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ORIGINAL ARTICLE

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Three novel mutations of the fibrillin-1 gene and ten single nucleotide polymorphisms of the fibrillin-3 gene in Marfan syndrome patients

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Abstract Marfan syndrome (MFS) is an autosomal dominant disorder of the extracellular matrix. Allelic variations in the gene for fibrillin-1 (*FBN1*) have been shown to cause MFS. To date, over 550 mutations have been identified in patients with MFS and related connective tissue diseases. However, about a half of MFS cases do not possess mutations in the *FBN1* gene. These findings raise the possibility that variants located in other genes cause or modify MFS. To explore this possibility, firstly we analyzed *FBN1* allelic variants in 12 Japanese patients with MFS, and secondly we analyzed fibrillin-3 gene (*FBN3*) in patients without *FBN1* mutations using conformation sensitive gel electrophoresis (CSGE) and direct sequencing analysis. We identified three novel *FBN1* mutations and ten *FBN3* single nucleotide polymorphisms (SNPs). In this report, we could not detect a responsible mutation of the *FBN3* gene for MFS. Although the number of the cases in this report is small, at least these results suggest that disease-causing mutations in exon regions of the *FBN3* gene are very rare in MFS.

Keywords Marfan syndrome · Fibrillin-3 · *FBN3* · Fibrillin-1 · *FBN1* · Connective tissue disorder

Nucleotide sequence data reported are available in the DDBJ/EMBL/GenBank databases under the accession numbers: AB177797, AB177798, AB177799, AB177800, AB177801, AB177802, AB177803

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Introduction

Marfan syndrome (MFS; MIM #154700) is an autosomal dominant disorder of connective tissue. The disease has an incidence of 1/5,000 with over 25% of sporadic cases. In 1991, the fibrillin-1 gene (*FBN1*; MIM 134797) was identified as a major disease-causing gene of MFS (Lee et al. 1991). *FBN1* on chromosome 15q21.1 codes for fibrillin-1, a main component of extracellular microfibrils. Since the identification of *FBN1*, over 550 mutations have been identified (Collod-Beroud et al. 2003). Although the mutation detection rate varies from 23.5 to 80%, the overall mutation detection rate is approximately 50% even in patients with classic MFS. *FBN1* mutations have been also found in individuals who do not satisfy the diagnostic criteria for MFS such as autosomal dominant ectopia lentis (EL; MIM 129600). Therefore, the wide spectrum of variability of MFS is not wholly explained solely by *FBN1* mutations. It is possible that another unknown candidate gene or genes causing or modifying the disease might exist. For example, the fibrillin-2 gene (*FBN2*; MIM 121050) on human chromosome 5q23–q31 shares a high degree of homology with *FBN1* and has been known to cause congenital contractural arachnodactyly (CCA; MIM 121050) (Lee et al. 1991). The fibrillin-3 gene (*FBN3*) on human chromosome 19p13 also has high homology to other fibrillin family members (Nagase et al. 2001). The *FBN3* gene is fragmented into 63 exons, transcribed in a 9 kb mRNA that encodes a 2,809 amino acid protein and has overall homology of greater than 60% with either *FBN1* or *FBN2*, and contains multiple EGF-like domains. Expression of *FBN3* is highest in brain tissue.

The spectrum of overlapping disorders presenting with *FBN1* mutations like EL define the molecular group of type 1 fibrillinopathies (Collod-Beroud and Boileau 2002). Weill-Marchesani syndrome (WMS; MIM 277600) is also a connective tissue disorder characterized by short stature, brachydactyly, joint stiffness, and eye anomalies including ectopia lentis.

Fibrillin immunofluorescence studies of skin biopsies from the patients with WMS show a decrease in immunostainable fibers compared to unaffected controls. Linkage analysis has mapped the autosomal dominant (AD) WMS locus to chromosome 15q21.1 (Wirtz et al. 1996). The *FBN1* gene was sequenced in an AD WMS family and in flame deletion of 24 nucleotides was found (Faivre et al. 2003a). These studies suggest that WMS is an allelic condition with MFS and one of a type 1 fibrillinopathies. Interestingly, the autosomal recessive (AR) WMS locus has recently been mapped to chromosome 19p13.3–p13.2, where the *FBN3* gene is located (Faivre et al. 2003b), suggesting that *FBN3* is a candidate gene for MFS as well as AR WMS. We analyzed allelic variants of the *FBN3* gene as well as *FBN1* gene using conformational sensitive gel electrophoresis (CSGE) and DNA sequencing in Japanese patients with MFS.

