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Impact of pathogenic *FBN1* variant types on the development of severe scoliosis in patients with Marfan syndrome

Yuki Taniguchi ,[1,2] Norifumi Takeda,[2,3] Ryo Inuzuka,[2,4] Yoshitaka Matsubayashi,[1] So Kato,[1] Toru Doi,[1] Hiroki Yagi,[2,3] Haruo Yamauchi,[2,5] Masahiko Ando,[2,5] Yasushi Oshima,[1] Sakae Tanaka[1]

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[1]Department of Orthopaedic Surgery, The University of Tokyo Hospital, Tokyo, Japan
[2]Marfan Syndrome Center, The University of Tokyo Hospital, Tokyo, Japan
[3]Department of Cardiovascular Medicine, The University of Tokyo Hospital, Tokyo, Japan
[4]Department of Pediatrics, The University of Tokyo Hospital, Tokyo, Japan
[5]Department of Cardiac Surgery, The University of Tokyo Hospital, Tokyo, Japan

Correspondence to
Dr Yuki Taniguchi, Department of Orthopaedic Surgery, The University of Tokyo Hospital, Tokyo, Japan; taniguchi-tky@umin.ac.jp

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ABSTRACT

Background Among the several musculoskeletal manifestations in patients with Marfan syndrome, spinal deformity causes pain and respiratory impairment and is a great hindrance to patients' daily activities. The present study elucidates the genetic risk factors for the development of severe scoliosis in patients with Marfan syndrome.

Methods We retrospectively evaluated 278 patients with pathogenic or likely pathogenic *FBN1* variants. The patients were divided into those with (n=57) or without (n=221) severe scoliosis. Severe scoliosis was defined as (1) patients undergoing surgery before 50 years of age or (2) patients with a Cobb angle exceeding 50° before 50 years of age. The variants were classified as protein-truncating variants (PTVs), which included variants creating premature termination codons and inframe exon-skipping, or non-PTVs, based on their location and predicted amino acid alterations, and the effect of the *FBN1* genotype on the development of severe scoliosis was examined. The impact of location of *FBN1* variants on the development of severe scoliosis was also investigated.

Results Univariate and multivariate analyses revealed that female sex, PTVs of *FBN1* and variants in the neonatal region (exons 25–33) were all independent significant predictive factors for the development of severe scoliosis. Furthermore, these factors were identified as predictors of progression of existing scoliosis into severe state.

Conclusions We elucidated the genetic risk factors for the development of severe scoliosis in patients with Marfan syndrome. Patients harbouring pathogenic *FBN1* variants with these genetic risk factors should be monitored carefully for scoliosis progression.

INTRODUCTION

Marfan syndrome (OMIM: #154700) was first described by Antoine Bernard Marfan in 1896 and is an autosomal dominant heritable disorder of the connective tissue.[1] Marfan syndrome is characterised by several clinical manifestations, including dilatation of the aortic root, ectopia lentis and characteristic skeletal features. Among the several musculoskeletal manifestations in patients with Marfan syndrome, spinal deformity causes pain and restrictive ventilatory impairment and is a great hindrance to patients' daily activities. Because patients with Marfan syndrome are potentially at high risk of further impairment of cardiopulmonary function following thoracotomy procedure and/or recurrent pneumothorax, it is essential to prevent the progression of spinal deformity and further deterioration of respiratory function. In addition, life expectancy of these patients has improved over the last few decades due to better medical and surgical management of cardiovascular conditions[2]; thus, appropriate control of spinal deformity is increasingly important. From the perspective of health economics, Marfan syndrome is reported to be the most common diagnosis among patients with syndromic scoliosis undergoing spinal deformity correction.[3] Recently, we have reported that female sex and positive wrist signs are predictive factors for the progression of scoliosis in Marfan syndrome[4]; however, predicting the progression of spinal deformity is challenging, which leads to inadequate management of spinal deformity in patients with Marfan syndrome.

Up to 97% of patients with Marfan syndrome who fulfil the Ghent criteria have pathogenic variants in the *FBN1* gene (OMIM: #134797), which contains 66 exons and encodes a major component of the extracellular matrix microfibril, namely fibrillin-1.[1 5] More than 3000 pathogenic variants, which are mostly unique among families, have been identified in the *FBN1* gene. The penetrance of *FBN1* variants in Marfan syndrome is generally high, but phenotype prediction from these variants has been a challenging task. To date, several studies have demonstrated genotype–phenotype correlations in Marfan syndrome. Pathogenic variants in exons 25–33 of *FBN1* were reported to be associated with neonatal Marfan syndrome,[6–8] which is characterised by severe emphysema and mitral and/or tricuspid valve insufficiency in early childhood. Strong correlations between ectopia lentis and *FBN1* variants affecting or creating cysteine residues have been repeatedly reported.[9 10] Regarding aortic manifestations, some recent studies have shown that patients with haploinsufficient (HI) type *FBN1* variants, such as nonsense and out-of-frame variants that presumably cause nonsense-mediated mRNA decay (NMD), have more severe aortic phenotypes than those with missense variants.[11–15] However, there have been very few reports that investigated

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genotype–phenotype correlations between musculoskeletal manifestations and variant types of *FBN1*. Recently, Arnaud *et al* reported that the premature termination codon (PTC) variants in *FBN1* are associated with the incidence of scoliosis with Cobb angle ≥20°.[15] De Maio *et al* also found an association between stop codon variants in *FBN1* and scoliosis with Cobb angle ≥20° or thoracolumbar kyphosis.[16] However, no study has investigated the actual impact of the pathogenic *FBN1* variant types on the progression of scoliosis into severe state requiring surgery. Scoliosis deteriorates patients' quality of life when it progresses to a severe spinal curve, which causes worsening respiratory functions and/or low back pain.[17 18] Hence, analyses focusing on patients with severe progressive spinal deformity are essential for eliciting clinically helpful information. The present study aimed to demonstrate, for the first time, the correlations between the pathogenic *FBN1* variant types and the development of severe scoliosis to identify the genetic risk factors for progression of spinal deformity in patients with Marfan syndrome.

METHODS

Patients and genetic tests

Data were retrospectively obtained from a prospective cohort from the Marfan syndrome center at our institute for a total of 175 months from September 2006 to March 2021. We enrolled consecutive patients with pathogenic or likely pathogenic *FBN1* variants detected by genetic analysis. The variants were classified as pathogenic or likely pathogenic based on *FBN1*-specific variant classification guidelines,[19] made by adapting 15 of the 28 general criteria of the American College of Medical Genetics and Genomics-Association for Molecular Pathology classification guidelines to better fit specific features of the *FBN1* gene and Marfan syndrome. Identification of the pathogenic *FBN1* variants was performed using Sanger sequencing for the *FBN1* gene or next generation sequencing (NGS)-based genetic tests.[14] NGS-based genetic tests included exome sequencing conducted using Japan's Initiative on Rare and Undiagnosed Diseases in Pediatrics research and hybridisation capture-based gene panel testing for aortopathies conducted at the Kazusa DNA Research Institute (Chiba, Japan).[14 20–22] In this study, seven patients with or suspected of having CNVs of the *FBN1* gene were also included.[22] Details of the genetic tests have been previously described.[14 22] The reference sequence used for *FBN1* was RefSeq NM_000138.4. Written informed consent was obtained either from the patients or from the guardians of minor patients.

Classification of pathogenic *FBN1* variants

The pathogenic variants were classified into two main categories based on their location and predicted amino acid alterations: protein-truncating variants (PTVs) or non-PTVs. PTVs were defined as single nucleotide variants predicted to introduce a premature stop codon or to disrupt a splice site, small insertions or deletions predicted to disrupt a transcript's reading frame, and larger deletions that remove the full protein coding sequence as previously described.[23] Hence, in addition to PTC-creating variants, variants predicted to induce inframe exon-skipping (IFES) caused by disruptions of the splice donor site (eg, intron +1G or +2T) or splice acceptor site (eg, intron −1G or −2A) were included in PTVs. Out-of-frame and inframe exon-skipping variants detected by CNV analysis were also categorised as PTVs. Non-PTVs included missense variants and small inframe insertion or deletion variants, which are expected to exert dominant-negative effects.

Definition of severe and control groups

Patients with severe scoliosis were classified into the 'severe' group, which was defined as (1) patients who underwent primary surgery for scoliosis before 50 years of age or (2) patients with Cobb angle exceeding 50° before 50 years of age. This definition is used because major curves exceeding 50° progress even after skeletal maturity due to biomechanical reason,[24] and thus in such patients prophylactic surgery is usually indicated to prevent progression of the curves to the severe level. Patients in the 'control' group were defined as those with a Cobb angle of 50° or less on the final X-ray which was taken at 15 years of age or older. To eliminate the impact of growth potential, which affects the progression of scoliosis, patients whose X-ray of the final follow-up was taken before 15 years of age were excluded from the control group. For all patients who did not undergo surgery for spinal deformity, posterior-anterior and lateral whole spine radiographs in standing position at final follow-up were evaluated and the Cobb angle was determined. For some patients who underwent surgery, the Cobb angle prior to surgery was unknown because preoperative X-rays were unavailable.

