Yuxin Sun[1], Di Zhou[2], Shouhua Wang[2], Jun Ding[3,*], Fei Ma[1,4,*]

[1] Department of Oncology, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China;
[2] Department of General Surgery, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China;
[3] Department of Biliary and Pancreatic Surgery, Shanghai Shuguang Hospital Affiliated to Shanghai University of T.C.M., Shanghai, China;
[4] Shanghai Institute for Pediatric Research, Shanghai, China.

SUMMARY Marfan syndrome (MFS) is an autosomal dominant connective tissue disease that affects multiple systems such as the ocular, skeletal, and cardiovascular systems. This disease is relatively rare and has no effective treatment except for symptomatic treatment. As a result, early detection, early intervention, and preventing the occurrence of adverse cardiovascular outcomes are crucial to the diagnosis and treatment of MFS. The rapid development of gene sequencing technology has facilitated the detection of MFS at the genetic level, allowing a more accurate and efficient diagnosis of the disease. Therefore, research on MFS-related genes has become a topic of interest. This article reviews the recent progress of genetic research on MFS in China.

Keywords Marfan syndrome (MFS), gene sequencing, *FBN1*, *TGFBR2*, base editor 3 (BE3)

Marfan syndrome (MFS) is an autosomal dominant connective tissue disease with a prevalence of 2-3/10,000 (*1*). According to an epidemiological survey in Taiwan, the total prevalence of MFS in the Chinese population is 10.2/100,000 (*2*), which is slightly different from the worldwide figure. MFS affects multiple organs and systems, including the eyes, cardiovascular system, musculoskeletal system, and possibly even the lungs, skin, and central nervous system. Cardiovascular diseases, including aortic dilation and dissection, are the leading cause of death in patients with MFS (*3*). Due to multi-organ involvement and the absence of an effective treatment except for symptomatic treatment, early detection and prevention of disease progression are crucial. The most common cause of MFS is a mutation in the fibrinogen 1 (*FBN1*) gene, which occurs in more than 90% of patients with MFS (*4*). Therefore, detection of mutations in the *FBN1* gene in patients with MFS, the relationship between gene mutation sites and phenotypes, and whether there are other gene mutations related to MFS have become hot topics in MSF research in China.

Since 1991, when the mutation of *FBN1* gene was identified as a pathogenic factor of MFS (*5*), approximately 2,900 mutated loci have been identified in the *FBN1* gene. Clinically, patients with *FBN1* mutations exhibit a range of phenotypes from mild to severe. A single mutation cannot fully explain the heterogeneity of clinical phenotypes in patients with MFS. In 2019, the Beijing Anzhen Hospital of Capital Medical University evaluated the genotype and phenotype in 180 patients by sequencing their DNA. The patients were divided into two groups according to clinical manifestations: an aortic dissection group and an aortic aneurysm group. Results indicated that frameshift mutations and nonsense mutations in *FBN1* were significantly more prevalent in patients with aortic dissection, while missense mutations in the *FBN1* gene were more prevalent in patients with aortic aneurysm (*6*). That said, a strong genotype-phenotype association between *FBN1* variants and MFS had not been reported. To resolve that situation, researchers at the Beijing Anzhen Hospital collected medical records and genetic tests from 131 patients with MFS in 2020 (*7*), and they found 82 low-frequency harmful loci in the *FBN1* gene, including 38 genes that had not been reported in the HGMD database. They also found that eight patients had two mutations (more than one SNP or INDEL locus) in the *FBN1* gene, and patients with two mutations in the *FBN1* gene exhibited a more significant MFS phenotype compared to other family members. They also found that patients with MFS had mutations in other genes, such as *PKD1*, *PKD2*, *FLNA*, *NKX2-5*, and *ACVRL1*. The frequency of mutations in the *PKD1* gene was much higher than that in other genes, and mutations in the *PKD1* gene were detected in a total of 27 people. This analysis also indicated that there are many genes associated with heart disease, such as *TTN, NEFH*, *PLEC*, *CASQ2*, and *SYNE1*. In the study, patients were divided into four groups depending on their aortic disease phenotype: 1): Aortic widening group; 2): Aortic aneurysm group; 3): Aortic dissection group; 4):

