あった。
解離を起こした時期は最も早い時期が妊娠12週であったが、大半は妊娠末期から分娩産褥期であり、妊娠の進行とともに解離のリスクが高まることが示唆された。中でも、産褥期（当日から5日目）に4例起こしており、分娩終了後は妊娠中と同様か、むしろリスクとしては大きい可能性がある。
解離部位はStanford A型が2例、B型が9例であった。B型が多いことは、妊娠時の特徴であるというよりは、Valsalva洞の拡張が見られる場合妊娠することのリスクが大きいとされ、妊娠中絶を勧められることも関係していると考えられる。
一方、大動脈解離を起こさなかった例では、僧房弁・大動脈弁異常の増悪などはみられていない。したがって、大動脈解離を起こした例と起こさなかった例の差を見出すことが、より良い管理を目指すことにつながると考えたが、患者背景、妊娠経過、産科異常、既往の心血管イベント、大動脈径、大動脈径の増大など、あらゆる要素を比較したときに、有意差は見出せなかった。

E．結論

「適切な管理」を「大動脈解離を起こさないこと」とするならば、今回の調査では、明らかなリスク因子を見出すことはできなかった。それは、取りも直さず、マルファン症候群患者の妊娠は余さず大動脈解離を念頭に置くべきである、ということである。突然死の家族歴、大動脈の拡張や妊娠中の増大、β遮断薬の服用、などこれまでに報告されてきたさまざまなリスク因子においても、大動脈解離を起こすリスクが下がるわけではないということである。せめて、解離が起きるときには病院内にいるという状況を提供することくらいでしか、リスクを下げることができない。しかしながら、これにはおそらく過剰な医療介入が行われていることが予想されるし、個別化された適切な管理方針を目指すためには、今後、より多くの症例の蓄積と詳細な情報の集約が必要であり、そのためには全国レベルで症例登録を行うなど、より積極的な調査研究の展開が必要である。

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なし

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G．知的所有権の取得状況

1．特許取得　該当なし

2．実用新案登録　該当なし

3．その他　該当なし

厚生労働省科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

マルファン症候群患者における歯周病罹患率と菌種別抗体値測定

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研究要旨

近年、種々の動脈硬化症の発生に歯周病菌の関与が示されている。大動脈瘤を易発症するマルファン症候群において歯周病の有無と菌種を同定した。その結果、本症患者では年齢の割に歯周病の存在が多く、歯周病菌と病態との関連性が注目される。

A.研究目的

マルファン症候群は、骨格変形や心血管病を高率に合併する全身性結合織疾患である。本症の生命予後を規定するのは大動脈瘤あるいは大動脈解離である。この疾患には歯周病の合併率が高い事が知られており、疾患責任遺伝子の一つである fibrillin-1 の calcium binding EGF-like ドメインの遺伝子変異などが影響していると考えられている。他の動脈硬化性心血管病変では、この歯周炎病巣から侵入した歯周病原細菌が心血管疾患に直接または間接的に影響していると考えられているが、これまでに、マルファン症候群の進展における歯周病の関与を、菌の種別毎に明らかにした報告はない。マルファン症候群の病態においてそれぞれの歯周病原細菌がどのように関与しているかを明らかにする事が本臨床試験の目的である。

B.研究方法

東京大学医学部附属病院マルファン症候群専門外来に受診した、Ghent クライテリアを満たしたマルファン症候群患者 45 名（平均年齢 35.0 歳）、および Ghent クライテリアを満たさなかった非マルファン症候群患者 35 名（平均年齢 32.5 歳）が対象である。通常の循環器診察、心エコー検査に加えて、歯周病スクリーニング検査（残存歯数; pocket depth, PD; bleeding on probing,

POD, community periodontitis index, CPI）を実施した上で、主要５種類の歯周病原細菌(*Porphyromonas gingivalis*, Pg; *Tannerella forsythia*, Tf; *Treponema denticola*, Td; *Aggregatibacter actinomycetemcomitans*, Aa; *Prevotella intermedia*, Pi)の血中抗体価を ELISA にて測定した。