Statistical analysis

Fisher's exact test was used to compare categorical data. Univariate and multivariate logistic regression analyses were performed to determine the risk factors associated with the progression of scoliosis to a severe state. Surgery-free curves were constructed using the Kaplan-Meier method and compared using the log-rank test. The threshold for significance was set at $p<0.05$. All statistical analyses were performed using JMP Pro (V.16.0.0; SAS Institute, Cary, North Carolina, USA).

RESULTS

Demographic data of severe and control groups

Among 376 cases with pathogenic or likely pathogenic *FBN1* variants, we identified a total of 278 eligible patients from 245 families, with 57 patients in the severe group and 221 patients in the control group. The remaining 98 cases were excluded for the following reasons: X-ray or clinical information was unavailable for 65 patients and final spinal X-ray was taken before 15 years of age in 33 patients. A total of 210 distinct *FBN1* variants were identified in the 278 cases studied (online supplemental table 1). The details of the identified *FBN1* variants are provided in online supplemental table 1. The profiles of the severe group are shown in table 1.

There were 20 male and 37 female patients in the severe group. Among the 57 patients in the severe group, 42 underwent

Table 1 Details of severe scoliosis cases with pathogenic *FBN1* variants

	n
Total number of patients	57
Sex (%)	
Male	20 (35.1)
Female	37 (64.9)
Details of 'severe' scoliosis	
Surgery conducted	42
Age at first surgery, years, mean (range)	15.2 (4–47)
Cobb ≥50° before 50 years old	15
Age at X-ray, years, mean (range)	29.8 (8–49)
Cobb angle, mean (SD)	86.3 (27.0)

Cobb is Cobb angle of scoliosis.

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Table 2 Demographic data of the severe scoliosis and control groups

	n	Severe group	Control group	P value*
Number of patients	278	57	221	
Age at X-ray (range)		N/A	33.7 (15–79)	N/A
Sex (male:female)	135:143	20:37	115:106	0.03
Cobb angle (°), mean (SD)		N/A	17.3 (12.7)	N/A
Type of *FBN1* variants				0.03
PTV (%)	132	35 (26.5)	97 (73.5)	
PTC-creating variants (%)	97	25 (25.8)	72 (74.2)	
Inframe exon-skipping (%)	35	10 (28.6)	25 (71.4)	
Non-PTV (%)	146	22 (15.1)	124 (84.9)	
Non-PTV affecting Cys residues (%)	112/146	16/22 (72.7)	96/124 (77.4)	0.60

*P<0.05.
Cys, cysteine; N/A, not applicable; PTC, premature termination codon; PTV, protein-truncating variant.

surgery, while 15 presented a Cobb angle exceeding 50° before 50 years of age (table 1). The mean age at first surgery for scoliosis in 42 patients was 15.2. All operations were identified as being performed for scoliosis or kyphoscoliosis. Demographic data comparing the severe and control groups are shown in table 2.

The mean age at X-ray in the control group was 33.7 and the mean Cobb angle was 17.3° (table 2). Female sex and PTVs were identified more often in the severe group (table 2). When assessing PTVs, we observed a similar tendency towards increase in the frequency of PTC-creating variants and IFES variants in the severe group (table 2). Furthermore, when we constructed surgery-free curves using the Kaplan-Meier method, excluding 15 non-surgical severe cases, the equivalent impact of PTC-creating variants and IFES variants on the development of severe scoliosis was visually verified (online supplemental figure 1). These results confirmed the validity of our strategy of adopting the concept of PTVs,[23] which included both PTC-creating variants and IFES variants. In contrast to the fact that PTVs were significantly more often identified in the severe group than non-PTVs, no significant difference was identified in the distribution of *FBN1* variant types in patients with mild scoliosis (10° ≤ Cobb angle <30°) or moderate scoliosis (30° ≤ Cobb angle ≤50°) (figure 1 and table 2). This result suggested that *FBN1* variant types may impact the progression rather than the onset of scoliosis (figure 1). Among the non-PTVs, there was no significant difference in the ratio of *FBN1* variants affecting or creating cysteine residues between the two groups (table 2).

Distribution of pathogenic *FBN1* variants

The detailed distribution of pathogenic *FBN1* variants is shown in table 3 and figure 2.

Among the 278 cases with pathogenic or likely pathogenic *FBN1* variants, 7 were suspected or confirmed to have CNVs[22]; 5 intragenic (multi-)exon deletions and 1 whole gene deletion were validated using chromosomal microarray analysis and multiplex ligation-dependent probe amplification analysis, respectively (table 3). In one patient (patient 330) clinically diagnosed with Marfan syndrome, heterozygous deletion of exons 64–66 was strongly suspected via a simple CNV prediction method that visually reviews the coverage tracks from the Integrative Genomics Viewer browser[22] (table 3). The distribution of pathogenic *FBN1* variants of the remaining 271 cases is summarised in figure 2.

Neonatal region (exons 25–33) as a hot spot for developing severe scoliosis

Because pathogenic variants in exons 25–33 of *FBN1* were reported to be associated with early-onset severe cardiovascular

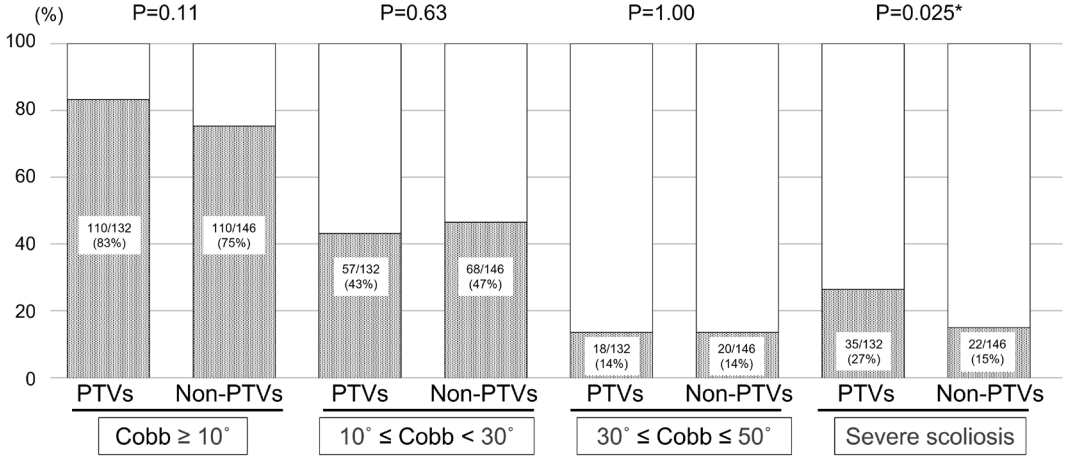

Figure 1 Distribution of *FBN1* variant types by severity of scoliosis. Significant difference in ratio for severe scoliosis was observed between the two variant types, while no significant difference in ratio for mild (10° ≤ Cobb angle <30°) or moderate (30° ≤ Cobb angle ≤50°) scoliosis was observed, suggesting that *FBN1* variant type may affect the progression rather than the onset of scoliosis. PTV, protein-truncating variant. *P<0.05.

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Table 3 Details of seven cases with exonic CNVs of *FBN1*

Number	Sex	Deleted exons	Type of *FBN1* variants	Affected 'hot spot'	Severe scoliosis or control	Surgery	Cobb angle
329	Male	20	PTV	No	Control	No	13
330	Female	64–66	PTV	C-terminal region	Severe	Yes	84*
331	Male	23–25	PTV	Neonatal region	Severe	Yes	80*
332	Female	3	PTV	No	Severe	No	80
333	Male	39–40	PTV	No	Severe	Yes	57*
334	Male	51–63	PTV	Exons 55–56	Control	No	9
335	Male	1–66	PTV	No	Severe	Yes	93*

*In cases of surgery, the Cobb angle prior to surgery is provided.
PTV, protein-truncating variant.

phenotype in patients with Marfan syndrome,[6–8] we hypothesised that there might be some 'hot spot' regions in the *FBN1* gene for the development of severe scoliosis. First, we investigated whether the variants in this so-called 'neonatal region' (exons 25–33) were associated with severe scoliosis. Among the 34 cases with pathogenic *FBN1* variants in this region, 13 (38.2%) developed severe scoliosis, while among the 244 cases with pathogenic *FBN1* variants in other regions only 44 (18.0%) developed severe scoliosis. This was a significant difference (p=0.01) (figure 2 and table 3). This result indicates that harbouring pathogenic variants in the neonatal region might be associated with the development of severe scoliosis.

Univariate and multivariate analyses for identification of risk factors for developing severe scoliosis

To determine the actual impact of genetic factors on the development of severe scoliosis in Marfan syndrome, we conducted univariate and multivariate logistic regression analyses. Univariate analysis revealed that female sex (OR, 2.01; 95% CI 1.11 to 3.73), PTVs of *FBN1* variants (OR, 2.03; 95% CI 1.13 to 3.73) and location of *FBN1* variants in the neonatal region (OR, 2.81; 95% CI 1.28 to 6.00) all had a significant correlation with the development of severe scoliosis (table 4).

