Table 1. Genotypic Manifestations

Mutations	Definite MFS group (n=51)	Non-definite MFS group (n=29)
FBN1 (n, %)	47/51 (92%)**	1/29 (3%)
TGFBR1 (n, %)	0/51 (0%)	2/29 (7%)
TGFBR2 (n, %)	1/51 (2%)	3/29 (10%)
TGFBR1 or *TGFBR2* (n, %)	1/51 (2%)	5/29 (17%)*
ACTA2 (n, %)	0/3 (0%)	4/23 (17%)
SLC2A10 (n, %)	0/3 (0%)	0/21 (0%)
MYH11 (n, %)	0/2 (0%)	0/6 (0%)
COL3A1 (n, %)	0/3 (0%)	0/6 (0%)
Undetermined (n, %)	3/51 (6%)	19/29 (66%)**

Data were expressed as mean±SD. *P<0.05, **P<0.01.

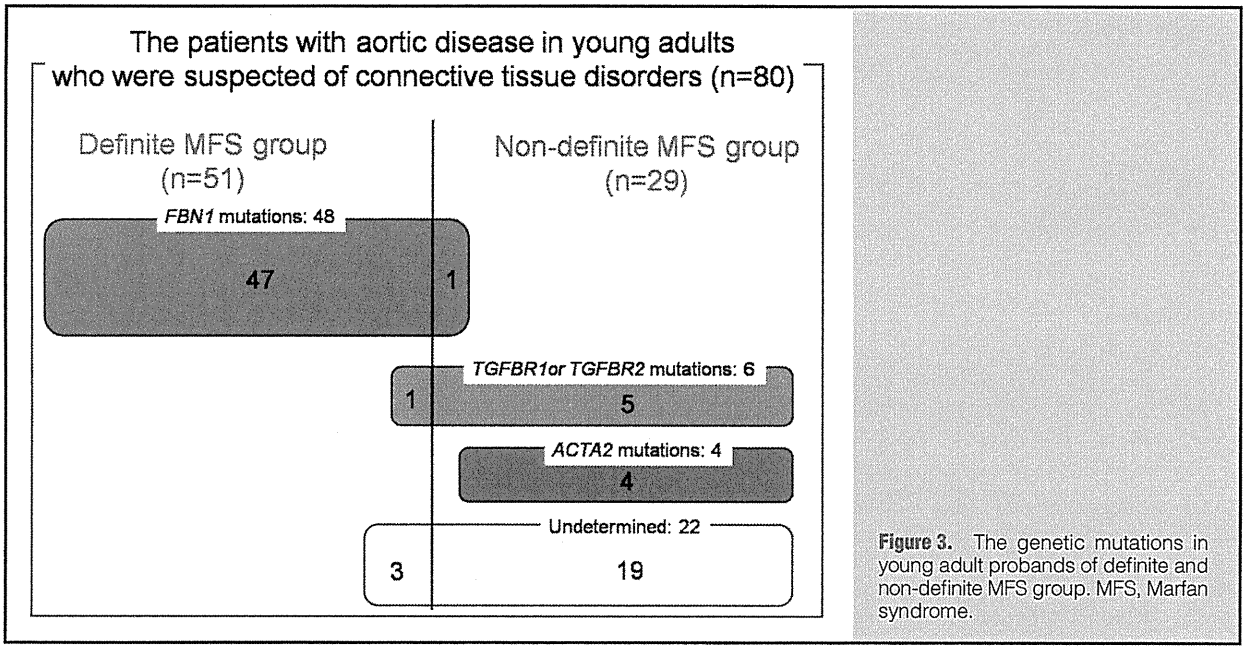

Figure 3. The genetic mutations in young adult probands of definite and non-definite MFS group. MFS, Marfan syndrome.

and *TGFBR2*. Mutations of *ACTA2* were examined in all of these 26 probands and 4 mutations were found. Mutations of *SLC2A10* were not found in 24 probands examined, and there were no *COL3A1* mutations out of 9 examined, and no *MYH11* mutations out of 8 examined. As a result, at least 58 (73%) mutations among all 80 probands were associated with aortic disease in young adults. The investigation flow chart is shown in Figure 1.

The results of genetic analysis of *FBN1* did not indicate any apparent phonotype–genotype correlation. All mutations of *TGFBR1* or *TGFBR2* were found in the exons corresponding to the kinase domain (data not shown). Also, all of these *TGFBR1* or *TGFBR2* mutations but one were a missense mutations, while the nonsense mutations found were not suggested to be a mutation causing nonsense mediated mRNA decay (data not shown).

Comparison of the Probands in Definite and Non-Definite MFS Groups

Genotypic Manifestations Genotypic manifestations in each group are shown in Table 1. Among 51 probands in the definite MFS group, 47 (92%) *FBN1* mutations and 1 (2%) *TGFBR2* mutations were found. *ACTA2* and *SLC2A10* mutations were investigated in the remaining 3 probands in the definite MFS group and no mutations were found.

Among 29 probands in the non-definite MFS group, 1 (3%) *FBN1*, 2 (7%) *TGFBR1*, and 3 (10%) *TGFBR2* mutations were found. In the remaining 23 probands, 4 *ACTA2* mutations were found. In total, at least 10 out of 29 (34%) probands in the non-definite MFS group had genetic mutations. Genetic mutations of both groups are summarized in Figure 3.

Comparing the probands in the definite and non-definite MFS groups, *FBN1* mutations were found more frequently in the definite MFS group than in non-definite MFS group (92% vs 3%, P<0.01). In contrast, *TGFBR1* or *TGFBR2* mutations were found more frequently in the non-definite than in the definite MFS group (17% vs 2%, P<0.05). *ACTA2* mutations were only found in the non-definite MFS group.

Phenotypic Manifestations The baseline clinical features are shown in Table 2. Comparing the probands in the definite and non-definite MFS groups, shorter height was observed more frequently in the non-definite MFS group (male; 176±6 cm vs 184±6 cm, P<0.01, female; 159±3 cm vs 174±8 cm,

Table 2. Baseline Clinical Features

	Definite MFS group (n=51)	Non-definite MFS group (n=29)
Age (years)	37±10	39±11
Male sex (n, %)	28 (38%)	20 (63%)
Height (cm)		
Male	184±6**	176±6
Female	174±8**	159±3
Obstructive sleep apnea	2/47 (4%)	3/24 (13%) (*ACTA2*:1)
Aortic dissection during pregnancy	2/23 female	1/9 female (*ACTA2*:1)
Family history (n, %)	25 (49%)	11 (40%)
Among Mutations (+) in each group	25/48 (52%)	7/10 (70%)
Among Mutations (–) in each group	0/3	4/19 (21%)

Data were expressed as mean±SD. **P<0.01.
Family history: family history of aortic dissection and/or sudden death at age <50 years or suspected.
Marfan syndrome: *ACTA2*:1, one patient was associated with *ACTA2* mutations.
Mutation (+): some mutations such as *FBN1*, *TGFBR1*, *TGFBR2*, *ACTA2*, were found.
Mutation (–): no mutations were found in *FBN1*, *TGFBR1*, *TGFBR2*, *ACTA2*, *SLC2A10*, *MYH11*, and *COL3A1* in the present study.

Table 3. Clinical Features Related to Ghent Nosology

	Definite MFS group (n=51)	Non-definite MFS group (n=29)
Skeletal system (n, %)		
Skeletal involvement	42/51 (82%)**	2/29 (7%) *TGFBR2*:1
Arm-span-to-height ratio >1.05	10/51 (20%)	1/27 (3%) *TGFBR2*:1
Thumb sign and wrist sign	33/51 (65%)**	2/28 (7%) *TGFBR2*:1
Joint hypermobility	26/50 (52%)**	3/25 (12%) *TGFBR2*:1
Cardiovascular system (n, %)		
Annulo-aortic ectasia	49/49 (100%)**	14/27 (52%)
Type A aortic dissection	11/51 (22%)	10/29 (34%)
Type B aortic dissection	18/51 (35%)	12/29 (41%)
Mitral valve prolapse	6/50 (52%)**	5/29 (17%)
Ectopia lentis (n, %)	13/50 (26%)**	0/26 (0%)
Dural ectasia (n, %)	35/51 (69%)**	4/29 (14%)
Lung involvement (n, %)	24/51 (47%)	7/29 (24%)
Skin involvement (n, %)	44/51 (24%)**	4/25 (7%)

Data were expressed as mean±SD. **P<0.01
Skeletal involvement: fulfilling 2 major criteria of Ghent nosology or one major and 2 minor criteria.

P<0.01). In the non-definite MFS group, the height of the patients with genetic mutations (n=10) was not significantly different from those without genetic mutations (n=19) (male; 177±6 cm vs 176±6 cm, female; 158±2 cm vs 159±3 cm). Obstructive sleep apnea was observed in 2 probands (2%) in the definite MFS group and 3 probands (13%) in the non-definite MFS group. Out of 3 probands with obstructive sleep apnea in the non-definite MFS group, one was associated with *ACTA2* mutations. Two probands in the definite MFS group and 1 proband in the non-definite MFS group presented with aortic diseases during pregnancy, and the latter proband had *ACTA2* mutations. Probands with hypertension from young age, and steroid use were not observed in either group. Although the number of the patients with family history of MFS or aortic disease did not differ between the definite and non-definite MFS group, some patients in the non-definite MFS group with no genetic mutations identified had a family history of MFS (4 out of 19; 21%).