Materials and methods

Genomic DNA extraction and polymerase chain reaction (PCR) amplification

Genomic DNA was collected from 12 patients of ten families containing two couples of parent–child that fulfilled the Ghent nosology revised diagnostic criteria (De Paepe et al. 1996) for the diagnosis of MFS and 50 Japanese volunteers who served as normal controls. Written informed consent for genomic examination was obtained from all participants and the Ethic Committee of Hirosaki University School of Medicine approved this study. Genomic DNA was extracted from peripheral blood leukocytes by the QIA amp DNA blood mini kit (Qiagen, Inc.). PCR primers used to amplify the individual exons of *FBN1* were derived from previously reported information (Korkko et al. 2002). PCR primers of *FBN3* on Table 1 were originally designed. PCR amplification was performed using 40 ng of genomic DNA, 0.25 mmol/l of forward and reverse primers, 2 mmol/l $MgCl_2$, 0.2 mmol/l dNTPs, and 1 U of Taq DNA polymerase. The PCR conditions consisted of initial denaturation at 95°C for 10 min, followed by 35 cycles at 95°C for 25 s, 58–68°C for 25 s, and 72°C for 35 s, with a final extension at 72°C for 10 min.

Heteroduplex analysis and sequencing

Heteroduplex analysis was performed using CSGE. CSGE was performed essentially using the same conditions as previously described (Ganguly et al. 1993; Korkko et al. 1998). Samples with abnormal CSGE band patterns were directly sequenced in both directions by means of the ABI PRISM Bigdye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). Forward and reverse primers were the same as those used for amplification.

Population studies

One hundred chromosomes from 50 unrelated controls were tested for the identified mutations by using restriction enzyme digestion or direct sequencing to determine recurrent mutations or polymorphisms, and to confirm their association with the pathologic condition under study.

Results

First, we screened *FBN1* allelic variants in MFS patients and identified three novel mutations in four MFS patients containing a parent–child couple. These three mutations consisted of the two nonsense variants and one missense variant shown in Table 2. These variants were completely absent from the genomes of 50 normal control samples. The missense mutation on exon 63 (c. 8121 G > C, p. Cys 2663 Ser) was detected in a parent–child couple. Although we tried to analyze the mutations of other family members, we could not obtain informed consent from them.

Next, we identified ten *FBN3* allelic variants in MFS patients without *FBN1* mutation using CSGE and sequence analysis. All of ten variants were analyzed by restriction enzyme digestion or direct sequencing and compared to 50 normal control samples. All variants of the *FBN3* gene were also detected in normal controls in either homozygous or heterozygous status (Table 2).

Discussion

Although mutations of the *FBN1* gene cause MFS, the disease-causing genes remain unknown in about half of MFS patients. In this study, mutations of the *FBN1* gene were detected in only four of 12 patients with MFS, even using very sensitive CSGE and sequence analysis. In order to investigate the possibility that a portion of MFS might be caused by mutation of another fibrillin gene, we screened mutations of the *FBN3* gene and identified multiple allelic variants. However, a responsible gene mutation for MFS was not detected in these patients. Although the number of the cases included in the present study was small, these results suggest that disease-causing mutations in exon regions of the *FBN3* gene are either very rare in MFS or that the *FBN3* gene is not a responsible gene for MFS. Functional variant analysis of the *FBN3* gene or more widespread screening of *FBN3* gene variants might determine a relationship between *FBN3* and several connective tissue disorders.