Multivariate analysis revealed that female sex (OR, 2.24; 95% CI 1.21 to 4.27), PTVs of *FBN1* variants (OR, 2.30; 95% CI 1.25 to 4.33) and location of *FBN1* variants in the neonatal region (OR, 3.11; 95% CI 1.38 to 6.88) were all significant independent predictive factors for the development of severe scoliosis in Marfan syndrome (table 4). To eliminate the impact of other genetic factors shared within the family members, we then conducted univariate and multivariate analyses for only 245 index cases as sensitivity analysis and achieved similar results (online supplemental tables 2 and 3). Moreover, to capture the time course impact of each risk factor on the development of severe scoliosis, we constructed surgery-free curves using the Kaplan-Meier method, excluding 15 non-surgical severe cases. The exclusion of the 15 non-surgical severe cases was necessary since the onset of 'severe scoliosis' with Cobb angle exceeding 50° could not be exactly determined due to lack of previous X-rays. Surgery-free curves constructed using the Kaplan-Meier method visually verified the similar impact of each risk factor on the development of severe scoliosis, although significance of the impact of PTVs on the development of severe scoliosis was marginal (online supplemental figure 2). This was probably due to the decreased number of severe cases. Furthermore, to capture the impact of the genetic factors more precisely, we constructed surgery-free curves using the Kaplan-Meier method for up to 20 years of age, excluding three cases who underwent surgery past the age of 20 who might have been modulated by other factors (online supplemental figure 3). Surgery-free curves up to 20 years of age visually verified the similar impact of each risk factor on the development of severe scoliosis as well (online supplemental figure 3).

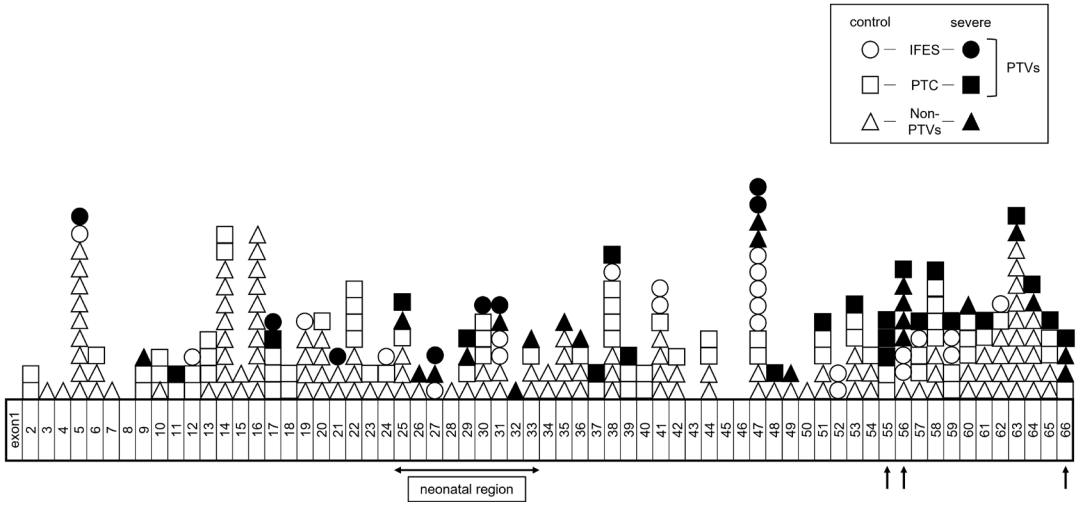

Figure 2 Detailed distribution of pathogenic *FBN1* variants of 271 cases other than the CNV cases. Black arrows indicate possible hot spot regions for developing severe scoliosis. Control cases are depicted as white shapes, while cases in the severe group are depicted as black shapes. Circle, rectangle and triangle represent IFES, PTC-creating variants and non-PTVs, respectively. IFES, inframe exon-skipping; PTC, premature termination codon creating variants; PTV, protein-truncating variant.

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Table 4 Univariate and multivariate logistic regression analyses for identifying the risk factors for the development of severe scoliosis in patients with pathogenic or likely pathogenic *FBN1* variants

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value*	OR (95% CI)	P value*
Sex		0.02		0.01
Female	2.01 (1.11 to 3.73)		2.24 (1.21 to 4.27)	
Male	Reference		Reference	
Type of *FBN1* variants		0.02		0.007
PTV	2.03 (1.13 to 3.73)		2.30 (1.25 to 4.33)	
Non-PTV	Reference		Reference	
Location of *FBN1* variants		0.01		0.007
Neonatal region (exons 25–33)	2.81 (1.28 to 6.00)		3.11 (1.38 to 6.88)	
Other regions	Reference		Reference	

*P<0.05.
PTV, protein-truncating variant.

Impact of the genetic factors on the progression of existing spinal deformity

We then conducted another sensitivity analysis in which we limited the control cases with Cobb angle ≥10° to determine the impact of genetic risk factors on the progression of existing scoliosis. By eliminating the 58 cases without scoliosis (Cobb angle <10°), we obtained 57 severe and 163 control cases. The demographic data of this cohort are provided in online supplemental file 1. Univariate and multivariate logistic regression analyses demonstrated that female sex, PTVs of *FBN1* variants and location of *FBN1* variants in the neonatal region were all significant independent predictive factors for progression of scoliosis into severe state as well (online supplemental table 5).

Possible hot spot regions other than the neonatal region for developing severe scoliosis

Finally, we attempted to identify possible hot spot regions other than the neonatal region for severe scoliosis by visual inspection (figure 2 and table 3). We focused on the region where at least three cases developed severe scoliosis and more than 50% of the cases involved were categorised in the severe group. In this way, we identified exons 55–56 and the C-terminal region (exon 66) as possible hot spot regions (figure 2). Eight out of 14 cases (57.1%) harbouring pathogenic variants in exons 55–56 and 4 out of 5 cases (80.0%) with pathogenic variants in the C-terminal region (exon 66) developed severe scoliosis (figure 2 and table 3).

DISCUSSION

This study provides two novel pieces of information. First, we demonstrated that the variant types of pathogenic *FBN1* variants have distinct impacts on the progression of scoliosis in patients with Marfan syndrome. Second, we showed that not only variant types but also the location of *FBN1* variants play an important role in the development of severe scoliosis.

There are several advantages in identifying patients at high risk of progression of spinal deformity. First, it can motivate patients at high risk of progression to seek regular medical help. Second, it may enable us to initiate timely interventions, including brace treatment and surgery. Because spinal deformity in Marfan syndrome is rapidly progressive and occasionally early onset, the timely initiation of brace treatment, which is usually suggested when the Cobb angle exceeds 20°, is sometimes difficult in these patients. This is probably one of the reasons for the lower success rate of brace treatment in Marfan syndrome than in idiopathic scoliosis.[25–27] Regarding surgical management, surgery for spinal deformity in Marfan syndrome is reported to be accompanied by a higher incidence of complications compared with that in idiopathic conditions.[28–30] Thus, it is crucially important to perform timely prophylactic surgery for scoliosis when the Cobb angle exceeds 45° or 50° to minimise perioperative complications, because surgery for progressed curves is known to be associated with a higher incidence of complications. Furthermore, impaired respiratory function due to progressive curves can be irreversible even after highly invasive surgery,[31] especially when they have been left for a certain period. Finally, identifying patients at high risk of progression can be beneficial in terms of health economics.

The present study demonstrated that PTVs in *FBN1* have distinct impacts on the development of severe scoliosis in patients with Marfan syndrome. In the current study, we adopted the concept of PTVs, which included PTC-creating variants and IFES variants. While most PTC-creating variants except those in the last exon result in haploinsufficiency through NMD mechanism, the actual functional effect of IFES remains to be determined. Furthermore, variants affecting splice sites in *FBN1* very often result in IFES, since the number of the nucleotides in most of the exons (exons 4–63) in *FBN1* is a multiple of 3. Thus, we first confirmed that PTC-creating variants and IFES variants have nearly equivalent impacts on the development of severe scoliosis. This finding is consistent with the findings of previous reports that demonstrated a significantly reduced amount of total mRNA of *FBN1* in the splice variants, which was in agreement with a mechanism of haploinsufficiency.[32] HI variants or PTC-creating variants in *FBN1* have been proven to be associated with a higher risk of aortic events or aggressive aortic dilatation than dominant-negative variants.[11–15] Hence, the present study proved that in Marfan syndrome, aortic manifestation and spinal deformity share common genetic risk factors for presenting severe phenotypes.

Pathogenic variants in the neonatal region (exons 25–33) have proven to be another genetic risk factor for developing severe scoliosis, regardless of the variant type (table 4). Faivre *et al*[7] reported that pathogenic variants in this region are associated with the presence of scoliosis.[7] However, no study has investigated the relationship between the severity of scoliosis and location of the *FBN1* variants. Patients harbouring pathogenic variants in the neonatal region are known to exhibit variable severity of cardiovascular phenotypes and do not always present with neonatal Marfan syndrome, which is usually lethal in the first 2 years of life.[7 8 33] Indeed, in the current case series of 13 cases with pathogenic *FBN1* variants in the neonatal region in the severe group, 6 cases were free from cardiovascular surgery until the final follow-up (data not shown). Hence, it is important to recognise that patients with pathogenic variants in the neonatal region are at high risk of severe scoliosis because they do not always develop severe cardiovascular manifestations early in life and can be candidates for aggressive care for spinal deformity.