Clinical features related to Ghent nosology are shown in Table 3. The following manifestations of Ghent nosology were less frequent in the non-definite than in the definite MFS group: skeletal system involvement (7% vs 82%, P< 0.01); thumb sign and wrist sign (3% vs 20%, P<0.01); joint hypermobility (12% vs 52%, P<0.01); AAE (52% vs 100%, P<0.01); mitral valve prolapse (17% vs 52%, P<0.01); ectopia lentis (0% vs 26%, P<0.01); dural ectasia (14% vs 69%, P<0.01); and skin involvement (7% vs 24%, P<0.01). The genetic background of each skeletal manifestation is also shown in Table 3. In the non-definite MFS group, Ghent skeletal manifestations were seen in some probands. However, one particular proband with mutations in *TGFBR2* gene fulfilled the criteria of "skeletal involvement", which means fulfilling 4 major skeletal manifestations, "arm-span-to-height ratio >1.05", "thumb sign and wrist sign", and "joint hypermobility", while the other probands of this group who fulfilled the skeletal criterion were not found to have any genetic mutations.

Clinical Features in Non-Definite MFS Group

The specific clinical features of the patients in the non-defi-

Table 4. Clinical Feature of Non-Definite MFS Group

	Mutations (+) (n=10)	Mutations (–) (n=19)
Features often found in the patients with genetic mutations other than *FBN1*		
Hyperterolism (n, %)	1 (*TGRBR1*)	0
Bifid uvula (n, %)	1 (*TGRBR2*)	0
Aortic branch aneurysm (n, %)	1 (*TGRBR1*)	0
Squint (n, %)	3 (*TGRBR2*:2) (*ACTA2*:1)	1 (FH–)
Arterial tortuosity (n, %)	2 (*TGRBR2*)	2 (FH–)
Livedo reticularis (n, %)	1 (*ACTA2*)	0
Iris flocculi (n, %)	1 (*ACTA2*)	0
Features listed in Ghent nosology		
Fulfilling 2 major criteria		
Cardiovascular+skeletal	1 (*TGFBR2*)	0
Cardiovascular+dural ectasia	0	1 (FH–)
Fulfilling 1 major criteria+2 involvement		
Dural ectasia+skin and cardiovascular involvement	0	1 (FH–)
Dural ectasia+skin and pulmonary involvement	0	1 (FH–)
Fulfilling 1 major criteria+1 involvement		
Cardiovascular+skeletal involvement	0	1 (FH–)
Cardiovascular+pulmonary involvement	1 (*TGFBR1*)	4 (FH+:1/FH–:3)
Cardiovascular+skin involvement	4 (*TGFBR1*:1) (*TGFBR2*:2) (*ACTA2*:1)	1 (FH+)

Mutation (+): some mutations such as *FBN1*, *TGFBR1*, *TGFBR2*, *ACTA2*, were found.
Mutation (–): no mutations were found in *FBN1*, *TGFBR1*, *TGFBR2*, *ACTA2*, *SLC2A10*, *MYH11* and *COL3A1* in the present study.
FH–: having no family history of aortic dissection and/or sudden death at age <50 years or suspected Marfan syndrome.
FH+: having a family history of aortic dissection and/or sudden death at age <50 years or suspected Marfan syndrome.

nite MFS group are shown in Table 4. Since easy bruising and thin and visible veins were not observed in the patients in the present study, no patient was strongly suspected of having Ehlers-Danlos syndrome. Patent ductus arteriosus was also not observed. Few specific skeletal features were observed in the patients with *ACTA2* mutations. In the Mutation (–) group, only 2 patients with tortuous aorta and one patient with squint, both without family history of MFS or aortic disease, were observed.

In addition, the extent of fulfilling the Ghent nosology in the non-definite MFS group is shown in Table 4. In the Mutation (+) group, some patients with *TGFBR1* or *TGFBR2* mutations fulfilled some criteria. In contrast, only one patient with *ACTA2* mutations fulfilled the criterion of skin involvement in addition to major criteria of cardiovascular system. In the Mutation (–) group, few patients fulfilled the Ghent criteria, even though some had a family history of MFS or aortic disease

Discussion

The results of the present study demonstrated that genetic mutations account for at least three-fourths of all causes of aortic disease in young adults. Especially in the non-definite MFS group, the genetic examination elucidated mutations of *TGFBR1* or *TGFBR2* and *ACTA2* in some probands, and genetic mutations accounted for at least one-third of all causes of aortic disease in the probands of the non-definite MFS group.

Among young patients with aortic disease, MFS associated with *FBN1* mutations was the most frequent cause of aortic disease. Recently, genetic mutations other than *FBN1* mutations were found in aortic disease. *TGFBR1* or *TGFBR2* mutations are known to cause LDS, Furlong syndrome and Shprintzen-Goldberg syndrome.[9,13,14] Among these diseases, phenotypic data of LDS are well documentd.[9] LDS is characterized by widely spaced eyes (hypertelorism), bifid uvula and/or cleft palate, and generalized arterial tortuosity with ascending aortic aneurysm and dissection. Although LDS was reported as MFS II initially, the phenotypic manifestations are often different from MFS.[2] In addition, the patients with *TGFBR1* or *TGFBR2* mutations do not always show the typical phenotype of LDS.[15] Therefore, we could not easily discriminate LDS from normal individuals only by clinical features.

ACTA2 mutations are reportedly the most common cause of TAAD without syndromatic characteristics, and they are responsible for 14% of TAAD, as compared with 5% and <2% for *TGFBR2* and *MYH11*, respectively.[4,16,17] The clinical features of the patients with *ACTA2* mutations were reported to be livedo reticularis and iris flocculi, but they are not always found in these patients, as we recently reported for a number of probands with *ACTA2* mutations.[4,5] *SLC2A10* mutations cause ATS, which is characterized by tortuousity and elongation of the large and medium-sized arteries, pulmonary arteries stenosis and aneurysm formation, often resulting in death at young age.[11] *MYH11* mutations are known as a cause of TAAD with patent ductus arteriosus.[6] Although patients

with mutations of *ACTA2*, *SLC2A10* or *MYH11* will develop characteristic abnormality in the aorta, their characteristic MFS-like features have not been described; therefore, we could not recognize their genetic disease by their readily observable physical features.

In the present study, *FBN1* mutations were found in 48 of 80 (60%) probands from patients with suspected connective tissue disorders, who had aortic diseases at a young age, and *TGFBR1* or *TGFBR2* mutations were found in 6 (8%) probands. *ACTA2* mutations were detected in 4 of the 26 probands examined. In total, more than 58 (73%) young probands with aortic disease had genetic mutations. Among 29 probands in the non-definite MFS group, there was 1 (3%) *FBN1* mutations and 5 (17%) *TGFBR1* or *TGFBR2* mutations. *ACTA2* mutations were found in 4 of the 22 probands examined. In total, more than 10 probands in the non-definite MFS group had genetic mutations. The remainder of the patients may have unknown genetic mutations, acquired factors or both. Indeed, some patients in the non-definite MFS group with no genetic mutations identified in the present study had a family history of MFS or aortic disease.

Acquired factors causing aortic diseases have not been fully elucidated. The causes of aortic dissection and those of aortic aneurysm should be different, and the causes of aortic disease in young individuals and those in old individuals should be also different. In elder individuals, aortic diseases were often associated with hypertension, smoking, atherosclerosis, and sleep apnea syndrome.[18–21] In contrast, in young individuals, the acquired factors causing aortic diseases are slightly different, including hypertension from young age, sleep apnea syndrome, pregnancy, steroid use, aortitis, etc.[22–27] In the present study, 3 cases of aortitis were observed among the first 129 patients before exclusion of some patients. Among the 29 probands in the non-definite MFS group, 3 obstructive sleep apnea cases including 1 with *ACTA2* mutations, and 1 pregnancy with *ACTA2* mutations, were found. Therefore, among the 29 probands in the non-definite MFS group, there are only 2 probands with aortic disease in young age whose aortic disease might be caused by acquired factors alone. Therefore, 12 probands had genetic mutations and/or acquired factors, and aortic disease in young age in 17 probands was still inexplicable through consideration of either genetic mutations or acquired factors.

Patients with MFS often develop aortic disease such as aortic dissection or AAE in young age. MFS is characterized by phenotypic abnormalities of the skeletal, ocular, and cardiovascular systems. Especially, skeletal abnormalities such as tall stature with long extremities are indicative of MFS. However, if young patients with aortic disease did not have MFS, we could not determine the cause of their aortic disease, because the characteristic features were often not observed in the patients with disorders other than MFS. The present study clearly showed that not only physical examination but also genetic study is needed to give a proper diagnosis, especially in young patients with aortic disease without MFS.

Some limitations of the present study must be taken into account. First, *COL3A1*, *MYH11*, *SLC2A10*, and *ACTA2* mutations were not examined for all 80 probands. We studied these mutations only in a maximum of 26 probands without *FBN1*, *TGFBR1*, and *TGFBR2* mutations. We have identified simultaneous two-gene mutations of *FBN1* and *TGFBR2* in one proband, although such double mutations seem to be rather rare. Therefore, we suspect the incidence of *ACTA2* mutations may be close to 4 out of 80 in the present study. Although we could not determine the exact incidence of the mutations, it is important to note that some patients with *ACTA2* mutations can be found in the patient population with aortic disease in young age. Second, we only showed the general characteristics in young patients with aortic disease without MFS. The presentation of the non-definite MFS could be heterogeneous. They may consist of various patients including patients with unknown genetic mutations, those with unknown acquired factors, etc. However, the present study showed that the non-definite MFS patients with aortic disease at a young age possess only a few obvious characteristic features, and it is difficult for us to discriminate them from normal individuals. Third, the method used to search genetic mutations in the present study might not capture all the causative mutations.

In conclusion, genetic mutations other than *FBN1* mutations were found in the non-definite MFS group with aortic disease in young age, and they accounted for one-third of all causes of aortic disease. If the etiology of aortic disease is not clear, we recommend genetic analysis with ethical considerations because these patients do not often exhibit characteristic features of MFS.