No aortic disease group. A statistical analysis with a *t*-test revealed a significant age difference between patients without an aortic disease phenotype and those with aortic aneurysm or aortic dissection ($P < 0.05$). Patients with aortic aneurysm or aortic dissection were mainly between 20 and 40 years of age, while patients without aortic disease were younger on average and the age distribution varied. The researchers ultimately created genotype-phenotype correlation maps and they combined sequencing results with various databases to screen and sequence affected genes. Genes were classified depending on the strength of the association between gene mutations and MFS. The top 10 candidate genes associated with a disease phenotype or the disease were *FBN1*, *MED12*, *TGFBR2*, *SMARD*, *FBN2*, *TP53*, *CDH1*, *FN1*, *COL4A3*, and *COL4a2*. These findings provide new ideas for further scientific research.

Although the *FBN1* gene is mutated in more than 90% of patients with MFS, *FBN1* mutations are not detected in 10% of patients clinically diagnosed with MFS, suggesting that atypical types of mutation or other genes may cause MFS. To look for atypical mutations or other mutations that cause MFS, the First Hospital Affiliated with Nanjing Medical University recruited 19 volunteers from three Han Chinese families between 2012 and 2016 to conduct a family-based study on 19 individuals using full exome sequencing (WES) (*8*). After DNA samples were collected, whole exon sequencing was performed, and quality control, mapping and variant calling were performed. Based on the literature regarding OMIM and MFS and the American Society of Medical Genetics (ACMG) standards and guidelines, genes were divided into eight previously reported MFS-related genes, 125 MFS-related genes from gene cards, and previously unknown genes. In the end, Sanger sequencing was performed after all remaining mutations were manually detected using an integrated genome viewer (IGV 2.3.80). A novel loss-of-function indel of *FBN1* (c. 5027 _5028insTGTCCTCC, p.D1677Vfs*8), a second novel loss-of-function indel (c.5856delG, p.S.1953Lfs*27), and a nonsense mutant (c. 8034C>A, p.Y2678 *) were found in those families. Moreover, all mutation sites were located in the highly conserved amino acid region (calcium-bounding epidermal growth factor (EGF) domain) across different species. These different types of loss-of-function (LOF) variations in *FBN1* are located in the cbEGF region and cross-species conserved domains and had not been previously reported.

Patients with type 2 MFS carry a mutation in the transforming growth factor β receptor 2 (*TGFBR2*) gene. In 2018, the Hypertension Diagnosis and Treatment Center of Fuwai Hospital, Chinese Academy of Medical Sciences conducted a study on two families with type 2 MFS, and for the first time researchers found that patients with MFS2 carried pathogenic mutations located in the *TGFBR2* gene transmembrane domain (*9*). They sequenced the *FBN1* gene and the *TGFBR2* gene. Their protein structures were predicted and a genotype-phenotype analysis was performed on the screened carriers of the *TGFBR2* gene transmembrane domain missense mutation. Results indicated that all carriers (100%, 8/8) of the *TGFBR2* gene transmembrane domain missense mutation met the main diagnostic criteria for cardiovascular involvement, with ascending aortic dilation, aortic root dilation, aortic dissection, or some other serious clinical phenotype of cardiovascular disease. Of the carriers, 75% (6/8) had abnormal skeletal involvement, and only 12.5% (1/8) of those met the main diagnostic criteria. None of the *TGFBR2* mutation carriers had ocular involvement. Results also indicated that carriers of two new *TGFBR2* gene missense mutations (p.137K(c.110T > A) and p. G43D (c.128G > A)) in families with type 2 MFS were more likely to develop aortic dilation or aortic dissection.