Exons 55–56 and the C-terminal region (exon 66) were also identified as possible hot spot regions for severe scoliosis. The reason for the correlation between severe patient phenotypes and these exon regions is unknown. Exons 55–56 encode two calcium-binding epidermal growth factor-like (cbEGF) domains:

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the cbEGF domains 34 and 35. Fibrillin-1 contains 43 cbEGF domains, each of which binds one calcium ion and is stabilised by three highly conserved disulfide bonds. Bound calcium stabilises cbEGF domains and cbEGF-cbEGF interdomain interfaces, extending tandem cbEGF domain repeats into rigid rod-like structures.[34] Thus, variants in cbEGF domain are presumed to interfere with calcium binding, thereby perturbing microfibril assembly, structure and function,[35] which may affect the structural strength of the spine. However, it remains to be elucidated why many patients in the severe group showed variations in this specific cbEGF region. The C-terminal region (exons 65–66) of *FBN1* encodes asprosin, which is a novel glucogenic adipokine and is known to be involved in lipid metabolism.[36] Furthermore, pathogenic variants in this region can cause Marfanoid-progeroid-lipodystrophy syndrome, a distinctive phenotype consisting of partial manifestations of Marfan syndrome, a progeroid facial appearance and clinical features of lipodystrophy.[37 38] Because the association between idiopathic scoliosis and lower body fat has recently been suggested,[39] variants in the C-terminal region of *FBN1* may affect the progression of spinal deformity through altered lipid metabolism. In any case, further investigation is needed to determine whether pathogenic variants in these regions are associated with the development of severe scoliosis.

Recently, rare variants in *FBN1* or *FBN2* have been identified in patients with severe idiopathic scoliosis who did not clinically meet the diagnostic criteria for Marfan syndrome or Beals syndrome.[40] Although there were not such cases in our present case series since at our institute genetic tests had been performed only in patients diagnosed as or highly suspected for Marfan syndrome with aortic or ocular manifestations according to the revised Ghent criteria,[5] our present study may provide new insights into the aetiology of idiopathic scoliosis.

The present study identified female sex as another predictive factor for the progression of scoliosis in Marfan syndrome, which was consistent with our previous study.[4] It has been repeatedly reported that male patients with Marfan syndrome have an increased risk of aortic events and root dilatation compared with female patients.[13 14 41 42] This difference may partially explain the discrepancy in the severity between physical manifestation and aortic manifestation observed in individual patients with Marfan syndrome despite the common genetic risk factors.

In this study, we used a logistic regression model for multivariate statistical analysis. There were two reasons for selecting this model instead of the Cox regression analysis. First, the onset of 'severe scoliosis' with a Cobb angle exceeding 50° could not be determined for 15 non-surgical severe cases because consecutive previous spine X-rays were unavailable for these cases. Second, the proportional hazard assumption was not expected for progression of scoliosis in general because spinal deformity rapidly deteriorates during the growth spurt period and then progresses very slowly. Hence, surgery for scoliosis is usually performed prophylactically in late adolescence when the Cobb angle exceeds 45°–50°. In the current study, the mean age at first surgery for scoliosis in 42 cases was 15.2, and the Kaplan-Meier surgery-free curves for scoliosis clearly demonstrated that most operations had been performed during adolescence (table 1 and online supplemental figures 1 and 2).

This study had a few limitations. First, it was retrospective in design. Second, there might be some selection bias. Due to the lack of whole spine X-rays, not all patients with pathogenic *FBN1* variants were analysed in this study. Third, in some patients undergoing surgery, the preoperative severity of spinal deformity is unknown due to the lack of preoperative X-rays. Finally, the effect of pathogenic variants on the stability and function of the protein product was not verified.

To the best of our knowledge, this study, for the first time, determined the genetic risk factors for progression to severe spinal deformity in patients with Marfan syndrome. PTVs in *FBN1* have distinct impacts on the development of severe scoliosis in patients with Marfan syndrome. Variants in the neonatal region were also independent genetic risk factors for the development of severe scoliosis. Exons 55–56 and the C-terminal region (exon 66) in *FBN1* were also identified as possible hot spot regions. Therefore, patients harbouring pathogenic *FBN1* variants with these genetic risk factors should be monitored carefully for scoliosis progression.

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ORCID iD

Yuki Taniguchi http://orcid.org/0000-0002-2329-123X

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Supplementary Table 1. Included *FBN1* variants

Variant code	Exon	Nucleotide Change	Predicted Protein Change	Type of *FBN1* variants	Classification of *FBN1* variants	Number of cases	Effect Group	Class	Reference
v1	2	NM_000138.4:c.111delC	p.(Arg38Glufs*70)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]
v2	2	NM_000138.4:c.2T>A	p.Met1Lys	PTC	PTV	1	Initiation codon variant	P	rs1057516934
v3	3	NM_000138.4:c.239G>A	p.(Cys80Tyr)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	rs397515767
v4	4	NM_000138.4:c.280T>C	p.(Cys94Arg)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	Takeda et al. [1]
v5	5	NM_000138.4:c.347-2A>G	Exon 5 deletion	IFES	PTV	2	splice-site ±1-2	P	rs1555405056
v6	5	NM_000138.4:c.364C>T	p.(Arg122Cys)	Missense	non-PTV	3	Cys outside cb-EGF or cb-site	LP	rs137854467
v7	5	NM_000138.4:c.385T>G	p.(Cys129Gly)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	rs199474693
v8	5	NM_000138.4:c.386G>A	p.(Cys129Tyr)	Missense	non-PTV	3	Cys outside cb-EGF or cb-site	LP	rs1566935517
v9	5	NM_000138.4:c.433T>C	p.(Cys145Arg)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	rs1555405031
v10	5	NM_000138.4:c.400T>G	p.(Cys134Gly)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	Attanasio et al. [2]
v11	6	NM_000138.4:c.493C>T	p.(Arg165*)	PTC	PTV	1	Nonsense	P	rs113905529
v12	6	NM_000138.4:c.502T>C	p.(Cys168Arg)	Missense	non-PTV	2	Cys outside cb-EGF or cb-site	LP	Jin et al.[3]
v13	7	NM_000138.4:c.718C>T	p.(Arg240Cys)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	rs137854480
v14	9	NM_000138.4:c.923_926del TCAG	p.(Val308Alafs*21)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]
v15	9	NM_000138.4:c.939C>G	p.(Cys313Trp)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs1555401007
v16	9	NM_000138.4:c.953delG	p.(Gly318Valfs*12)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]
v17	10	NM_000138.4:c.1129T>G	p.(Cys377Gly)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	Takeda et al. [1]
v18	10	NM_000138.4:c.1090C>T	p.(Arg364*)	PTC	PTV	2	Nonsense	P	rs794728165
v19	11	NM_000138.4:c.1285C>T	p.(Arg429*)	PTC	PTV	2	Nonsense	P	rs112645512
v20	12	NM_000138.4:c.1416C>G	p.(Tyr472*)	PTC	PTV	2	Nonsense	P	Takeda et al. [1]
v21	12	NM_000138.4:c.1468+5G>A	Exon 12 deletion	IFES	PTV	1	splice-site non±1-2	LP	rs397515757
v22	13	NM_000138.4:c.1495T>C	p.(Cys499Arg)	Missense	non-PTV	1	Cys in cb-EGF	LP	Schrijver et al. [4]
v23	13	NM_000138.4:c.1585C>T	p.(Arg529*)	PTC	PTV	2	Nonsense	P	rs137854476
v24	13	NM_000138.4:c.1477G>T	p.(Glu493*)	PTC	PTV	1	Nonsense	P	Takeda et al. [1]
v25	14	NM_000138.4:c.1623C>A	p.(Cys541*)	PTC	PTV	1	Nonsense	P	Takeda et al. [1]
v26	14	NM_000138.4:c.1637G>T	p.(Cys546Phe)	Missense	non-PTV	4	Cys in cb-EGF	LP	Takeda et al. [1]
v27	14	NM_000138.4:c.1709G>C	p.(Cys570Ser)	Missense	non-PTV	2	Cys in cb-EGF	LP	Ogawa et al. [5]
v28	14	NM_000138.4:c.1693C>T	p.(Arg565*)	PTC	PTV	1	Nonsense	P	rs113422242
v29	14	NM_000138.4:c.1664G>A	p.(Cys555Tyr)	Missense	non-PTV	2	Cys in cb-EGF	LP	rs794728172
v30	15	NM_000138.4:c.1786T>G	p.(Cys596Gly)	Missense	non-PTV	2	Cys in cb-EGF	LP	rs1057520131
v31	16	NM_000138.4:c.1879C>T	p.(Arg627Cys)	Missense	non-PTV	1	Cys in cb-EGF (creating Cys)	LP	rs727503057
v32	16	NM_000138.4:c.1904A>G	p.(Tyr635Cys)	Missense	non-PTV	5	Cys in cb-EGF (creating Cys)	LP	rs1555399816
v33	16	NM_000138.4:c.1910G>A	p.(Cys637Tyr)	Missense	non-PTV	1	Cys in cb-EGF	LP	Zhao et al. [6]
v34	16	NM_000138.4:c.1949G>A	p.(Arg650His)	Missense	non-PTV	1	Other missense	LP	Takeda et al. [1]
v35	16	NM_000138.4:c.1955G>A	p.(Cys652Tyr)	Missense	non-PTV	1	Cys in cb-EGF	LP	Comeglio et al. [7]
v36	16	NM_000138.4:c.1911T>G	p.(Cys637Trp)	Missense	non-PTV	1	Cys in cb-EGF	LP	Ogawa et al. [5]
v37	17	NM_000138.4:c.1980delA	p.(Cys661Alafs*57)	PTC	PTV	2	Frameshift	P	Takeda et al. [1]
v38	17	NM_000138.4:c.2025_2026d elTTG	p.(Phe675Valfs*42)	PTC	PTV	1	Frameshift	P	na
v39	17	NM_000138.4:c.2111C>G	p.(Ser704*)	PTC	PTV	1	Nonsense	P	na
v40	17	NM_000138.4:c.2113+3A>C	Exon 17 deletion	IFES	PTV	1	splice-site non±1-2	LP	Ogawa et al. [5]
v41	18	NM_000138.4:c.2153dupC	p.(Ser719Valfs*5)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]
v42	18	NM_000138.4:c.2121T>G	p.(Tyr707*)	PTC	PTV	1	Nonsense	P	na
v43	19	NM_000138.4:c.2201G>T	p.(Cys734Phe)	Missense	non-PTV	2	Cys in cb-EGF	LP	rs794728187
v44	19	NM_000138.4:c.2237A>G	p.(Tyr746Cys)	Missense	non-PTV	2	Cys in cb-EGF (creating Cys)	LP	rs1555399372
v45	19	NM_000138.4:c.2293+1G>C	Exon 19 deletion	IFES	PTV	1	splice-site ±1-2	P	Ogawa et al. [5]
v46	20	NM_000138.4:c.2413T>G	p.(Cys805Gly)	Missense	non-PTV	1	Cys in cb-EGF	LP	Takeda et al. [1]
v47	20	NM_000138.4:c.2413T>C	p.(Cys805Arg)	Missense	non-PTV	2	Cys in cb-EGF	LP	na
v48	20	NM_000138.4:c.2306G>A	p.(Cys769Tyr)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs794728190
v49	20	NM_000138.4:c.2302G>T	p.(Glu768*)	PTC	PTV	1	Nonsense	P	na
v50	21	NM_000138.4:c.2447G>T	p.(Cys816Phe)	Missense	non-PTV	2	Cys in cb-EGF	LP	rs397515770
v51	21	NM_000138.4:c.2538_2539+ 5delAGGTATT	Exon 21 deletion	IFES	PTV	1	splice-site ±1-2	P	Takeda et al. [1]
v52	22	NM_000138.4:c.2586T>G	p.(Cys862Trp)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	Takeda et al. [1]
v53	22	NM_000138.4:c.2638G>A	p.(Gly880Ser)	Missense	non-PTV	1	Other missense	LP	rs794728194
v54	22	NM_000138.4:c.2561G>A	p.(Trp854*)	PTC	PTV	1	Nonsense	P	rs1597568968
v55	22	NM_000138.4:c.2651delG	p.(Gly884Glufs*28)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]
v56	22	NM_000138.4:c.2569_2570d elGT	p.(Val857Hisfs*2)	PTC	PTV	1	Frameshift	P	na
v57	22	NM_000138.4:c.2645C>T	p.(Ala882Val)	Missense	non-PTV	1	Other missense	LP	rs794728195
v58	22	NM_000138.4:c.2581C>T	p.(Arg861*)	PTC	PTV	1	Nonsense	P	rs140583
v59	23	NM_000138.4:c.2722T>C	p.(Cys908Arg)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	rs1060501021
v60	23	NM_000138.4:c.2702C>G	p.(Ser901*)	PTC	PTV	1	Nonsense	P	na
v61	24	NM_000138.4:c.2729-1G>C	Exon 24 deletion	IFES	PTV	1	splice-site ±1-2	P	Takeda et al. [1]
v62	24	NM_000138.4:c.2753delC	p.(Pro918Glnfs*24)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]