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Evaluating Japanese Patients With the Marfan Syndrome Using High-Throughput Microarray-Based Mutational Analysis of Fibrillin-1 Gene

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Marfan syndrome (MS) is an inherited connective tissue disorder, and detailed evaluations of multiple organ systems are required for its diagnosis. Genetic testing of the disease-causing fibrillin-1 gene (FBN1) is also important in this diagnostic scheme. The aim of this study was to define the clinical characteristics of Japanese patients with MS and enable the efficient and accurate diagnosis of MS with mutational analysis using a high-throughput microarray-based resequencing system. Fifty-three Japanese probands were recruited, and their clinical characteristics were evaluated using the Ghent criteria. For mutational analysis, an oligonucleotide microarray was designed to interrogate FBN1, and the entire exon and exon-intron boundaries of FBN1 were sequenced. Clinical evaluation revealed more pulmonary phenotypes and fewer skeletal phenotypes in Japanese patients with MS compared to Caucasians. The microarray-based resequencing system detected 35 kinds of mutations, including 23 new mutations. The mutation detection rate for patients who fulfilled the Ghent criteria reached 71%. Of note, splicing mutations accounted for 19% of all mutations, which is more than previously reported. In conclusion, this comprehensive approach successfully detected clinical phenotypes of Japanese patients with MS and demonstrated the usefulness and feasibility of this microarray-based high-throughput resequencing system for mutational analysis of MS. © 2011 Elsevier Inc. All rights reserved. **(Am J Cardiol 2011;108:1801–1807)**

The Marfan syndrome (MS) is an inherited connective tissue disorder with an autosomal dominant inheritance, primarily involving the skeletal, ocular, and cardiovascular systems, caused by mutations in fibrillin-1 gene (FBN1).[1] Diagnosis of the MS has been made using the Ghent criteria[2] on the basis of data from European and American populations, but the Ghent criteria may not be completely suitable for the Japanese population.[3] Therefore, epidemiologic and genetic surveys in the Japanese population are mandatory to establish more Japanese-specific (or Asian-specific) diagnostic criteria for the MS. The Ghent criteria were recently further revised.[4] More weight is now given to FBN1 testing, and a diagnosis can be made if a patient has the FBN1 mutation plus either an aortic phenotype or ectopia lentis. These new criteria are much simpler than the original criteria. Thus, genetic testing of MS is becoming more important. FBN1 spans a 230-kb genomic region and contains 65 exons. More than 1,000 reported mutations are spread throughout the gene and are mostly unique in each affected family.[5,6] Classic genetic analysis methods such as direct sequencing are very time consuming. Thus, the introduction of a more efficient genetic analysis tool is needed. Custom-designed resequencing microarrays enable the analysis of multiple genes spanning 30 to 300 kb on a single array. The microarray identifies individual nucleotides by comparative, high-fidelity hybridization using oligonucleotide probes[7–9] (Figure 1). In the present study, we comprehensively evaluated the clinical characteristics of Japanese patients with suspected MS and also conducted mutational analysis of these patients by adopting a high-throughput genetic diagnosing system to achieve more efficient and accurate diagnoses.

Methods

Fifty-three consecutive probands suspected of having MS who visited the MS clinic at our hospital were enrolled. All patients were assessed using the original Ghent criteria.[2,10] This study was conducted according to the Declara-

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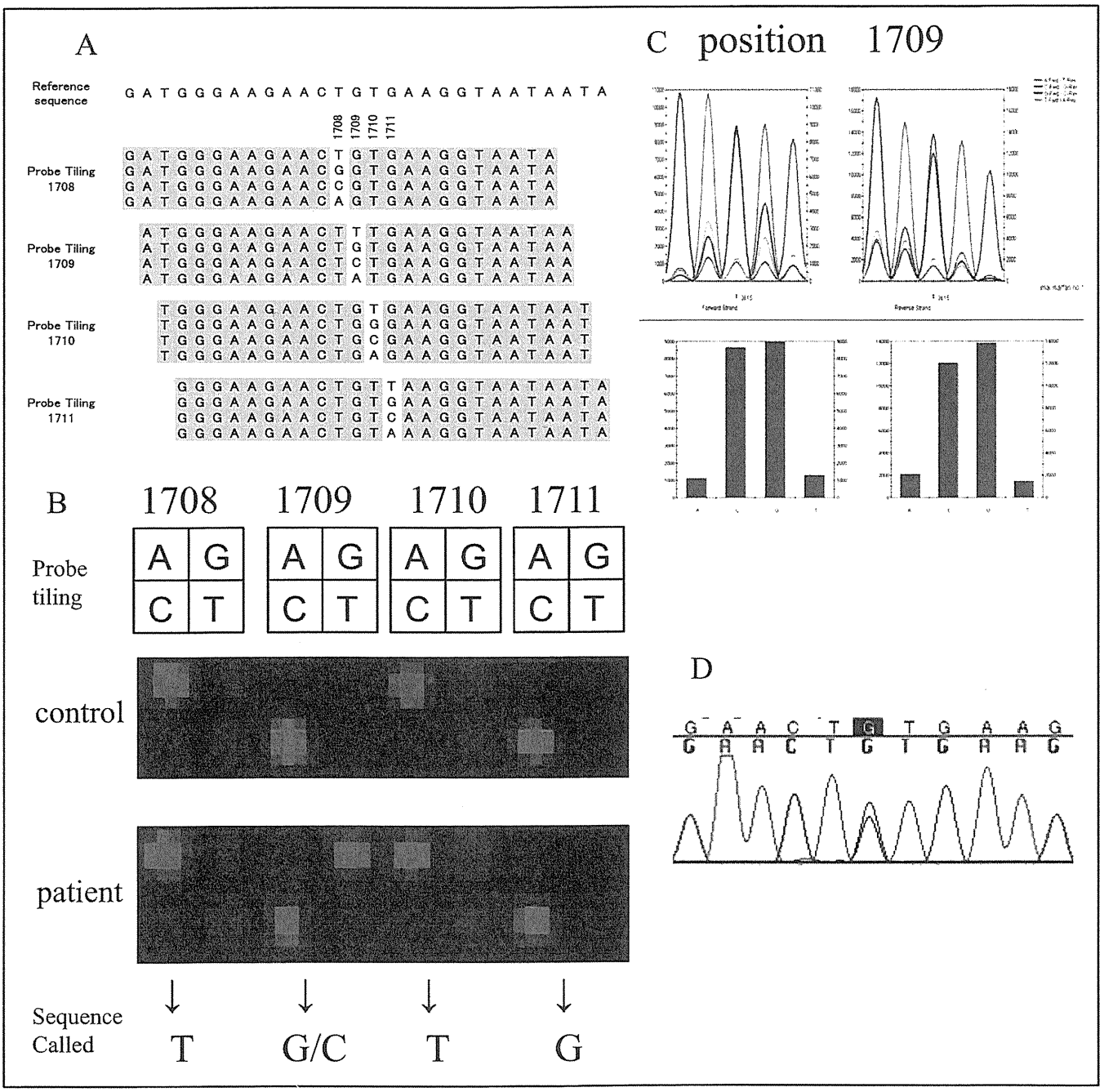

Figure 1. Representative example of mutational analysis using the present microarray-based resequencing system. *(A)* The microarray identifies individual nucleotides by comparative, high-fidelity hybridization using oligonucleotide probes that are synthesized in situ by photolithography and solid-phase DNA synthesis. For each base position, 8 unique 25-mer probes (4 oligonucleotide probes for each strand) are tiled on the array, and each 25-mer probe is varied at the central position to incorporate each possible nucleotide (A, G, C, or T), allowing the detection of all possible nucleotide substitutions. *(B)* Scan images of the probes around the nucleotide position 1709. In patients with the FBN1 c.1709G>C mutation, high signal intensities can be observed in probe G and C at nucleotide 1709 compared to control. *(C,D)* Signal intensity data at nucleotide position 1709. The intensity data for each base position can be also displayed as traces and bar graphs. The missense mutation (c.1709G>C) was successfully detected *(C)* and was verified by direct sequencing *(D)*.

tion of Helsinki and was approved by the institutional ethics committee. Written informed consent was obtained after providing a detailed explanation of the study. Genomic deoxyribonucleic acid (DNA) was extracted from buffy coat using a Genomix DNA extraction kit (Talent, Trieste, Italy). For amplification of the 65 exons of FBN1, polymerase chain reaction (PCR) primers were designed by referring to previous reports.[11–13] After performing the PCRs according to the standard protocol, the PCR products were subjected to hybridization on the microarray.

The resequencing microarray was designed on the basis of the reference sequences from the Ensembl database. Because highly homologous sequences lead to cross-hybridization, FBN1 was checked for possible repetitive sequences using RepeatMasker software (http://repeatmasker.org/chi-bin/webrepeatmasker). No repetitive elements were

Table 1
Background of participants who underwent genetic analysis (n = 53)

Variable	Total (n = 53)	Ghent-Positive Patients (n = 45)
Age (years)	33.1 ± 9.8	33.1 ± 10.4
Men	35/53 (66%)	30/45 (67%)
Ghent positive	45/53 (85%)	45/45 (100%)
Skeletal major criteria	12/49 (25%)	12/41 (29%)
Skeletal minor criteria	19/49 (39%)	17/41 (42%)
Ectopia lentis	25/53 (47%)	25/45 (56%)
Cardiovascular major criteria	48/53 (91%)	44/45 (98%)
Cardiovascular minor criteria	36/48 (75%)	32/41 (78%)
Pulmonary	22/49 (45%)	19/42 (45%)
Skin	26/49 (53%)	23/42 (55%)
Dural ectasia	34/47 (72%)	33/40 (83%)
Family history of MS	31/55 (56%)	28/45 (62%)

Data are expressed as mean ± SD or as number (percentage).

observed. The microarray contained sense and antisense sequences for the 65 exons of FBN1 and ≥12 flanking base pairs of the splice junctions. The PCR product was fragmented, end-labeled with biotin, and hybridized to the array. Washing and staining with streptavidin-phycoerythrin were performed on automated fluidic stations according to the manufacturer's protocol (Affymetrix, Santa Clara, California). Hybridization signals were read by a high-resolution laser scanner, and the data collection and interpretation were carried out using GeneChip Operating Software and GeneChip Sequence Analysis Software (Affymetrix), respectively.