C.研究結果

心血管疾患を合併している率は、マルファン症候群患者で 95.6%に対し、非マルファン症候群患者では 37.1%であった。心エコーでは、Valsalva 洞径がマルファン症候群患者で 39.9+0.8mm と拡大していたのに対し、非マルファン症候群患者では 36.5+1.3mm ($p<0.05$)であった。歯垢内 Aa 菌陽性率はマルファン症候群患者で 8.9%であったのに対し、非マルファン症候群患者では 2.9%であった。その他の菌においては両群で有意差を認めなかった。

D.考察

近年、種々の動脈硬化症の発生に歯周病菌の関与が示されている。大動脈瘤でも同様の報告が既にある。しかし具体的な病原菌ならびにその役割までは明らかにされていない。同様に大動脈瘤を形成しやすいマルファン症候群に関しては報告が限られている。本症は顎が小さいことが多く、そのための歯列異常により歯周病が年齢の割に多いことが知られているが、最近では歯槽骨そのものにも易発症性と関連するとの報告もある。

以上のことより本研究ではフィブリリン 1 の遺伝子異常の確定された患者における歯周病の有無とその菌の同定を試みた。これまでの日本人統計と比較して、マルファン症候群患者においては、年齢の割に歯周病の罹患率は高率であり、歯周病菌陽性率も高率であることが推定される。また、Aa 菌感染の有無は、歯周病の状態のみならず、全身性変化にも影響している可能性がある。

E.結論

マルファン症候群における歯周病の罹患は、全身状態に影響している可能性が示唆される。

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H. 知的所有権の出願・取得状況

該当無し

I. 班友

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研究成果の刊行に関する一覧表

雑誌

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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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This work was supported by Health Labor Science's Research Grants from the Japanese Ministry of Health, Labor, and Welfare (Grant 10103493 to Dr. Hirata), the Human Resources Development Program of the Japanese Ministry of Education, Culture, Sports and Technology and JSPS through its FIRST Program (Drs. Yamazaki and Nagai).

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doi:10.1016/j.amjcard.2011.07.053