v63	24	NM_000138.4:c.2854G>C	p.(Asp952His)	Missense	non-PTV	1	Other missense	LP	na
v64	25	NM_000138.4:c.2886C>A	p.(Tyr962*)	PTC	PTV	1	Nonsense	P	rs772108557
v65	25	NM_000138.4:c.2953G>A	p.(Gly985Arg)	Missense	non-PTV	2	Other missense	LP	rs794728199
v66	25	NM_000138.4:c.2965_2966delGG	p.(Gly989Tyrfs*2)	PTC	PTV	1	Frameshift	P	na
v67	25	NM_000138.4:c.2942G>A	p.(Cys981Tyr)	Missense	non-PTV	2	Cys outside cb-EGF or cb-site	LP	rs727505110
v68	26	NM_000138.4:c.3093G>C	p.(Glu1031Asp)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	Takeda et al. [1]
v69	26	NM_000138.4:c.3095G>A	p.(Cys1032Tyr)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs137854481
v70	27	NM_000138.4:c.3209-1G>A	Exon 27 deletion	IFES	PTV	1	splice-site ±1-2	P	Takeda et al. [1]
v71	27	NM_000138.4:c.3302A>G	p.(Tyr1101Cys)	Missense	non-PTV	1	Cys in cb-EGF (creating Cys)	LP	rs1555398625
v72	27	NM_000138.4:c.3337+1G>A	Exon 27 deletion	IFES	PTV	1	splice-site ±1-2	P	rs397515789
v73	28	NM_000138.4:c.3463G>C	p.(Asp1155His)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	Stheneur et al. [9]
v74	29	NM_000138.4:c.3559delC	p.(His1187Ilefs*17)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]
v75	29	NM_000138.4:c.3584G>A	p.(Cys1195Tyr)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs886038802
v76	29	NM_000138.4:c.3544T>G	p.(Cys1182Gly)	Missense	non-PTV	1	Cys in cb-EGF	LP	na
v77	29	NM_000138.4:c.3524_3525delTA	p.(Ile1175Argfs*17)	PTC	PTV	1	Frameshift	P	na
v78	30	NM_000138.4:c.3650G>A	p.(Gly1217Asp)	Missense	non-PTV	1	Other missense	LP	rs1555398397
v79	30	NM_000138.4:c.3603C>A	p.(Cys1201*)	PTC	PTV	1	Nonsense	P	Ogawa et al. [5]
v80	30	NM_000138.4:c.3622delT	p.(Cys1208Valfs*22)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]
v81	30	NM_000138.4:c.3670C>T	p.(Gln1224*)	PTC	PTV	1	Nonsense	P	Takeda et al. [1]
v82	30	NM_000138.4:c.3712G>A	p.(Asp1238Asn)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	rs794728208
v83	30	NM_000138.4:c.3712+1G>A	Exon 30 deletion	IFES	PTV	1	splice-site ±1-2	P	rs794728209
v84	31	NM_000138.4:c.3713-1G>A	Exon 31 deletion	IFES	PTV	3	splice-site ±1-2	P	Takeda et al.[1]
v85	31	NM_000138.4:c.3746G>C	p.(Cys1249Ser)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs137854458
v86	31	NM_000138.4:c.3781T>G	p.(Tyr1261Asp)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	Arbustini et al. [10]
v87	31	NM_000138.4:c.3725G>A	p.(Cys1242Tyr)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs137854471
v88	32	NM_000138.4:c.3919T>C	p.(Cys1307Arg)	Missense	non-PTV	1	Cys in cb-EGF	LP	Takeda et al. [1]
v89	33	NM_000138.4:c.4021A>C	p.(Asn1341His)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	Takeda et al. [1]
v90	33	NM_000138.4:c.4027G>T	p.(Ala1343Ser)	Missense	non-PTV	1	Other missense	LP	na
v91	33	NM_000138.4:c.4066G>T	p.(Gly1356*)	PTC	PTV	1	Nonsense	P	Takeda et al. [1]
v92	33	NM_000138.4:c.4017T>G	p.(Cys1339Trp)	Missense	non-PTV	1	Cys in cb-EGF	LP	na
v93	34	NM_000138.4:c.4096G>A	p.(Glu1366Lys)	Missense	non-PTV	2	Cys outside cb-EGF or cb-site	LP	rs763449629
v94	35	NM_000138.4:c.4313G>A	p.(Ser1438Asn)	Missense	non-PTV	2	Other missense	LP	rs587782945
v95	35	NM_000138.4:c.4280A>G	p.(Tyr1427Cys)	Missense	non-PTV	1	Cys in cb-EGF (creating Cys)	LP	rs1555397548
v96	35	NM_000138.4:c.4331G>T	p.(Cys1444Phe)	Missense	non-PTV	1	Cys in cb-EGF	LP	Takeda et al. [1]
v97	35	NM_000138.4:c.4222T>C	p.(Cys1408Arg)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs397515802
v98	36	NM_000138.4:c.4411_4420dupGAGTGTGAGA	p.(Ile1474Argfs*3)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]
v99	36	NM_000138.4:c.4408T>C	p.(Cys1470Arg)	Missense	non-PTV	1	Cys in cb-EGF	LP	Tjeldhorn, et al. [11]
v100	36	NM_000138.4:c.4382G>C	p.(Cys1461Ser)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs1057522902
v101	36	NM_000138.4:c.4459G>A	p.(Asp1487Asn)	Missense	non-PTV	1	Other missense	LP	rs113693945
v102	37	NM_000138.4:c.4567C>T	p.(Arg1523*)	PTC	PTV	1	Nonsense	P	rs397515812
v103	37	NM_000138.4:c.4507_4508delGT	p.(Val1503Glnfs*9)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]
v104	38	NM_000138.4:c.4621C>T	p.(Arg1541*)	PTC	PTV	4	Nonsense	P	rs794728228
v105	38	NM_000138.4:c.4588C>T	p.(Arg1530Cys)	Missense	non-PTV	3	Cys outside cb-EGF or cb-site	LP	rs111401431
v106	38	NM_000138.4:c.4583-1G>A	Exon 38 deletion	IFES	PTV	1	splice-site ±1-2	P	rs1555397176
v107	38	NM_000138.4:c.4615C>T	p.(Arg1539*)	PTC	PTV	1	Nonsense	P	rs111231312
v108	39	NM_000138.4:c.4750G>T	p.(Glu1584*)	PTC	PTV	1	Nonsense	P	Loeys et al. [12]
v109	39	NM_000138.4:c.4786C>T	p.(Arg1596*)	PTC	PTV	1	Nonsense	P	rs113871094
v110	39	NM_000138.4:c.4777G>T	p.(Glu1593*)	PTC	PTV	1	Nonsense	P	Ogawa et al. [5]
v111	40	NM_000138.4:c.4930C>T	p.(Arg1644*)	PTC	PTV	1	Nonsense	P	rs140630
v112	40	NM_000138.4:c.4834G>T	p.(Glu1612*)	PTC	PTV	1	Nonsense	P	Takeda et al. [1]
v113	41	NM_000138.4:c.4973delG	p.(Cys1658Leufs*24)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]
v114	41	NM_000138.4:c.5060G>A	p.(Cys1687Tyr)	Missense	non-PTV	1	Cys in cb-EGF	LP	Takeda et al. [1]
v115	41	NM_000138.4:c.4993A>T	p.(Asn1665Tyr)	Missense	non-PTV	2	Cys outside cb-EGF or cb-site	LP	Takeda et al. [1]
v116	41	NM_000138.4:c.4973G>A	p.(Cys1658Tyr)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs1555396859
v117	41	NM_000138.4:c.5065+1G>A	Exon 41 deletion	IFES	PTV	2	splice-site ±1-2	P	Ogawa et al. [5]
v118	42	NM_000138.4:c.5177delG	p.(Gly1726Alafs*167)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]
v119	42	NM_000138.4:c.5162G>A	p.(Cys1721Tyr)	Missense	non-PTV	2	Cys outside cb-EGF or cb-site	LP	Takeda et al. [1]
v120	44	NM_000138.4:c.5371T>C	p.(Cys1791Arg)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs1555396427
v121	44	NM_000138.4:c.5368C>T	p.(Arg1790*)	PTC	PTV	2	Nonsense	P	rs113249837
v122	44	NM_000138.4:c.5372G>A	p.(Cys1791Tyr)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs886038848
v123	47	NM_000138.4:c.5672-2A>G	Exon 47 deletion	IFES	PTV	2	splice-site ±1-2	P	rs1060501053
v124	47	NM_000138.4:c.5725_5740delinsCAGTTGAA	p.(Ile1909Glnfs*16)	PTC	PTV	2	Frameshift	P	Takeda et al. [1]
v125	47	NM_000138.4:c.5743C>A	p.(Arg1915Ser)	Missense	non-PTV	1	Other missense	LP	na
v126	47	NM_000138.4:c.5726T>C	p.(Ile1909Thr)	Missense	non-PTV	1	Other missense	LP	rs794728333
v127	47	NM_000138.4:c.5729G>T	p.(Gly1910Val)	Missense	non-PTV	1	Other missense	LP	na
v128	47	NM_000138.4:c.5740T>C	p.(Cys1914Arg)	Missense	non-PTV	1	Cys in cb-EGF	LP	na
v129	47	NM_000138.4:c.5788+1G>A	Exon 47 deletion	IFES	PTV	1	splice-site ±1-2	P	rs1555395819