Candidate nucleotide substitutions detected by the microarray-based resequencing system were subsequently validated by fluorescent dideoxy DNA sequencing using BigDye terminator version 3.1 on an ABI PRISM 3100xl genetic analyzer (Applied Biosystems, Foster City, California).

Some patients underwent cardiovascular surgery, and written informed consent for research use of surgical specimens was obtained from each patient. Total ribonucleic acid (RNA) was extracted using an RNeasy Fibrous Tissue Mini Kit (Qiagen, Venlo, The Netherlands). For patients whose aortic tissues were not available, total RNA was extracted from blood using a QIAamp RNA Blood Mini Kit (Qiagen). The RNA was converted to complementary DNA using SuperScript III First-Strand Synthesis SuperMix (Invitrogen, Carlsbad, California). PCR analyses were performed with specific primers designed for the target regions. PCR samples or subcloned plasmids after TA cloning of PCR products using a TOPO-TA vector (Invitrogen) were subjected to fluorescent dideoxy DNA sequencing.

DNA from patients whose mutations were not found by the aforementioned methods was screened by multiplex ligation-dependent probe amplification using a SALSA MLPA kit P065/P066 (MRC-Holland, Amsterdam, The Netherlands)[14] for large deletions and duplications.

All quantitative data are expressed as mean ± SD. Statistical comparisons of distributions between groups were made using the chi-square test. Significance was taken as $p < 0.05$.

Table 2
Detailed clinical findings of Ghent-positive patients (n = 45)

Criterion	n (%)
Skeletal major criteria	
Pectus carinatum	9/42 (21%)
Pectus excavatum, requiring surgery	7/44 (16%)
Arm span/height ratio >1.05	8/41 (20%)
Wrist and thumb signs	32/43 (74%)
Scoliosis of >20% or spondylolisthesis	21/44 (48%)
Reduced extension at the elbows (<170°)	2/41 (5%)
Medial displacement of medial malleolus, causing pes planus	16/41 (39%)
Protrusio acetabuli	8/39 (21%)
Skeletal minor criteria	
Pectus excavatum of moderate severity	10/44 (23%)
Joint hypermobility	7/41 (17%)
Highly arched palate with crowding of teeth	31/40 (78%)
Facial appearance	15/40 (38%)
Cardiovascular major criteria	
Dilatation/dissection of the ascending aorta	44/45 (98%)
Cardiovascular minor criteria	
Mitral valve prolapse	23/42 (55%)
Dilatation of main pulmonary artery	9/20 (45%)
Calcification of mitral annulus	0/34 (0%)
Dilatation/dissection of descending thoracic/abdominal aorta	12/43 (28%)
Pulmonary minor criteria	
Spontaneous pneumothorax	13/43 (30%)
Apical blebs	15/44 (34%)
Skin minor criteria	
Striae atrophicae	24/42 (57%)
Recurrent or incisional herniae	0/41 (0%)

Results

Of the 53 probands enrolled, 45 were diagnosed with MS according to the original Ghent criteria. Because our Marfan clinic offers cardiac surgery and some patients were referred for aortic surgery from other hospitals, most of the patients had aortic phenotypes (Table 1). Dural ectasia and ectopia lentis were common findings, and positive family histories were seen in about half of the probands. We confirmed a lower frequency for some of the skeletal manifestations in Japanese patients with MS compared to that reported in a Western database, such as an arm span/height ratio >1.05 (20% in our study vs 55% in Western populations) and reduced extension at the elbows (<170°) (5% vs 15%), findings that were similar to the report of Akutsu et al[3,6] (Table 2). However, the frequency of major skeletal criteria (29%) was higher than a previous Japanese report (15%), which is partially due to a lack of evaluation of protrusio acetabuli in the earlier study. We found a higher frequency of spontaneous pneumothorax (30% vs 7%) in our Japanese population compared to a previous study conducted in Western patients. Calcification of the mitral annulus and frequency of dilatation of the main pulmonary artery were rarely reported. Actually, mitral annular calcification was not detected at all. However, pulmonary artery dilatation was relatively frequent (45% [9 of 20]) in our study, after excluding those patients whose main pulmonary artery diameters were difficult to evaluate.

Table 3
Mutations found in this study

Exon	Complementary DNA	Protein
Missense mutations		
4	c.386G>A	p.Cys 129 Tyr
13	c.1709G>C*	p.Cys 570 Ser
14	c.1786T>G*	p.Cys 596 Gly
15	c.1911T>G*	p.Cys 637 Trp
18	c.2171T>G*	p.Ile 724 Arg
18	c.2201G>T	p.Cys 734 Phe
21	c.2638G>A	p.Gly 880 Ser
24	c.3043G>A	p.Ala 1015 Thr
26	c.3263A>G*	p.Asn 1088 Ser
28	c.3503A>G	p.Asn 1168 Ser
34	c.4280A>G*	p.Tyr 1427 Cys
43	c.5371T>C*	p.Cys 1791 Arg
47	c.5873G>A*	p.Cys 1958 Tyr
50	c.6296G>T	p.Cys 2099 Phe
53	c.6518G>A*	p.Gly 2173 Ser
57	c.7015T>G*	p.Cys 2339 Gly
60	c.7466G>A*	p.Cys 2489 Tyr
62	c.7754T>C	p.Ile 2585 Thr (2 probands)
Nonsense mutations		
8	c.945T>A*	p.Cys 315 X
12	c.1585C>T	p.Arg 529 X
29	c.3603C>A*	p.Cys 1201 X
37	c.4709G>A*	p.Trp 1570 X
38	c.4777G>T*	p.Glu 1593 X
38	c.4786C>T	p.Arg 1596 X
54	c.6658C>T	p.Arg 2220 X
58	c.7240C>T	p.Arg 2414 X
65	c.8521G>T*	p.Glu 2841 X
Splicing mutations		
11–12	c.IVS11+5G>A	p.Cys474Tyr Glu475_Asp490del
15–16	c.IVS15-3T>G*	
16–17	c.IVS16+3A>C*	
18–19	c.IVS18+1G>C*	
34–35	c.IVS34-1G>A*	p.Asp1446ValfsX21
40–41	c.IVS40+1G>A*	
52–53	c.6453C>T*	p.Cys2151Tyr, Glu2152_Asp2166del
56–57	c.IVS56+5G>A*	
Deletion mutations		
54	c.6665delT*	p.Val2222GlyFsX69
54	c.6703-6704delGG*	p.Gly2235IlefsX7
55	c.6837delG*	p.Tyr2280IlefsX10
57	c. 7071_7079delCGTCACCAA*	p.Val2358SerfsX511
65	c. 8532_8delTACAACT*	p.Thr2785X
3	Exon 3 deletion*	

* Newly found mutation.

In our mutational analysis, the base call rate of this system for FBN1 was >96% when examining 5 representative cases, and resequencing as many as 12,688 bp per patient was easily accomplished in 3 working days, demonstrating the high fidelity and high throughput of this system.

In the 53 probands, 35 kinds of FBN1 mutations were found in 36 probands using this system (Table 3). There were 18 missense and 9 nonsense mutations. Eight other mutations located near the exon-intron boundaries were thought to alter the splicing patterns. Supplemental direct sequencing in probands with no mutation detected by the microarray-based method revealed 5 deletion mutations in FBN1 (Table 3). Furthermore, multiplex ligation-dependent probe amplification assay revealed a large deletion mutation (exon 3) in 1 proband. Finally, novel mutations were found in 23 probands using microarray and in 29 probands in total. All possible mutations found by the microarray-based resequencing system were verified by direct sequencing, and thus the microarray detected point mutations with 100% accuracy. A representative example of genetic analysis using the microarray-based resequencing system is shown in Figure 1. Of 18 missense mutations, 11 were either affecting or creating cysteine residues. For other novel missense mutations, none of the mutations were found in ≥200 ethnically matched control subjects. The mutation detection rate

Table 4
Number of mutations detected

Mutation Detection Method	Total (n = 53)	Ghent Positive (n = 45)	Other (n = 8)
Microarray	36 (68%)	32 (71%)	4 (50%)
Direct sequencing	5 (9%)	5 (11%)	0
Multiplex ligation-dependent probe amplification	1 (2%)	1 (2%)	0
Total of all 3 modalities	42 (79%)	38 (84%)	4 (50%)

of the microarray-based resequencing system for the Ghent-positive patients was 71%. The overall mutation detection rate after additional analysis by fluorescent dideoxy DNA sequencing and multiplex ligation-dependent probe amplification reached 84% (Table 4).

Eight possible splicing mutations were identified, and these mutations constituted 19% of all mutations, which was more than the 11% currently reported in the UMD-FBN1 mutation database.[5] One patient and his 2 relatives with MS had the same silent mutation in FBN1 exon 52 (c.6453C>T, p.Cys2151Cys; Figure 2). Therefore, we re-sequenced complementary DNA from his aortic tissue and verified an alternation of the splicing pattern between FBN1 exon 52 and 53. The C at nucleotide position 6453 of FBN1 complementary DNA was substituted with a T, which resulted in the creation of a new splicing donor site, causing abnormal shorter messenger RNA. Another patient had a mutation at the fifth nucleotide of the beginning of intron 11 (c.IVS11+5G>A), although it is well known that the first 2 nucleotides at the beginning of the intron are very important as a splice donor site. We found by sequencing the complementary DNA that the latent splice donor site within exon 11 was activated and created the frame-shift mutation (Figure 2).