The recently developed base editor (BE) system is a novel technique involving the creation of a BE by fusing the protein dCas9 to deaminase. In contrast to traditional genome technologies based on CRISRP/Cas9, a BE edits specific loci through C-to-T or G-to-A transformations without requiring the formation of double-strand breaks (DSBs). The BE system provides a safer genome editing tool with low off-target effect (*10,11*). The Third Hospital Affiliated with Guangzhou Medical University conducted an experiment using BE3 to correct mutated MFS-related genes (*12*). They modeled the T7498C mutation of *FBN1* using CRISPR/Cas9 in combination with ssODN in HEK293T cells and then designed a specific single guide RNA (sgRNA). Mutant cells in this model were transfected with correctional sgRNA and BE3 expression plasmids. Three days later, the cells were collected, and genomic DNA was extracted and used as template to amplify the target sequence. PCR was performed, and the results were compared to those for the wild type to verify allele correction. Correction occurred in 10 of the 20 models, and ideal C-to-T correction at T7498C was performed in eight, demonstrating the high efficiency of the BE technique in correcting *FBN1*T7498C. The researchers then assembled immature oocytes and single sperm donated by patients with MFS and a heterozygous *FBN1*T7498C mutation after *in vitro* maturation intracytoplasmic sperm injection (ICSI) and partially injected BE3 mRNA and corrected sgRNA 16-18 hours later. Control samples were injected with BE3 mRNA and scrambled sgRNA and cultured. A total of 7 experimental embryos and 7 control embryos were obtained. Genome-wide amplification was performed, and genotyping was performed using Sanger sequencing. Results indicated that the rate of allele correction in the tested embryos was close to 100%. The researchers then performed deep sequencing and a comprehensive analysis of the test samples, the results of which revealed the high efficiency and precision of gene correction. Finally, the researchers tested non-target mutations

by deep sequencing and whole genome sequencing to demonstrate the safety of the aforementioned procedures, providing a theory and direction for further research. Studies and the significance of their findings are summarized in Table 1.

FBN1 gene research is also a hot topic in Europe, but European studies have reported slightly different results.

In a French study (*13*), the investigators took the mRNA of the *FBN1* gene from adventitial fibroblasts from multiple sites in 5 patients with thoracic aortic aneurysm or dissection. RT-PCR was then used to study the differences in levels of expression of *FBN1* subtypes between normal people and patients with MFS and differences in levels of expression of the three subtypes *FBN1_001* (ENST00000316623.5, NM_000138), *FBN1_004* (ENST00000559133.1), and *FBN1_009* (ENST00000561429.1) in relation to clinical phenotypes. This was the first time that *FBN1* alternative splicing was identified as a potential mechanism of clinical variability in MFS. In a Dutch study (*14*), 14 individuals from 2 families underwent next-generation sequencing (NGS) gene panel diagnostics. An *FBN1* mutation at site c.1453C>T, p.(Arg485Cys) was found to be a pathogenic mutation that leads to autosomal