v130	47	NM_000138.4:c.5788+2T>G	Exon 47 deletion	IFES	PTV	1	splice-site ±1-2	P	na
v131	47	NM_000138.4:c.5788+5G>A	Exon 47 deletion	IFES	PTV	2	splice-site non±1-2	LP	rs193922219
v132	47	NM_000138.4:c.5788+5G>C	Exon 47 deletion	IFES	PTV	1	splice-site non±1-2	LP	Takeda et al. [1]
v133	48	NM_000138.4:c.5873G>A	p.(Cys1958Tyr)	Missense	non-PTV	1	Cys in cb-EGF	LP	Ogawa et al. [5]
v134	48	NM_000138.4:c.5886T>G	p.(Tyr1962*)	PTC	PTV	1	Nonsense	P	Takeda et al. [1]
v135	49	NM_000138.4:c.5966G>T	p.(Cys1989Phe)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs1597531796
v136	49	NM_000138.4:c.5950T>C	p.(Cys1984Arg)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs1555395659
v137	50	NM_000138.4:c.6113G>A	p.(Cys2038Tyr)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs363804
v138	51	NM_000138.4:c.6296G>T	p.(Cys2099Phe)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	rs1131691803
v139	51	NM_000138.4:c.6181T>C	p.(Cys2061Arg)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	rs1555395267
v140	51	NM_000138.4:c.6169C>T	p.(Arg2057*)	PTC	PTV	2	Nonsense	P	rs763091520
v141	51	NM_000138.4:c.6268G>T	p.(Glu2090*)	PTC	PTV	1	Nonsense	P	na
v142	52	NM_000138.4:c.6379+1G>A	Exon 52 deletion	IFES	PTV	2	splice-site ±1-2	P	rs397515833
v143	53	NM_000138.4:c.6388G>T	p.(Glu2130*)	PTC	PTV	1	Nonsense	P	rs794728334
v144	53	NM_000138.4:c.6448delC	p.(Arg2150Alafs*10)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]
v145	53	NM_000138.4:c.6487G>T	p.(Glu2163*)	PTC	PTV	1	Nonsense	P	rs1555395191
v146	53	NM_000138.4:c.6388G>A	p.(Glu2130Lys)	Missense	non-PTV	3	Cys outside cb-EGF or cb-site	LP	rs794728334
v147	54	NM_000138.4:c.6518G>A	p.(Gly2173Asp)	Missense	non-PTV	1	Other missense	LP	Ogawa et al. [5]
v148	54	NM_000138.4:c.6577G>T	p.(Glu2193*)	PTC	PTV	1	Nonsense	P	Biggin et al.[13]
v149	54	NM_000138.4:c.6542G>A	p.(Cys2181Tyr)	Missense	non-PTV	1	Cys in cb-EGF	LP	Waldmuller et al.[14]
v150	55	NM_000138.4:c.6703_6704delGG	p.(Gly2235Ilefs*8)	PTC	PTV	1	Frameshift	P	Ogawa et al. [5]
v151	55	NM_000138.4:c.6665delT	p.(Val2222Glyfs*69)	PTC	PTV	3	Frameshift	P	Ogawa et al. [5]
v152	55	NM_000138.4:c.6658C>T	p.(Arg2220*)	PTC	PTV	1	Nonsense	P	rs113001196
v153	56	NM_000138.4:c.6740-2A>G	Exon 56 deletion	IFES	PTV	2	splice-site ±1-2	P	Takeda et al. [1]
v154	56	NM_000138.4:c.6806T>C	p.(Ile2269Thr)	Missense	non-PTV	3	Other missense	LP	rs193922228
v155	56	NM_000138.4:c.6837delG	p.(Tyr2280Ilefs*11)	PTC	PTV	1	Frameshift	P	Ogawa et al. [5]
v156	56	NM_000138.4:c.6752G>C	p.(Cys2251Ser)	Missense	non-PTV	1	Cys in cb-EGF	LP	na
v157	56	NM_000138.4:c.6748G>A	p.(Glu2250Lys)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	rs1597520789
v158	57	NM_000138.4:c.6874G>T	p.(Glu2292*)	PTC	PTV	1	Nonsense	P	Takeda et al. [1]
v159	57	NM_000138.4:c.6947G>A	p.(Cys2316Tyr)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs1555394629
v160	57	NM_000138.4:c.6982C>T	p.(Gln2328*)	PTC	PTV	1	Nonsense	P	rs371097218
v161	57	NM_000138.4:c.6997+5G>A	Exon 57 deletion	IFES	PTV	1	splice-site non±1-2	LP	Ogawa et al. [5]
v162	57	NM_000138.4:c.6915delG	p.(Arg 2306 Alafs*92)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]
v163	58	NM_000138.4:c.7039_7040delAT	p.(Met2347Valfs*19)	PTC	PTV	2	Frameshift	P	rs794728319
v164	58	NM_000138.4:c.7090T>C	p.(Cys2364Arg)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	Takeda et al. [1]
v165	58	NM_000138.4:c.7184G>T	p.(Gly2395Val)	Missense	non-PTV	1	Other missense	LP	rs397515849
v166	58	NM_000138.4:c.7141C>T	p.(Gln2381*)	PTC	PTV	2	Nonsense	P	rs869025414
v167	58	NM_000138.4:c.7015T>G	p.(Cys2339Gly)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	Ogawa et al. [5]
v168	58	NM_000138.4:c.7180C>T	p.(Arg2394*)	PTC	PTV	1	Nonsense	P	rs397515848
v169	59	NM_000138.4:c.7266_7267delAG	p.(Gly2423Ilefs*7)	PTC	PTV	1	Frameshift	P	Stheneur et al. [9]
v170	59	NM_000138.4:c.7240C>T	p.(Arg2414*)	PTC	PTV	2	Nonsense	P	rs112550005
v171	59	NM_000138.4:c.7330+3_6delAAGT	Exon 59 deletion	IFES	PTV	1	splice-site non±1-2	LP	Takeda et al. [1]
v172	59	NM_000138.4:c.7327_7330+2delGTAGGT	Exon 59 deletion	IFES	PTV	1	splice-site ±1-2	P	na
v173	60	NM_000138.4:c.7406_7407insTGTT	p.(Cys2470Valfs*19)	PTC	PTV	2	Frameshift	P	Takeda et al. [1]
v174	60	NM_000138.4:c.7339G>A	p.(Glu2447Lys)	Missense	non-PTV	2	Cys outside cb-EGF or cb-site	LP	rs137854464
v175	60	NM_000138.4:c.7408T>C	p.(Cys2470Arg)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs1555394399
v176	60	NM_000138.4:c.7339G>T	p.(Glu2447*)	PTC	PTV	1	Nonsense	P	Arbustini et al. [10]
v177	61	NM_000138.4:c.7466G>A	p.(Cys2489Tyr)	Missense	non-PTV	3	Cys in cb-EGF	LP	rs1060501077
v178	61	NM_000138.4:c.7565delG	p.(Cys2522Serfs*160)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]
v179	61	NM_000138.4:c.7545delT	p.(Phe2515Leufs*167)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]
v180	62	NM_000138.4:c.7582T>C	p.(Cys2528Arg)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs1566891701
v181	62	NM_000138.4:c.7664G>T	p.(Gly2555Val)	Missense	non-PTV	1	Other missense	LP	rs1566891654
v182	62	NM_000138.4:c.7636G>A	p.(Gly2546Arg)	Missense	non-PTV	1	Other missense	LP	Bustamante-Aragones et al. [15]
v183	62	NM_000138.4:c.7664delG	p.(Gly2555Aspfs*127)	PTC	PTV	1	Frameshift	P	na
v184	62	NM_000138.4:c.7606G>A	p.(Gly2536Arg)	Missense	non-PTV	1	Other missense	LP	rs397515854
v185	62	NM_000138.4:c.7669+1G>A	Exon 62 deletion	IFES	PTV	1	splice-site ±1-2	P	Baetens et al. [16]
v186	63	NM_000138.4:c.7754T>C	p.(Ile2585Thr)	Missense	non-PTV	9	Other missense	LP	rs727503054
v187	63	NM_000138.4:c.7792dupC	p.(Gln2598Profs*10)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]
v188	63	NM_000138.4:c.7784G>T	p.(Gly2595Val)	Missense	non-PTV	1	Other missense	LP	na
v189	64	NM_000138.4:c.7831T>C	p.(Cys2611Arg)	Missense	non-PTV	1	Cys in cb-EGF	LP	Takeda et al. [1]
v190	64	NM_000138.4:c.7864T>C	p.(Cys2622Arg)	Missense	non-PTV	1	Cys in cb-EGF	LP	Takeda et al. [1]
v191	64	NM_000138.4:c.7936T>C	p.(Cys2646Arg)	Missense	non-PTV	1	Cys in cb-EGF	LP	rs1555393863
v192	64	NM_000138.4:c.7938C>A	p.(Cys2646*)	PTC	PTV	1	Nonsense	P	Takeda et al. [1]