Six additional mutations possibly causing a splicing aberration were also found (Table 3). Although aortic tissue was unavailable for these patients, splicing aberrations were successfully confirmed in 2 whose complementary DNA was clinically available by resequencing FBN1 complementary DNA obtained from peripheral blood (Figure 2).

In published research, it has been suggested that mutations causing the in-frame loss or gain of the central coding sequence through deletions, insertions, or splicing errors are thought to be associated with more severe disease phenotypes. In contrast, nonsense mutations that result in rapid degradation of mutant transcripts are reported to be potentially associated with milder conditions. However, we could not find any associations between mutation types and clinical severity in our study subjects. A higher incidence of ectopia lentis in patients who carried a missense mutation involving a cysteine substitution or splicing mutation has been reported.[15] However, these correlations were not observed in our study. Among 4 patients who had mutations located between FBN1 exons 24 and 32, the so-called "neonatal region," none had the neonatal or early-onset form of MS.

Discussion

The Ghent criteria for MS diagnosis are based on data obtained mainly from European and American populations. Our clinical evaluations revealed that there were more pulmonary phenotypes and fewer skeletal phenotypes in Japanese patients with MS compared to Western patients. Therefore, the criteria for systemic and orthopedic features in the Ghent nosology may not be entirely suitable for application to Japanese and perhaps other Asian populations. Further epidemiologic and genetic studies in the Japanese population should be conducted to establish Asian- or Japanese-specific diagnostic criteria for MS.

The present microarray-based resequencing system is an efficient method for rapid and affordable mutation analysis of heterogenous disorders such as MS. The mutation detection rate is influenced by the accuracy of the clinical diagnosis of MS, the type of mutation, and the ability of the testing method. It ranged from 55% to 91% in previous reports.[11,16–18] The mutation detection rate of our system was concordant with previous reports. Its greatest advantages are high throughput and digitalized sequencing data. The digitally retrieved sequencing data are easily computable and can be displayed in various ways. In most of the cases, we could identify the mutations within a few minutes of data collection. Several other causative genes, such as transforming growth factor receptor types 1 and 2 (TGFBR1 and TGFBR2),[19] smooth muscle α-actin (ACTA2),[20] myosin heavy chain 11 (MYH11),[21] and SMAD3,[22] have been identified for syndromic or nonsyndromic aortic aneurysms and dissection. Such additional candidate genes can also be included on the same array because 1 array can resequence up to 300 kb.

Our system can detect point mutations with 100% accuracy and thus is a reliable first screening method for detecting single nucleotide substitutions. In contrast, it is difficult to detect heterozygous deletion or insertion mutations, because an abnormal allele containing a deletion or an insertion mutation is difficult to hybridize to probes. For patients with MS with no mutation detected by the microarray system, conventional direct sequencing and multiplex ligation-dependent probe amplification was helpful for searching for possible deletion or insertion mutations. Because there is a certain number of patients with MS without mutations in FBN1,[12,19] the 7 probands without any mutations may have possessed mutations in undiscovered disease-causing genes.

Eight splicing mutations that accounted for 19% of all the mutations were found. Because this type of mutation represented a greater proportion than that of previous reports, every exon-intron boundary should be resequenced. It is also advisable to obtain messenger RNA in addition to DNA for analyzing the splicing pattern. We successfully demonstrated altered splicing patterns using FBN1 messenger RNA extracted from peripheral leukocytes. Thus, we also recommend the extraction of RNA as well as genomic DNA from peripheral blood, if a surgically retrieved specimen is not available.

We also assessed patients using the recently published revised Ghent criteria. Forty-two of the 45 original Ghent-positive patients were also diagnosed with MS using the revised criteria. One patient, who was positive according to the original Ghent criteria, did not satisfy the revised criteria and was diagnosed with ectopia lentis syndrome. Two patients (aged 20 and 30 years) failed to meet the revised Ghent criteria because their z scores of aortic diameter were not

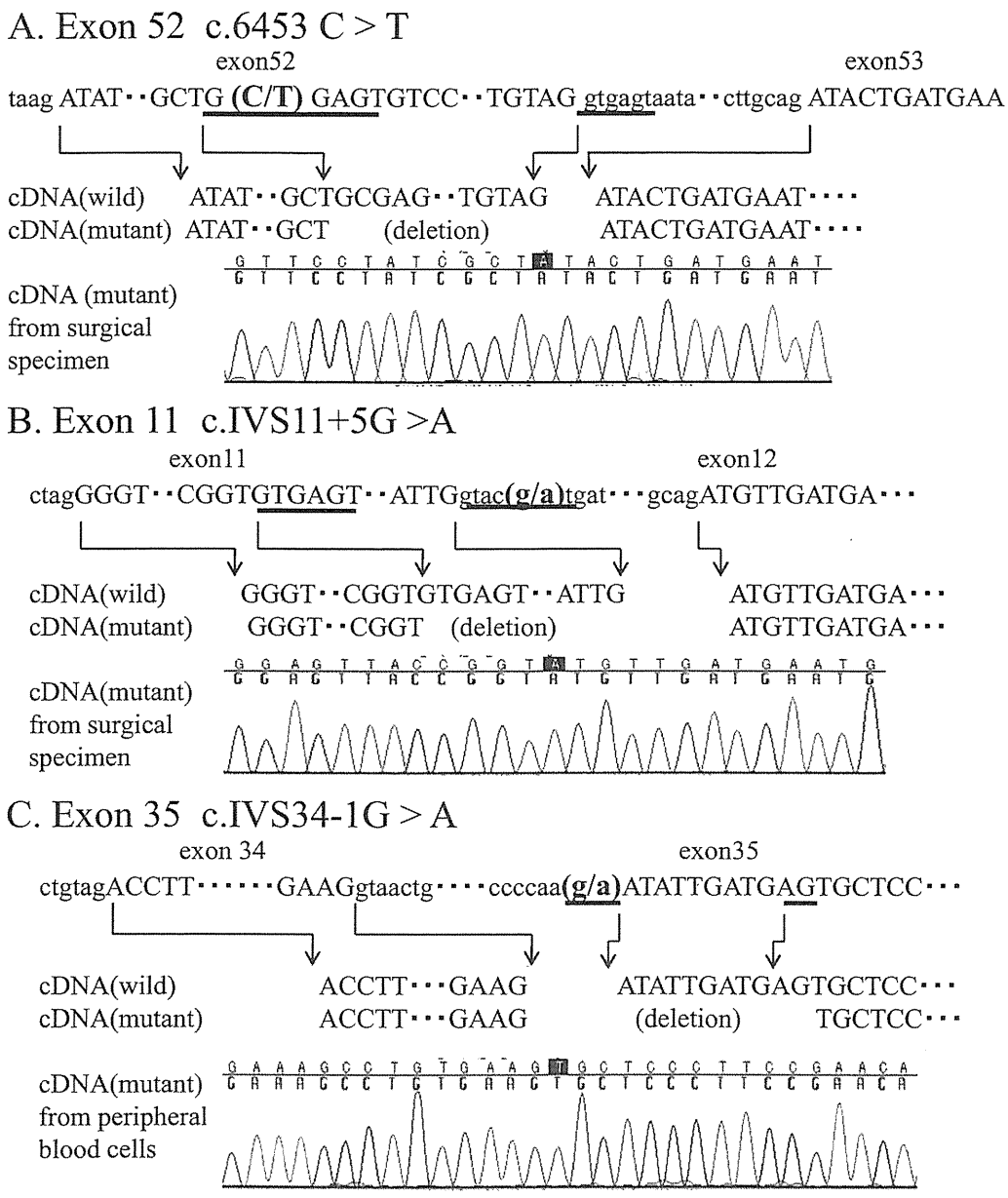

Figure 2. Representative splicing mutations in our study. *(A)* C-to-T substitution at nucleotide position 6453 produced a 6–nucleic acid sequence (c.6452-6457) *(underlined)* identical to that of IVS52+1 to +6 (gtgagt) *(underlined)*, and this was recognized as the splicing donor site. The following nucleotides of exon 52 from this point were deleted in the mutant allele. *(B)* The fifth nucleotide at the beginning of intron 11 was substituted from G to A (c.IVS11+5G>A). Resequencing of complementary DNA revealed that the latent splice donor site within exon 11 *(underlined)* was activated and became a new splice donor site and created the frame-shift mutation. *(C)* The last nucleotide of intron 34 was substituted from G to A (c.IVS34-1G>A). Resequencing FBN1 complementary DNA obtained from the peripheral blood demonstrated the splicing aberration, resulting in the deletion of 11 nucleotides of exon 35.

satisfactory, although their aortas dilated gradually later on. One patient met the revised Ghent criteria after genetic analysis of FBN1. Although further studies are warranted to clarify the properties and usefulness of the novel Ghent criteria, genetic testing is more important, although it is not mandatory. In such a setting, a high-throughput resequencing method such as the present microarray-based resequencing system can be a powerful tool for making an accurate diagnosis of MS.