Table 1 Primer sequences

Exon	Sense primer	Antisense primer	Product size (bp)
1	5′-GCCTCCTCTGACTGTTCACC	5′-ACAGCCCAATCCACACCGAA	405
2	5′-GCAGCCCAGGCATCTTGCAG	5′-CCTGGCTAAGTCCCCGTGTAAAC	329
3	5′-AGACCCCTTCGGTGTGGATTGG	5′-ACAGGAAGGCAGGACGCATAGTAA	345
4	5′-ATTACTATGCGTCCTGCCTTCCT	5′-AAGAGACTGTGGCAATAAACGTCC	271
5	5′-CCTCAGTCTTATTCAACCTGGGCC	5′-TCCATCGAGTCCCCTGTCTACCT	262
6	5′-CGCTGAGAAGGTGTTAGAGACGCA	5′-GGAGGGAAGAACCAAGGCAGCTAA	346
7	5′-CTTAGCTGCCTTGGTTCTTCCCTC	5′-TCCAGTCACCCACCTTCACATGC	203
8	5′-AGGTGTGAGCTACTGCATCTGGAC	5′-GCAGGCTGCAGCTCTTACTCAC	369
9	5′-GCCTCAACCCTGCTTCCTTG	5′-ATCCTTCAAGTCTTGGAATGTTCCT	250
10	5′-TCCATGAGAAAGAGGGCAGGATC	5′-GGTGCATTCGTCTACATCTGAGG	360
11	5′-TCTGCCGACACTTCACCAACCTGT	5′-CCCCAACAACATCACCCCTACTCT	353
12	5′-ATCCAAGAGTAGGGGTGATG	5′-GGCACAGTGAAGATTTGAGGT	300
13	5′-GGACGGACTAATATACAAGCTCAC	5′-TCTTCTCCTTCTCTCATGCTCGT	406
14	5′-GAGGACAGAGACACAGACAACAGG	5′-AGGCATTCAGACCAAGGAGGCGA	481
15	5′-CGGGGTTGGAGTTGTGTCTTAGTC	5′-AACTCCTACAGCCTCCCACCACA	266
16	5′-CTCGGACACACCCTCCTTCT	5′-GGGCACACTCATCCACGTCT	282
17	5′-ACCGCTGTGTCTGCAACCTG	5′-CTTTGAGCGTGTGCATGCCT	348
18	5′-AGTGCCCTCTGCATGCTTGT	5′-ACCTGCACCCTGACCCATAG	300
19	5′-GCTGGACCCCTCTGGTACCT	5′-CCCCATGGAGGGCTTTTCCCA	328
20	5′-TCTCTTCACTCTTGTTCTCCCAGT	5′-TCACACTCGTTCACATCTGGGTA	375
21	5′-CTCCTTACCCAGATGTGAACGA	5′-GGGAACAAAGGAAGAACGTG	190
22	5′-CTGGCCCTCGACCTGACTAC	5′-TTCTTAGTGCCTCGCCTTACCCA	470
23	5′-TGGTTTGATGCCCCTGTACTTCA	5′-TGTCTCTACGTCTTATTTGAGGCC	225
24	5′-CCTCTAGCCCACACCTCCATCAAA	5′-ATCCACGTCCACACAGCAACAGC	305
25	5′-CCAGCATGAGCCTCATCACC	5′-CACATTCACCAGACATCCACTCAC	278
26	5′-GGGTGTGTATATGGGTGTCTGTGC	5′-TGGGAGCAGAGTGGAGTTGAGGT	430
27	5′-TGCATCCTGCAGACATCAACGAATG	5′-GGCTGGCTTCACTATGCTGT	242
28	5′-AGTGACCATCGCTATCCTCACCT	5′-AATTCTGACACTGCCTCCTCCAC	297
29	5′-CTCAGACCTCATGCTCTCTTC	5′-GCACTGTATAGGACTCTGTTCTTG	298
30	5′-AGAGGAAGAGTCCCTGCCCTTTC	5′-CCCCACCTACCTCTACTGCT	253
31	5′-AGGGTTGGAGGGTTGATGGGTCT	5′-AGAAGGAGTCAGGAGGTCGAGTG	313
32	5′-GAATCTTGGGGCCCCTCAGACA	5′-TGCGCACACTCGTCCACATC	255
33	5′-AGAGGACAGAGTGGGTAGGTC	5′-CATGTCCCCTGCCCCAACTC	252
34	5′-AGAACAGAGCAGGAGAGGGAATG	5′-CAGGAGCAGGTAGATTTGAACACG	428
35	5′-GCTTTCCCTCTGACACCTACTAAC	5′-TCTTGGTTTTCCTGTCGCCTAAGC	300
36	5′-TCCTGCTGTTGCTCCCCTGTTTC	5′-GCATAGTGTGGTCCTGCTGCTCA	298
37	5′-GTCCTCTGCATGGGTTTAGCTCC	5′-CCTTCCCTCCTCTACCCTTGTGA	349
38	5′-GAAGTGGCCTTGTGCTGGGTCTC	5′-TGACTGCCAGTTCCCTAGGTCATC	252
39	5′-AGTTCCTATCTCTTTGGCTCCCT	5′-ACTTCAACTCTCTGGCTCTCATCA	355
40	5′-TAAGTGGAAGGAGGGAAAGGGTG	5′-GAAAAGAGCTGTTGAGGAGGGGA	291
41	5′-GGCCACAACAGCCTCAACCA	5′-GGGAGAGGTGGCACCTTCAC	247
42	5′-GCCTCCATCTCATATAACCACCCT	5′-GACAGAGCCATACCCCATCTAAGT	349
43	5′-TAGGATGTGATAGAGGTGAGAGCT	5′-TGAACTAGGATGGACAGAAACACC	393
44	5′-GTAAATGGTTGATCTGCCTCCCTG	5′-GGACTTTCTGGGCATCTTTCTGTG	431
45	5′-GTGGACAGAAGTACAAGGAAGGTT	5′-GTAAATGGATCTATGCTCACCCAG	277
46	5′-ACAGTGATTGCAGGTGTTGGTCC	5′-TCCACCTTCATCACAACCCTAGC	305
47	5′-CCTGACCATGACCTTTACTCTGGA	5′-ACACACCTAGCTCAGAATGGACC	432
48	5′-ATCAGCACAACCCATTCACCAGGA	5′-TGTGCAGGGAAGGATGAGAGAAG	340
49	5′-TCTCTCATCCTTCCCTGCACACA	5′-CACACTCATTCACGTCTGCGGAT	381
50	5′-AGCCCCTCACCCACATGCTAAGAG	5′-GGGAGAGAGGCAGTGGTTAAGGAC	475
51	5′-GACTAGAGGTGCATTCGATCTGGC	5′-GTATTGTGGCAGCGGAAGGCACA	441
52	5′-TTACTCCCACCCAGACACAGACG	5′-CCAGGTTCAAGCGATTCTCCTGTC	496
53	5′-GAATCCAGGTCCCCACAGCCATA	5′-ACTGCCTGTCCCTCCCATTCATTG	383
54	5′-CCCCTCCCATCTCAGATGAC	5′-CAGTGGGTGCCACATGGTAG	198
55	5′-AGTGACCGTTGTCTTTCCCA	5′-AGACACATGACCCTGAGCAAAC	310
56	5′-AGTGGCCGGTGTGGAAGGGA	5′-GGCTCACCCAGGCAGGTAGT	319
57	5′-CTGCTTACCCTCAATGGCTGCTTT	5′-AGAGGGGTGACAAGAGAAAGACCT	492
58	5′-TCCGAGGTCTTTCTCTTGTC	5′-AAATAACTAATGCTGGGGTCATGG	289
59	5′-AGGCACAGAGAGGGTAGGTAAGT	5′-AGAGAGAGGGAGAGAGGGTTTAC	467
60	5′-ACCCTGCCTGATCTCTCTCCTCT	5′-CATCATCTTGCAGGGTGGCGTCT	401
61	5′-TTGGCATTGGACTTGGGCTCTGAA	5′-GCGGGGCATGGAGTCTGCTT	389
62	5′-CCTCTTTCCCACACTCACCCATT	5′-AGGGACACACATGCACACCCTTAT	386
63A	5′-AACTCTTGTCTCCCTCCCTCTCTC	5′-CCAGGTGTGAGAGGTTCAGG	397
63B	5′-TGAACCTGGCCACCCTTGAC	5′-GTCCTTCCCAGTTCCAGAATCC	404
63C	5′-CTACCGGCTGGAGGTGGTGA	5′-GCCTCTTCCTGACCTTGGCT	384
63D	5′-GGAAGAGTGAAATGCTACAC	5′-ACTTTATTATCGTCTCCACT	396
63E	5′-GCCCAGACTCCACAGTGACTTG	5′-AATTAGCTGGGTGTGGTGGCGTG	430