v193	64	NM_000138.4:c.7982A>G	p.(Tyr2661Cys)	Missense	non-PTV	1	Cys in cb-EGF (creating Cys)	LP	rs112196241
v194	64	NM_000138.4:c.7906G>T	p.(Gly2636Cys)	Missense	non-PTV	1	Cys in cb-EGF (creating Cys)	LP	Takeda et al. [1]
v195	64	NM_000138.4:c.7828G>A	p.(Glu2610Lys)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	rs111984349
v196	65	NM_000138.4:c.8063C>G	p.(Ser2688Cys)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	Takeda et al. [1]
v197	65	NM_000138.4:c.8090delC	p.(Pro2697Glnfs*55)	PTC	PTV	1	Frameshift	P	Takeda et al. [1]
v198	65	NM_000138.4:c.8188C>T	p.(Arg2730Trp)	Missense	non-PTV	1	Other missense	LP	na
v199	65	NM_000138.4:c.8135delC	p.(Pro2712Glnfs*40)	PTC	PTV	2	Frameshift	P	Takeda et al. [1]
v200	66	NM_000138.4:c.8377T>G	p.(Tyr2793Asp)	Missense	non-PTV	1	Other missense	LP	na
v201	66	NM_000138.4:c.8521G>T	p.(Glu2841*)	PTC	PTV	1	Nonsense	LP	rs587782948
v202	66	NM_000138.4:c.8378A>G	p.(Tyr2793Cys)	Missense	non-PTV	1	Cys outside cb-EGF or cb-site	LP	rs397515863
v203	66	NM_000138.4:c.8326C>T	p.(Arg2776*)	PTC	PTV	1	Nonsense	LP	rs137854466
CNVs									
v204	20		Exon 20 deletion	IFES	PTV	1	Copy number variation causing exon(s) skipping	P	Takeda et al. [17]
v205	64-66		Exons 64-66 deletion		PTV	1	Copy number variation causing exon(s) skipping	P	na
v206	23-25		Exons 23-25 deletion	IFES	PTV	1	Copy number variation causing exon(s) skipping	P	Takeda et al. [17]
v207	3		Exon 3 deletion	PTC	PTV	1	Copy number variation causing exon(s) skipping	P	Takeda et al. [17]
v208	39-40		Exons 39-40 deletion	IFES	PTV	1	Copy number variation causing exon(s) skipping	P	Takeda et al. [17]
v209	51-63		Exons 51-63 deletion	IFES	PTV	1	Copy number variation causing exon(s) skipping	P	Takeda et al. [17]
v210	1-66		Exons 1-66 deletion		PTV	1	Copy number variation causing exon(s) skipping	P	na
						Total=	278		

PTC, variant creating a premature termination codon; IFES, in-frame exon-skipping; PTV, protein-truncating variant; P, pathogenic; LP, likely-pathogenic; CNV, copy number variation.