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研究

マルファン症候群では歯周病は極めて高頻度に認められる*

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要旨

マルファン症候群は，骨格異常，眼異常，心血管異常など多くの器官に病変を引き起こす常染色体優性遺伝の全身性結合組織疾患である．以前より口腔内所見として，高口蓋，歯列不正などが知られている．近年，諸外国においてマルファン症候群と歯周病との関係が注目されてきており，日本人におけるマルファン症候群の実態調査としてGhent基準陽性20名のマルファン症候群症例につき歯周病罹患状態を評価した．現在歯数は27歯とほぼ保たれていたが，歯周ポケットの深さ(PD)は2.815±0.624 mm，PD測定部位での出血の有無(BOP)は11.567±8.394%，地域歯周疾患指数(CPI)は中等度・重度に該当するコード3，4の症例が15名(75%)も認められた．以上よりマルファン症候群では，中等度から重度の歯周病が高頻度に認められマルファン症候群における歯周組織の脆弱性が示唆された．

キーワード　マルファン症候群，歯周病，地域歯周疾患指数(CPI)

マルファン症候群は，1896年にパリの小児科医Antoine Marfanにより初めて報告された常染色体優性遺伝性の疾患である[1]．全身において骨格異常，眼異常，心血管異常など多くの器官に病変を引き起こし，また，口腔においては高口蓋，歯列不正，歯の形態異常などがみられることが知られている[2,3]．今日その診断には，Ghentの基準[4]を採用することが一般的であり，骨格異常や眼異常，心血管異常といった多彩な病態の表現型ごとに設定された大基準と小基準および家族歴や遺伝的要素を加味したものとなっている．

近年，諸外国においてマルファン症候群と歯周病との関係が注目されている[5]．以前より国内ではマルファン症候群を有する顎変形症症例に対する外科処置の報告は散見されるものの，マルファン症候群の口腔内所見に関する報告は少なく，また骨格系に関する表現型は同じマルファン症候群であっても欧米人と日本人では相違点が少なくないことが知られている[6]．そこで，今回マルファン症候群の口腔内の状態を把握する目的で歯周病罹患状態を調査し，またマルファン症候群の表現型と歯周病所見との関係にも注目し検討したので，文献的考察を加えて報告する．

対象と方法

東京大学マルファン症候群専門外来を受診し，Ghent基準においてマルファン症候群と診断された症例で，本研究の主旨に同意が得られた患者20名(男性11名，女性9名，平均年齢35.7歳)を対象とした．

患者には事前に研究の目的を十分に説明し，同意を書面で確認後，口腔内診査を行った．本研究は，東京大学医学部研究倫理審査委員会で承認を得た．

1. 歯周組織の評価

各対象者について現在歯数をはじめ以下の項目について歯周組織検査を実施した．

1) Probing Depth (PD)

歯周病の現在の進行度を表すため歯周ポケットの深さを測定した．カラーコードポケット探針(PCP-11，Hu-Friedy社製)を用い，約20 g前後の力で1点法にて測定した．被験者の1歯あたりの平均値をmm単位で算出した．

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* The High Prevalence of Periodontitis in Patients with Marfan Syndrome(2011年6月6日受付)
[1] 東京大学医学部附属病循環器内科(〒113-8655 東京都文京区本郷7-3-1)　Mieko Aoki, Yasushi Imai, Daishi Fujita, Naomi Ogawa, Masayoshi Kato, Hiroshi Nishimura, Jun-ichi Suzuki, Yasunobu Hirata, Ryozo Nagai: Department of Cardiovascular Medicine, University of Tokyo Hospital

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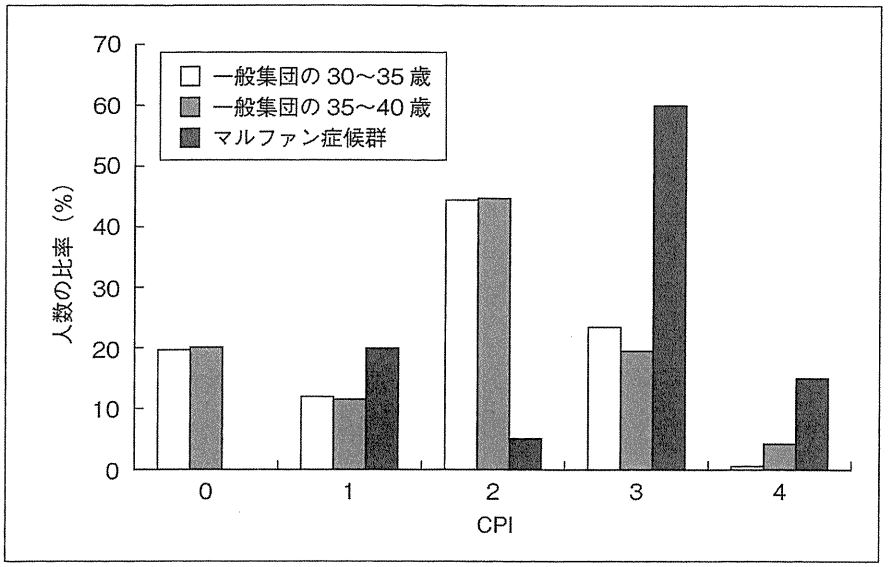

図1 本研究対象者と同年代男性および女性との間でのCPIの比較

2) Bleeding on Probing (BOP)

歯周ポケット内の現在の炎症を調べるためPD測定部位での出血の有無を測定し，被験者の全被験歯に対する検出率(%)を算出した．

3) 歯の動揺度

Millerら[7]の方法により0度～3度の4段階で測定し全被験歯に対する平均値を算出した．

4) Community Periodontal Index (CPI)

1982年にAinamoら[8]がWHOの提案として発表した地域歯周疾患指数CPIにて歯周組織の評価を行った．口腔内を6群に分割し，それぞれの分画の代表歯を被験歯として評価した．

2. マルファン症候群の表現型と歯周病所見との関連性

マルファン症候群の診断基準であるGhent基準の各臓器所見，すなわち骨格系，眼，心血管系，肺，皮膚，硬膜のどの表現型，あるいは表現型の合計数と歯周病罹患状態との間に相関があるか否かを検証した．

3. 統計解析

統計処理は，一元配置分散分析を用いた(SPSS11.0 J for Windows, SPSS Japan. 東京)．特に指示がなければp値が0.05未満のものを有意とし，全体として有意差を認めたものはpost hoc解析を追加した．

結 果

1. 歯周病所見

20名の現在歯数の平均は27歯であった．PDは2.815±0.624 mm，BOPは11.567±8.394%，動揺度はすべての症例において0であった．さらにCPI codeは，CPI code0の者が0名，code1もしくはcode2の者(歯肉炎)5名(25%)，code3の者(軽～中等度歯周炎)が12名(60%)，code4の者(中～重度歯周炎)が3名(15%)であった．すなわち4 mm以上の歯周ポケットを有する者(CPI=3または4の者)は15名(75%)と非常に高頻度であった(CPI 2.70±0.98)(図1)．

このことは平成17年歯科疾患実態調査による報告における30～35歳(CPI:1.73±1.05)，35～40歳(CPI:1.76±1.09)の年齢層(本研究の対象集団の平均年齢は35歳)と比較して統計的に明らかな有意差をもってCPIが高値を示している．一元配置分散分析にて3群を比較するとp値=0.001，post hoc解析(Scheffe法)にてわれわれの症例と30～35歳，35～40歳の一般集団と比較してp<0.001と有意にCPIの値が高値であることが示された．

2. 表現型

20名にみられた表現型は心血管系が20名(100%)と全症例に認められた．次いで皮膚が12名(60%)，眼が11名(55%)となった(表1)．

3. 表現型と歯周病所見との関係

表現型の数とPDの比較を行った結果，表現型3つではPDが2.808 mm，表現型4つではPDが2.819 mm，表現型5つではPDが2.822 mmと表現型が多くなるにつれてPDは深くなる傾向であったが，両者の間に有意差は認めなかった．さらに表現型の数とBOPの比較を行った結果，表現型3つではBOPが12.81%，表現型4つではBOPが10.98%，表現型5つではBOPが10.23%と表現型とBOPの間に有意な差はみられなかった．

考　察

マルファン症候群は5,000人～10,000人に1人の確率で発症するといわれている[9]．特徴的な表現型として，クモ状指，側弯症，後弯症，胸郭変形，バルサルバ洞を含め大動脈弁逆流，大動脈解離，水晶体亜脱臼，硬膜拡張などが挙げられる．本症例でもバルサルバ洞を含む上行大動脈の拡大は全症例においてみられた．また約半分に眼症状がみられた．

治療にあたってはβ遮断薬，アンジオテンシンⅡ受容体拮抗薬による血圧のコントロール，運動制限，妊娠出産時の厳格な管理，大動脈径の定期的な評価と人工血管置換術などが挙げられる．このように多臓器に表現型を呈する全身疾患であり，集学的な検査および治療体制が必要とされる．そのため当院では，診療科の枠を越えて循環器内科，心臓外科，小児科，整形外科，眼科，放射線科，臨床ゲノム情報部・診療部がチーム体制を作り，マルファン症候群専門外来を開設して対応している[10]．

歯科的な特徴として，下顎後退症，高口蓋，口蓋垂裂，口蓋正中部の偏位，舌の奇形，歯列不正，歯の先天欠如，形態異常や形成不全などが挙げられる．

近年，マルファン症候群に有意に歯周病罹患率が高いことが指摘されている[5]．しかしマルファン症候群の口腔内所見の報告は少なく，特に国内において歯周病罹患状態に関した報告は皆無に近いのが現状である．今回の結果，本症例ではPDが4 mm以上の部位を有する者(CPI＝3または4の者)は15例(75%)であった．これは平成17年歯科疾患実態調査[11]によると35～39歳で23.7%であり，全国調査に比較して非常に高いことが明らかになった．また，今回の結果ではCPIの最も多い値はCPIが3であったのに対し，平成17年歯科疾患実態調査によるとCPI2が最も多く，マルファン症候群は歯周炎が重度の傾向を示した．このように高頻度に認められる歯周病は，あわせて存在する心臓弁膜疾患(大動脈弁閉鎖不全，僧帽弁逸脱症など)において口腔内細菌を起因菌とする感染性心内膜炎の発症母地となり得るとともに，最近ではこのような口腔内の慢性炎症によって大動脈解離や拡大といった血管病変の進行に寄与する可能性も十分に考えられる．