Table 2 *FBN1* and *FBN3* allelic variants identified in 12 MFS patients and 50 normal controls

Exon	Nucleotide change	Amino acid change	Patient frequency genotype (allele)	Control frequency genotype (allele)
FBN1 allelic variants				
6	c.719 C > T	p.Gln 136 X	1/12 (1/24)	0/50 (0/100)
33	c.4229 T > C	p.Gln 1366 X	1/12 (1/24)	0/50 (0/100)
63	c.8121 G > C	p.Cys 2663 Ser	2/12 (2/24)	0/50 (0/100)
FBN3 allelic variants				
11	c.1833 G > A	p.Arg 473 Gln	3/12 (3/24)	7/50 (14/100)
22	c.3219 G > T	p.Arg 935 Leu	1/12 (1/24)	16/50 (18/100)
22	c.3227 G > T	p.Val 938 Phe	1/12 (1/24)	2/50 (2/100)
25	c.3662 C > T	p.Arg 1083 Trp	3/12 (3/24)	15/50 (19/100)
30	c.4318 A > G	p.Pro 1301 Pro	3/12 (3/24)	45/50 (71/100)
38	c.5255 G > A	p.Gly 1614 Ser	2/12 (2/24)	1/50 (1/100)
44	c.6022 C > G	p.Asn 1869 Lys	2/12 (2/24)	15/50 (19/100)
45	c.6125 C > T	p.Leu 1911 Phe	2/12 (2/24)	18/50 (21/100)
48	c.6592 G > A	p.Glu 2059 Glu	1/12 (1/24)	8/50 (9/100)
61	c.8245 T > G	p.Asp 2610 Glu	2/12 (2/24)	5/50 (5/100)

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