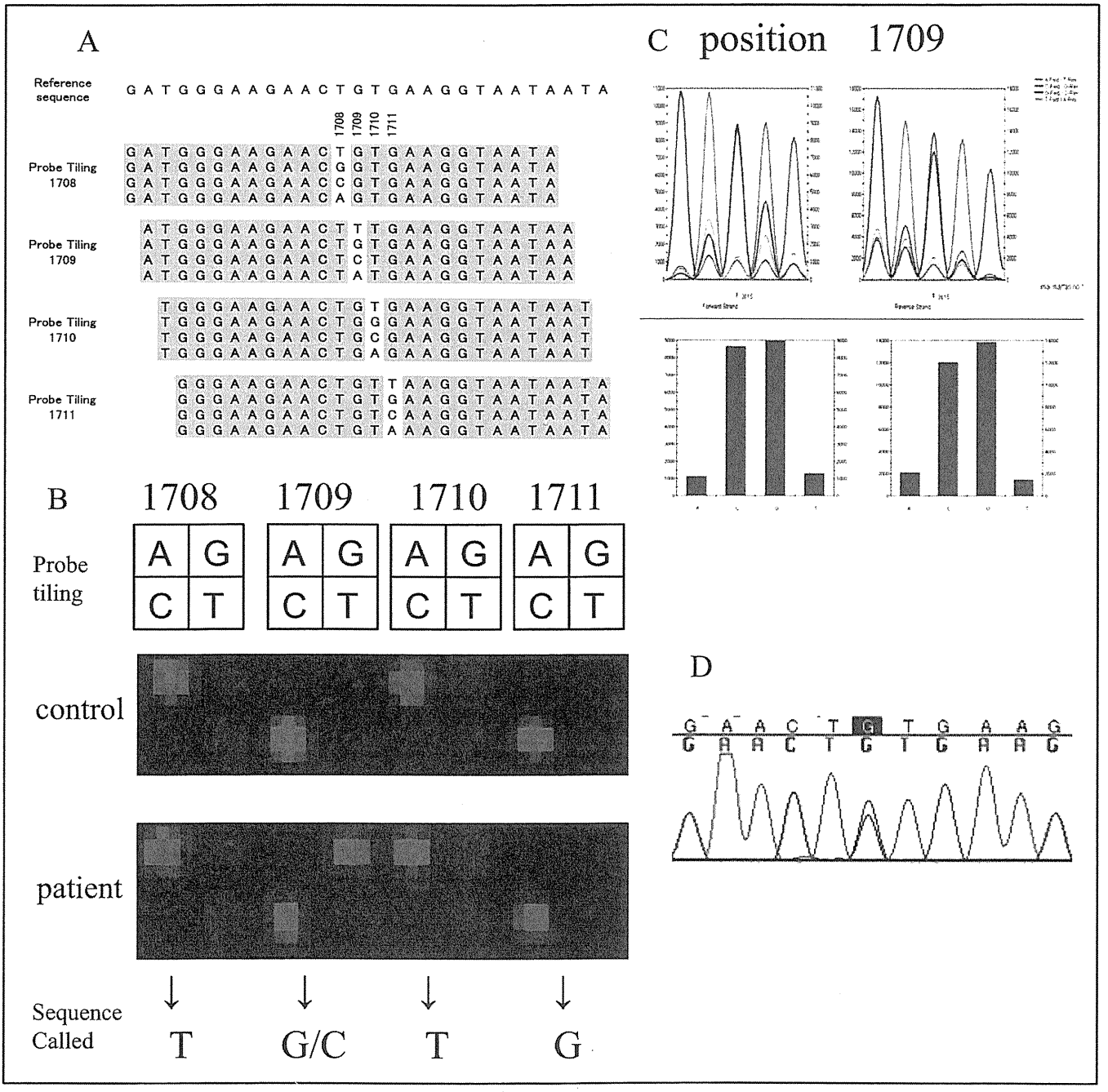

Figure 1. Representative example of mutational analysis using the present microarray-based resequencing system. *(A)* The microarray identifies individual nucleotides by comparative, high-fidelity hybridization using oligonucleotide probes that are synthesized in situ by photolithography and solid-phase 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