マルファン症候群の原因として1991年に15q21.1に座位を有する*FBN1*遺伝子が発見された[12,13]．その後2004年には*TGFBR2*遺伝子[14]，さらには*TGFBR1*が新たにマルファン症候群の原因遺伝子として特定され，最近ではフィブリリン異常とTGF-βシグナルとの関連性がマルファン症候群の病態生理に重要であることが明らかになりつつある．*FBN1*遺伝子は全身の結合組織の構成要素となる主要蛋白のフィブリリンをコードする．フィブリリンは歯周組織の歯根膜にも存在する．歯周組織は歯の支持組織で，セメント質，歯根膜，歯槽骨，歯肉の一部によって構成されている．特に歯根膜は特殊化した線維性結合組織であり，フィブリリンを主成分とする微細線維が集まって構成されたオキシタラン線維から成る．オキシタラン線維は歯根膜以外にも血管外膜，神経上皮，神経周膜，腱などほとんどの結合組織に存在する．歯根膜でのオキシタラン線維は，歯根を歯軸方向に三次元的に囲み，しばしば血管やリンパ管の複合体に終わるか近接している．機能は脈管周囲や圧力のかかる部分に分布していることから，脈管の機械的支持と血流調整作用が考えられている．また，歯の萌出方向をガイドしているという報告もある[15]．よってフィブリリンの異常は，オキシタラン線維の正常な働きを阻害する．すなわち，*FBN1*遺伝子の異常は歯根膜の機能異常を来している可能性があり，マルファン症候群における歯周病の重症度と関係があるかもしれない．また，*TGFBR1*および*2*遺伝子は*TGF-βI*またはⅡ型受容体をコードしており，この異常は結合組織の脆弱性を引き起こすといわれているが，歯周組織との関連性は不明である．

表1　本症例における各表現型

	大基準	小基準	合計
骨格系症状	3例	2例	5例（25%）
眼症状	11例	0	11例（55%）
心血管系症状	19例	1例	20例(100%)
肺症状	—	4例	4例（20%）
皮膚症状	—	12例	12例（60%）
硬膜拡張	8例	—	8例（40%）

マルファン症候群の表現型である眼症状や心血管系異常と歯周病との関連に関する報告は検索する限りではみられず，本研究でも明確な示唆は得られなかったが，今後さらに症例を重ねることで他臓器の表現型との関連性について検証が可能と考える．

マルファン症候群は突然死の恐れのある予後不良な病気と認識されていたが，最近の治療成績の向上およびマルファン症候群の早期診断により予後は改善している．これは一方ではマルファン症候群の長期生存を意味し，今後ますますこれらの患者が歯科を受診する機会が増加することが予想される．よってマルファン症候群の口腔症状を理解し，歯周病のマネージメントを行うことは患者のQOLの維持の点からも急務であ

る．

結　語

本研究から，マルファン症候群の患者は中等度から重度の歯周病に罹患している確率が非常に高く，歯周組織の脆弱性が示唆された．

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Summary

The High Prevalence of Periodontitis in Patients with Marfan Syndrome

by

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Marfan syndrome is a connective tissue disorder with autosomal dominant inheritance.

The disease affects mainly the skeletal, cardiovascular, and ocular systems. Patients with this syndrome often demonstrate oral and maxillofacial manifestations including highly arched palate with crowding of teeth. In order to evaluate the clinical characteristics in Japanese Marfan syndrome patients, we evaluated the periodontal status of those patients who were diagnosed as Marfan syndrome according to the Ghent nosology (n=20). The results showed that the number of teeth present was 27. Probing pocket depth were 2.815±0.624 mm, bleeding on probing 11.567±8.394%, and percentages of CPI (community periodontal index) codes 3 or 4 75%. Our results demonstrate the significantly high prevalence of severe periodontitis in patients with Marfan syndrome. The connective tissue disorder in Marfan syndrome may also increase susceptibitity to inflammatory breakdown of periodontal tissue.

Key words　Marfan syndrome, periodontitis, CPI

非動脈硬化性遺伝性疾患
1. Marfan 症候群と関連疾患

藤田大司*　今井　靖*　平田恭信**

要　旨

Marfan 症候群は，大動脈病変，眼症状，骨格異常を主徴とする常染色体優性遺伝疾患である．原因として結合組織を構成する fibrillin 1 の遺伝子異常が同定されており，また TGFβ の活性化の関与も判明してきている．従来の身体的特徴をもととした診断基準から，原因遺伝子や分子メカニズムを考慮した新基準に移行しつつあり，類縁疾患の概念の確立や診断・治療方法の進歩が期待される．

はじめに

Marfan 症候群およびその類縁疾患は，近年の遺伝子・分子生物学的研究の進展により原因遺伝子および分子メカニズムの解明が進み，それらの成因に基づいて今後疾患概念が再構成される方向にあると思われる．本稿では，Marfan 症候群およびその類縁疾患における大動脈病変に関して概説する．

Marfan 症候群

1．病態生理

Marfan 症候群は，常染色体優性遺伝の結合組織形成不全性疾患であり，骨格異常（高身長，長く細い四肢・手指など），眼異常（水晶体脱臼，高度近視など），心血管系異常（大動脈瘤，大動脈解離，大動脈弁閉鎖不全症，僧帽弁逸脱症）などを主症状とする．心血管系に関しては，病理学的に大動脈の嚢胞状中膜壊死（cystic medial necrosis）や弾性線維の配列異常，弁の粘液変性が認められる．発症頻度は 5,000～10,000 人に 1 人程度である．約 3/4 に家族歴が認められ，約 1/4 の症例は家族歴の認められない孤発例である．結合組織の構成要素である microfibril の主要成分である fibrillin 1 の異常が原因である．fibrillin 1 遺伝子（FBN1）の変異が多数報告されており，遺伝子変異に基づく fibrillin の量および組み合わさり方の異常による結合織異常が種々の症状を引き起こすと考えられている．ただし，FBN1 の遺伝子型と表現型との間の相関は乏しい．また，FBN1 の異常に関連してサイトカインの 1 つである TGFβ の活性化が関与していることも判明しており，Marfan 症候群の症例および動物モデルにおいて血中 TGFβ 濃度が上昇していると報告さ

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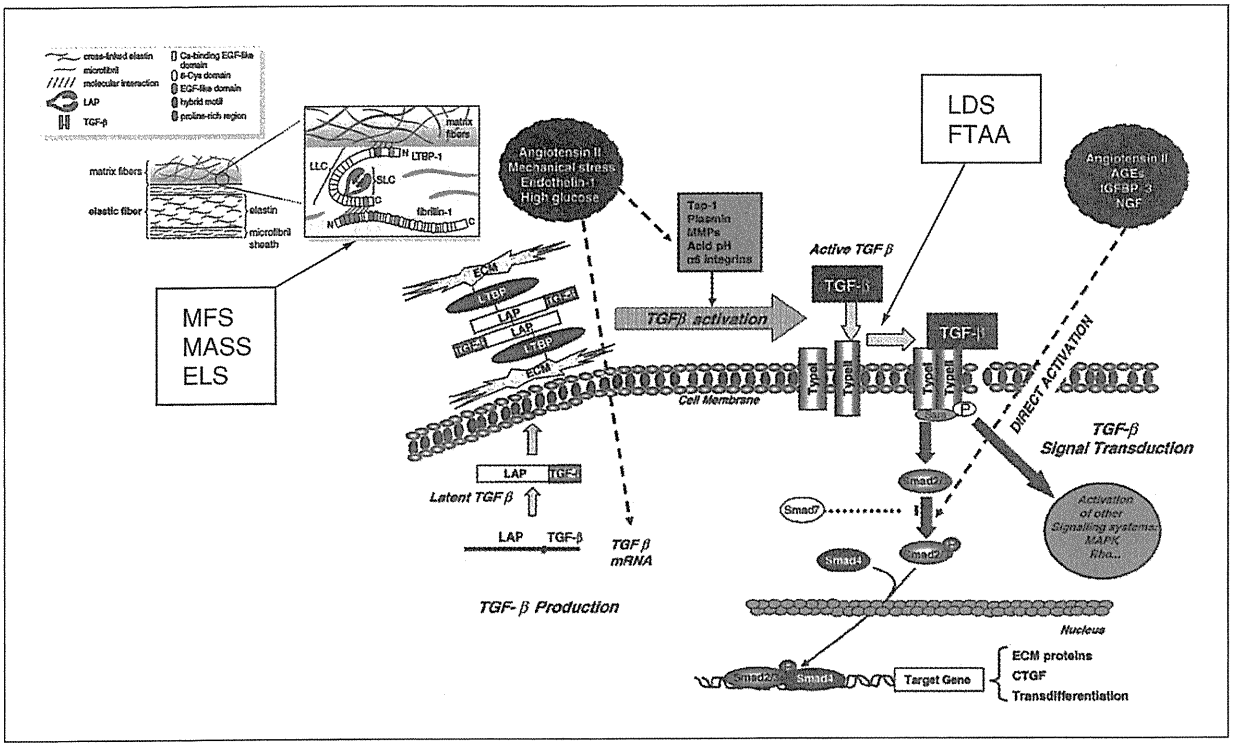
キーワード：大動脈瘤，大動脈解離，Marfan 症候群，fibrillin，TGFβ

図1　Fibrillin と TGFβの相関図
(Ruiz–Ortega M, et al: TGF–β signaling in vascular fibrosis. Cardiovasc Res 74: 196–206, 2007
Kaartinen V, et al: Fibrillin controls TGF–β activation. Nat Genet 33: 331–332, 2003 より改変引用)