Table 1. Research in China and several European countries

Lead author	Results	Significance
Shijun Xu (2019)	Frameshifts and nonsense mutations of the *FBN1* gene significantly more prevalent in patients with aortic dissection, while missense mutations of the *FBN1* gene were more frequent in patients with aortic aneurysm.	These results have laid the foundation for the study of the genotype-phenotype association between *FBN1* variation and MFS and may have guiding significance for the treatment of patients with MFS.
Yuduo Wu (2020)	1). 82 low-frequency harmful loci were identified in the *FBN1* gene, including 38 novel loci. 2). Patients with two mutations in the *FBN1* gene exhibited a more significant MFS phenotype. 3). Patients with MFS also have mutations in other genes, such as *PKD1* and *PKD2*; mutations in the *PKD1* gene were the most prevalent. 4). Many heart disease-related genes such as *TTN* and *NEFH* were noted. Patients without an aortic disease phenotype and patients with aortic aneurysm or aortic dissection differed significantly in age.	Many new MFS mutation sites and double mutation sites were identified, further confirming the pathogenicity of the *FBN1* gene in patients with MFS. A mutation in the *FBN1* gene is the main factor leading to aortic dissection or aneurysm in patients, and a double mutation site is the key factor aggravating the phenotype of patients
Zhening Pu (2018)	1). Two new sites of *FBN1* gene dysfunction (c.5027_5028insTGTCCTCC, p.D1677Vfs*8; c.5856delG, p.S1953Lfs*27) and a nonsense mutant (c.8034C>A, p.Y2678*) were identified. 2). All mutation sites were located in the highly conserved amino acid region (calcium binding epidermal growth factor (EGF) domain) in different species.	For the first time, different types of loss-of-function (LOF) variants of *FBN1* were identified in the cbEGF region and in the cross-species conserved domain.
Lin Zhang (2018)	The carriers of two new *TGFBR2* gene missense mutations p.137K(c.110T>A) and p.G43D(c.128G>A) in families with type 2 Marfan syndrome were more likely to develop aortic dilation or aortic dissection.	For the first time, results revealed that patients with MFS2 carried pathogenic mutations located in the transmembrane domain of the *TGFBR2* gene.
Yanting Zeng (2018)	In HEK293T cells, CRISPR/Cas9 combined with ssODN was used to establish the T7498C model of an *FBN1* mutation, and then the BE3 system was used for gene correction. Correction occurred in 10 of the 20 models, and ideal C-to-T correction at 7,498 was performed in eight. The safety of these procedures was verified with deep sequencing and whole genome sequencing to detect non-target mutations.	This study proved the safety of gene editing in patients with MFS and provided a theory and direction for further research
Louise Benarroch (France) (2019)	RT-PCR was used to study the differences in levels of expression of FBN1 subtypes between normal people and patients with MFS. Three subtypes were identified: *FBN1_001*, *FBN1_004*, and *FBN1_009*. The main isoform was *FBN1_001*, and it was significantly reduced in skin and adventitial fibroblasts of patients with MFS	This was the first time that *FBN1* alternative splicing was identified as a potential mechanism of clinical variability in MFS.
Eline Overwater (Netherlands) (2018)	An *FBN1* mutation caused by site c.1453C>T, p.(Arg485Cys) was found to be a pathogenic mutation that leads to autosomal dominant MFS, which is characterized by high clinical variability and apparently isolated early onset familial abdominal aortic aneurysms.	This study confirmed the high degree of clinical variability associated with *FBN1* variation and it provided new insights into the genetic pattern of *FBN1* variation c.1453C>T, p.(Arg485Cys)
Sinem Yalcintepe (Turkey) (2020)	Three mutated loci were identified: NM_000138.4(*FBN1*):c.229G>A(p.Gly77Arg), NM_000138.4(*FBN1*):c.165-2A>G(*novel*), NM_000138.4(*FBN1*):c.399delC (p.Cys134ValfsTer8) (*novel*).	The two novel pathogenic mutations have been added to the genotype-phenotype spectrum of clinical features of MFS. This case report emphasized the role of molecular analysis in the diagnosis of MFS.
Fatemeh Bitarafan (Iran) (2020)	5 mutations in *FBN1* and *2* mutations in *TGFBR2* were identified in 7 patients with MFS. *Novel* mutation sites were NM_000138.4(*FBN1*):c.3833G>A(p.Cys1278Tyr), NM_000138.4(*FBN1*):c.6288C>A(p.Cys2096*), and NM_003242.6(*TGFBR2*):c.1085A>G (p.His362Arg).	Early and accurate molecular diagnosis leads to better management and improved life expectancy, so the results of this study should greatly improve genetic counselling for families with MFS in Iran.

dominant MFS, which is characterized by high clinical variability and apparently isolated early onset familial abdominal aortic aneurysms. In a case report from Turkey (*15*), three patients with a prediagnosis MFS underwent gene sequencing, and three mutated loci were identified: NM_000138.4(*FBN1*):c.229G>A(p.Gly77Arg),NM_000138.4(*FBN1*):c.165-2A>G(*novel*), NM_000138.4(*FBN1*):c.399delC (p.Cys134ValfsTer8) (*novel*). The two latter mutations, which were not detected in the parents' genes, have been termed new pathogenic mutations and were added to the genotype-phenotype spectrum of clinical features of MFS. This case report emphasized the role of molecular analysis in the diagnosis of MFS. In Iran (*16*), 7 patients with MFS were screened for 14 genes (including *FBN1* and *TGFBR2*), and 5 mutations in *FBN1* and 2 mutations in *TGFBR2* were found. The novel mutation sites were NM_000138.4(*FBN1*):c.3833G>A(p.Cys1278Tyr), NM_000138.4(*FBN1*):c.6288C>A(p.Cys2096*), and NM_003242.6(*TGFBR2*):c.1085A>G (p.His362Arg). In addition, *FBN1* gene testing in patients with MFS has been reported in Italy, Spain, and elsewhere.

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**Address correspondence to:*
Fei Ma, Department of Oncology, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China, No. 1665 Kongjiang Road, Shanghai 200092, China.
E-mail: mafei@xinhuamed.com.cn

Jun Ding, Department of Biliary and Pancreatic Surgery, Shanghai Shuguang Hospital Affiliated to Shanghai University of T.C.M., Shanghai, China; Shanghai 201203, China.
E-mail: doctording0916@126.com

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