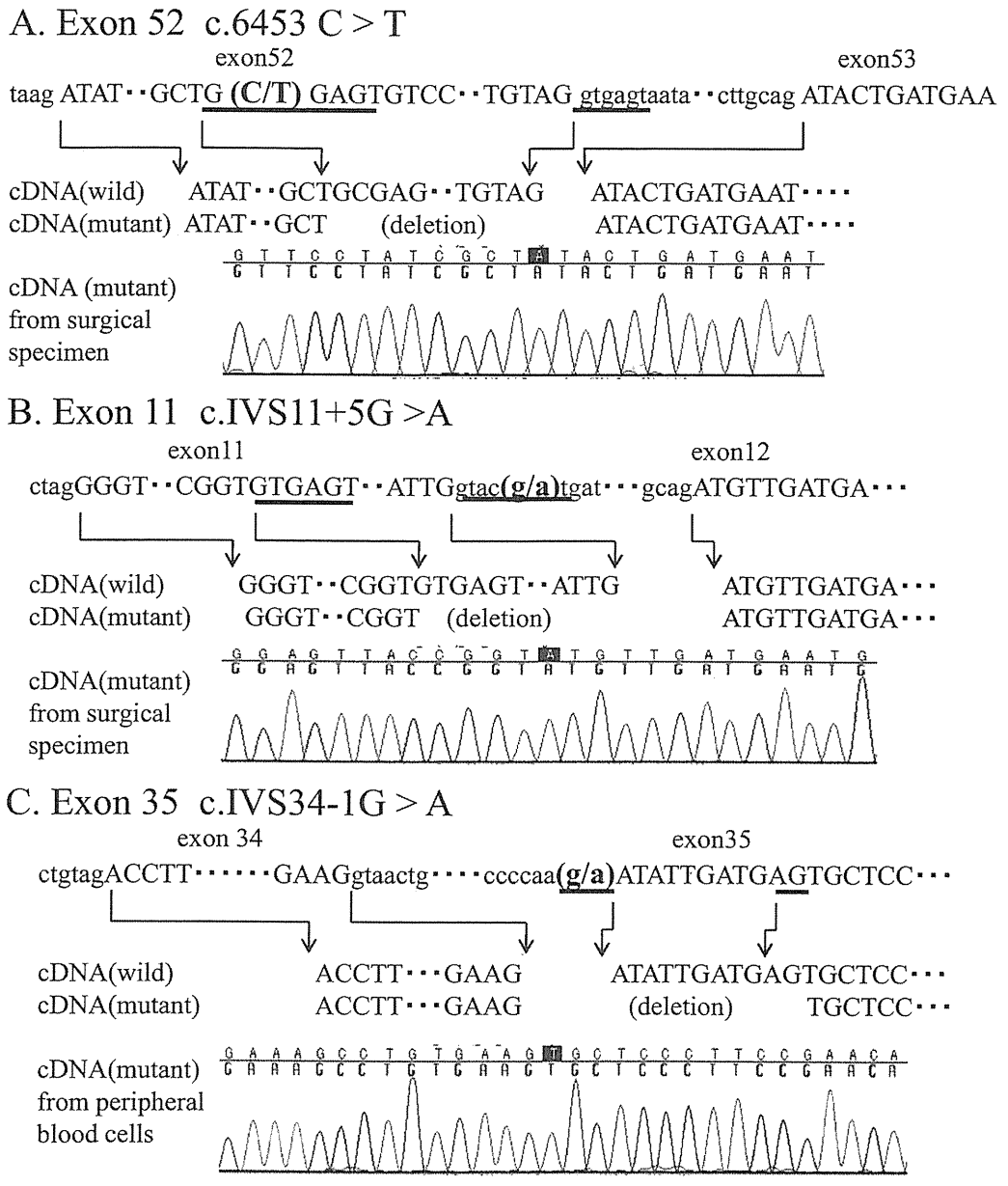

Figure 2. Representative splicing mutations in our study. *(A)* C-to-T substitution at nucleotide position 6453 produced a 6-nucleic acid sequence (c.6452-6457) *(underlined)* identical to that of IVS52+1 to +6 (gtgagt) *(underlined)*, and this was recognized as the splicing donor site. The following 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.

Acknowledgments: We would like to express our sincere gratitude to the patients and their families for agreeing to participate in this work and to all the doctors in the MS clinic for their valuable advice. We also thank Wu Zhenghong, Eri Kasukawa, Ryohei Kataoka, Keiko Hori, Yumiko Fujimaki, and Takako Kawamura for their excellent technical assistance.

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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．

* The High Prevalence of Periodontitis in Patients with Marfan Syndrome(2011年6月6日受付)
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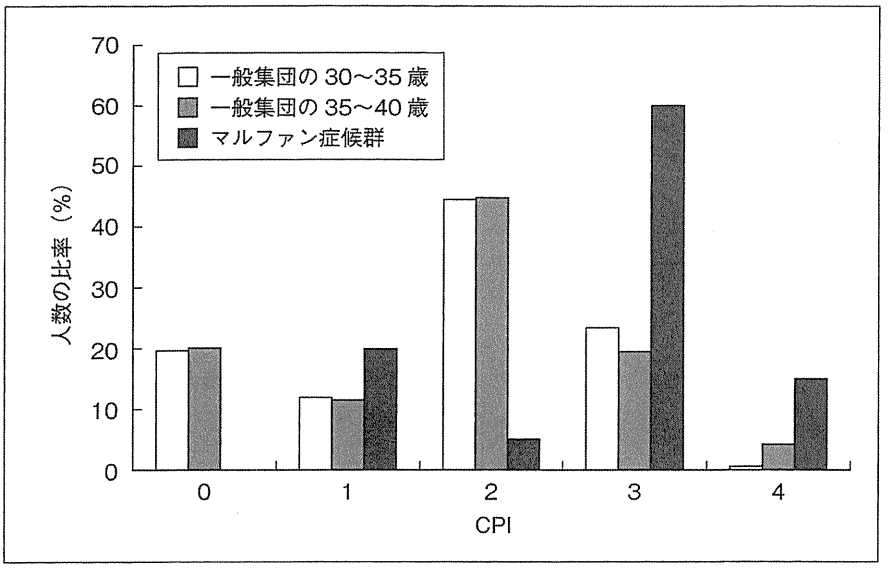

図1 本研究対象者と同年代男性および女性との間でのCPIの比較

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