TGFβ は潜在化タンパク質（LAP）と結合し，不活性型として細胞外マトリックス（ECM）の fibrillin に結合している．刺激に対して，同部位より切り離され，活性型となり，細胞内シグナルを引き起こす．Marfan 症候群をはじめ，MASS や ELS では fibrillin 遺伝子異常が，LDS や FTAA では TGFBR の遺伝子異常が報告されている．MASS：MASS 表現型，ELS：水晶体脱臼症候群，FTAA：家族性胸部大動脈瘤症候群，
LDS：Loeys–Dietz 症候群

れている．図1に，fibrillin と TGFβ の関連について示す．

2．診　断

Marfan 症候群においては，① 定期的な心血管系の検査，② 適切なタイミングでの内科的・外科的介入，③ 身体的負荷の大きい仕事やスポーツの制限，④ 女性の場合妊娠出産時の管理，⑤ 子どもへの遺伝の可能性に対するコンサルテーションなどが必要であり，診断は慎重かつ適切に行われなくてはならない．主要徴候をすべて満たす場合には診断は容易であるが，そのような症例は決して多くない．多臓器にわたる種々の項目を評価するために，診断基準に基づいて評価・診断を行う．1986 年のベルリン基準を経て，現在は 1996 年に改定されたゲント基準が一般的に用いられている（表1）．ゲント基準では，整形外科的特徴，眼異常，心血管系異常などの主症状に加え，硬膜拡張や皮膚病変，肺病変などを評価項目としている．家族歴を有する場合には大項目1つ＋他臓器の異常あり1つで，家族歴を有さない場合あるいは不明である場合には2臓器における大項目陽性＋他臓器の異常あり1つで診断に至る．心血管系異常は，心エコー検査を行い，上行大動脈の拡張，大動脈弁閉鎖不全症，僧帽弁逸脱症などの有無を確認する．Marfan 症候群においてはバルサルバ洞の拡張が特徴的である（図2）．整形外科的項目は診察所見およびX線所見より判断する．体格に関する基準で arm span/身長比 1.05 以上という基準が

表1 Marfan 症候群 ーゲントの診断基準ー

家族歴がない場合には 2 つ以上の臓器の大項目が陽性で，かつそのほかの 1 つ以上の臓器の異常あり

家族歴がある場合には 1 臓器の大項目 1 つと他臓器の異常あり

(1) 骨格系

大項目（このうち 4 つ以上を満たせば大項目ありとする）：鳩胸，手術を要する漏斗胸，上腕長/前腕長比の低下または両手を広げた長さと身長の比が 1.05 以上，手指徴候（片方の手で他方の手首を握ったときに母指と第 5 指が重なる）および母指徴候（図 4），20 度以上の脊椎側弯症または脊椎すべり症，肘関節の伸展制限（< 170 度），内踵の内側偏位による扁平足，寛骨臼突出

小項目：中等度の漏斗胸，関節過伸展，高口蓋，顔貌（長頭，頬骨低形成，眼球陥凹，下顎後退症，眼瞼裂外下方傾斜）

大項目 2 つ以上＋小項目 2 つ以上で骨格系異常ありとする．

(2) 眼

大項目：水晶体偏位

小項目：扁平角膜，眼球軸長増加，縮瞳低下を引き起こす虹彩あるいは毛様体筋の低形成

小項目 2 つ以上で眼異常ありとする．

(3) 心血管系

大項目：バルサルバ洞を含む上行大動脈の拡張（大動脈弁逆流の有無は問わない）または上行大動脈解離

小項目：僧帽弁逸脱（逆流の有無は問わない），40 歳以前で肺動脈弁狭窄，末梢肺動脈狭窄，またはほかの明確な原因がない主肺動脈の拡張，40 歳以前の僧帽弁輪石灰化または 50 歳以前の下行大動脈，腹部大動脈の拡張あるいは解離

大項目 1 つまたは小項目 1 つで心血管系異常ありとする

(4) 肺

小項目：自然気胸，または肺尖部ブラ

小項目 1 つで肺の異常ありとする

(5) 皮膚

小項目：皮膚線条（妊娠線，急な体重変化，反復するストレスは除外），繰り返すヘルニア

小項目 1 つで皮膚の異常ありとする

(6) 硬膜

大項目：CT または MRI によって確認された腰椎仙椎部の硬膜拡張

(7) 家族歴・遺伝歴

大項目：この診断基準を満たす親，子ども，あるいは兄弟を持っていること，Marfan 症候群を引き起こすことが知られている FBN1 遺伝子変異，または Marfan 症候群の遺伝歴が明らかな FBN1 遺伝子近辺のハプロタイプ

あるが，日本人にはこの基準は厳しすぎるという意見が多い．また側弯などのために低～平均身長のことも少なくないため，身長に惑わされてはいけない．眼異常では水晶体脱臼が最も多く，そのほかに高度近視や眼軸異常，網膜剥離などが認められるが，眼科医の診察が望ましい．肺病変として挙げられる気胸はX線撮影においても評価可能であるが，ブラの有無は最終的には CT において確認する．硬膜拡張に関しても，CT ないしは MRI による評価を要する（図 3）．

ゲント基準の特異度は高く，本基準で陽性とされた症例では，最も好成績の報告では実に 97% に FBN1 の遺伝子異常を検出するこ

図2 Marfan 症候群におけるバルサルバ洞拡大
（心エコー胸骨左縁長軸断面）

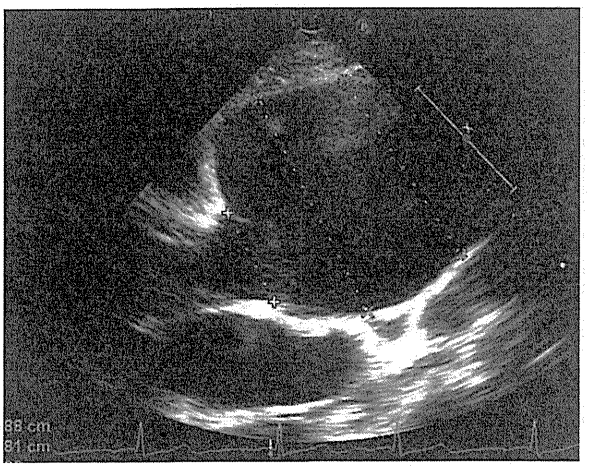
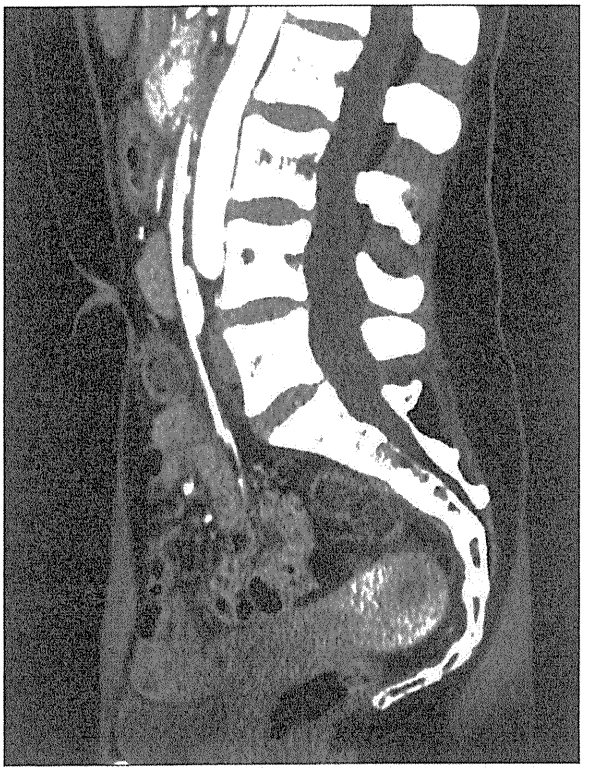


図3 Marfan 症候群における硬膜拡張



とができたと報告されている．一方，表現型が顕在化していない小児例では診断が困難であることや，低頻度の評価項目や病態生理的に本質的でない評価項目も含まれていること，複数科での診察・検査を要すること，また本基準を満たさないが実質 Marfan 症候群と言わざるをえない症例が一定数認められることなどが問題として指摘されてきた．

上述の問題に加え，原因遺伝子および鑑別疾患に関する研究の結果，2010 年に新基準（revised Ghent nosology）が提唱された（表2）．新基準では，眼症状と大動脈拡張がより重要視されるようになった．その他の整形外科的特徴，硬膜拡張，皮膚病変，肺病変は全身性徴候として一括され，点数化される．この基準においては，大動脈拡張は体表面積で標準化したZスコアで評価されるため，成長期の小児例においても評価がしやすい．FBN1 の遺伝子異常に関しても，大動脈拡張に関与する変異が重視され，それ以外の遺伝子異常は他の類縁疾患として分類されている．また，Marfan 症候群の診断を確定する前に，後述する他の類縁疾患（表3）を鑑別するよう喚起している．もちろん実臨床の場面で全例に遺伝子検査を行うことは現実的ではないが，診断に迷う場合の一助として役割が明文化されるようになった．ただし新基準であっても若年者では表現型がそろわないこともあるため，定期的な経過観察が必要なことは言うまでもない．

3．治　療

Marfan 症候群に対する根本的治療法はなく，各身体的異常に個別に対応が必要であるが，何よりも生命予後を規定する心血管系表現型に注意を注ぐべきである．Marfan 症候群における主な予後規定因子は大動脈瘤/大動脈解離であり，適切な加療により良好な予後が期待できる．

近視は眼鏡などで矯正されるが，矯正困難な場合もある．水晶体脱臼に対しては手術が選択される．骨格異常に対しては装具などによる矯正や手術が選択される．大動脈拡張は進行性であり，生命予後に直結するため定期的な経過観察が必要である．仮に大動脈拡張が顕著でなくても，Marfan 症候群と診断された場合には年1回の心エコー検査が推奨さ