<Supplementary Reference>

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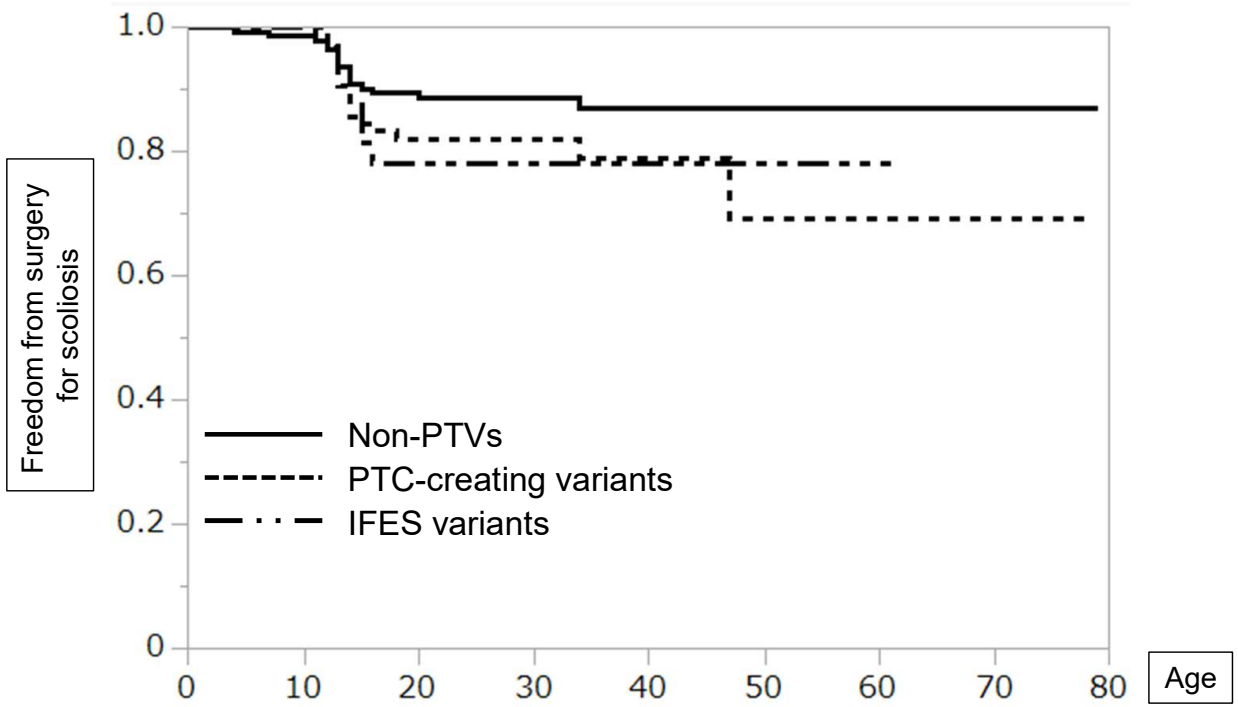
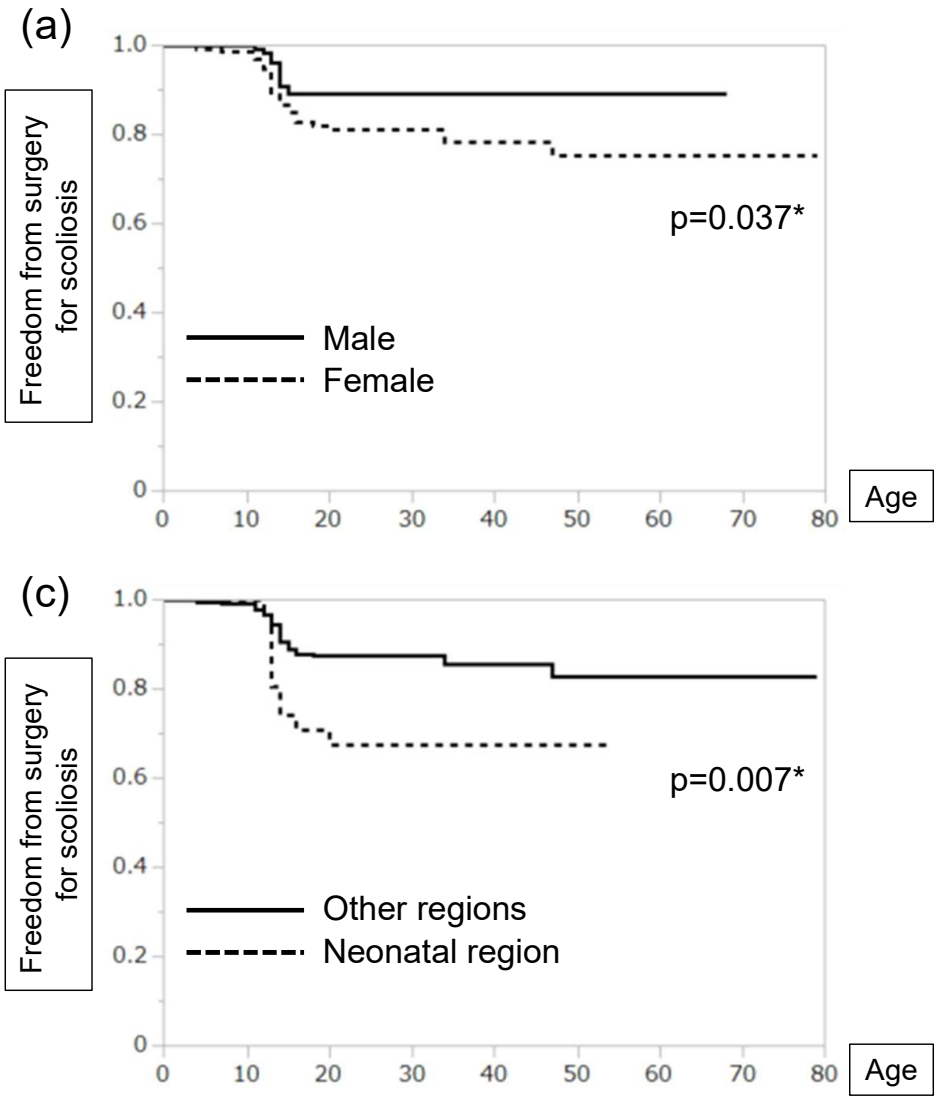
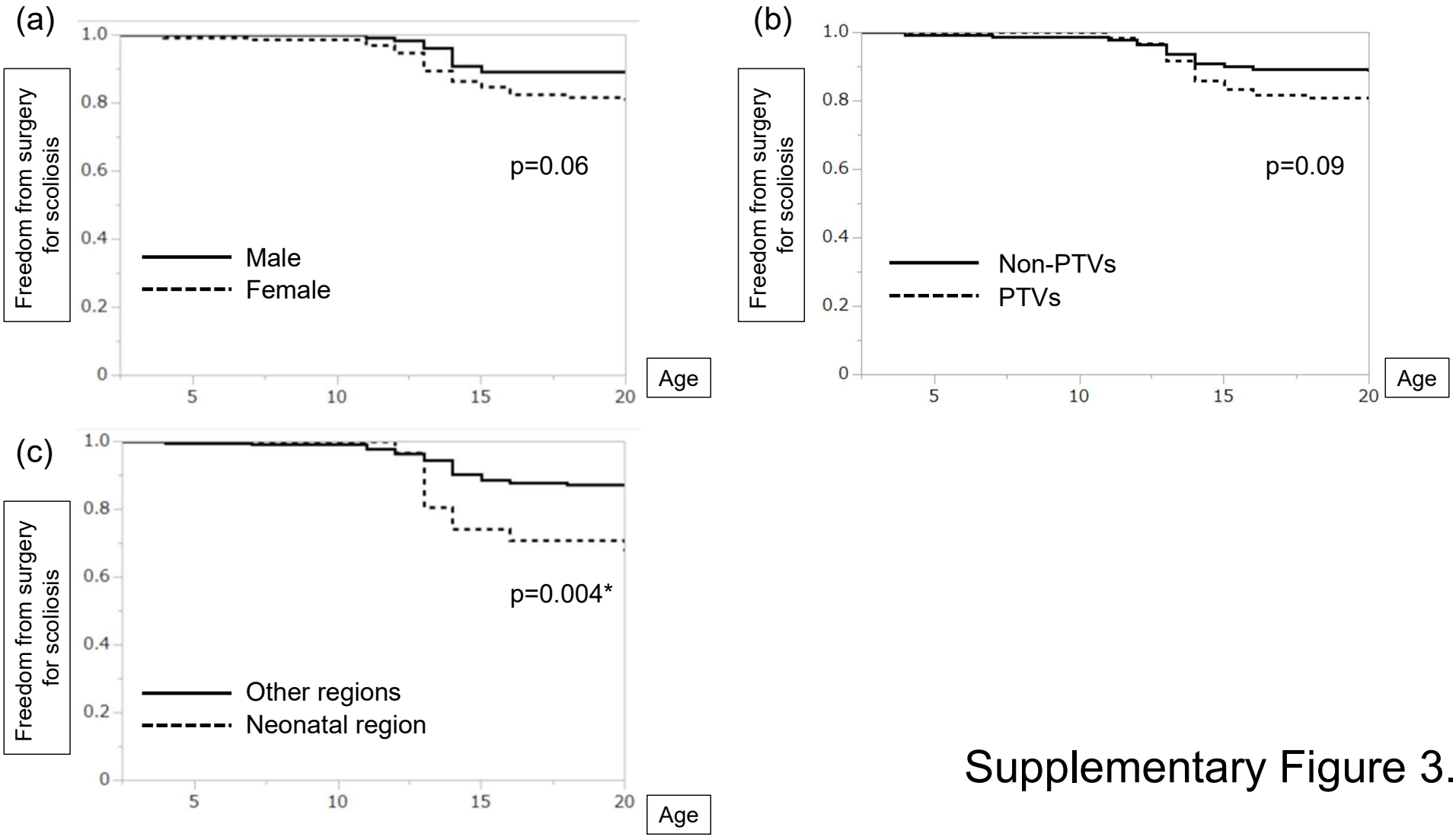


Supplementary Figure 1.



Supplementary Figure 2.



Supplementary Figure 3.

Supplementary Figure Legends

Supplementary Figure 1.

A Kaplan–Meier surgery-free curve for scoliosis by *FBN1* variant types, excluding 15 non-surgical severe cases visually indicated the equivalent impact of PTC-creating variants and IFES variants on the development of severe scoliosis. PTV, protein-truncating variant; IFES, in-frame exon-skipping; PTC, premature termination codon

Supplementary Figure 2.

Kaplan–Meier surgery-free curves for scoliosis, excluding 15 non-surgical severe cases. Kaplan–Meier curves by (a) sex, (b) *FBN1* variant types, and (c) location of *FBN1* variant were constructed. PTV, protein-truncating variant

Supplementary Figure 3.

Kaplan–Meier surgery-free curves for scoliosis for up to 20 years of age, excluding 15 non-surgical severe cases and three cases underwent surgery past the age of 20. Kaplan–Meier curves by (a) sex, (b) *FBN1* variant types, and (c) location of *FBN1* variant were constructed. PTV, protein-truncating variant

Supplementary Table 2. Detail of the index cases

		N	Severe group		Control group		*p*
Number of patients		245	53		192		
Sex (male:female)		120:125	19:34		101:91		0.04*
Type of *FBN1* variants							0.06
PTVs	(%)	120	32	(26.7)	88	(73.3)	
Non-PTVs	(%)	125	21	(16.8)	104	(83.2)	
Location of *FBN1* variants							0.04*
Neonatal region (exon 25–33)	(%)	32	12	(37.5)	20	(62.5)	
Other regions	(%)	213	41	(19.2)	172	(80.8)	

PTV, protein-truncating variant. *: $p < 0.05$

Supplementary Table 3. Univariate and multivariate logistic regression analysis limited to 245 index cases of severe scoliosis development

	Univariate analysis		Multivariate analysis	
	OR 95% [CI]	*p*	OR 95% [CI]	*p*
Sex		0.03*		0.02*
Female	1.99 [1.07–3.78]		2.15 [1.14–4.18]	
Male	Reference		Reference	
Type of *FBN1* variants		0.06		0.03*
PTVs	1.80 [0.98–3.38]		2.05 [1.09–3.96]	
Non-PTVs	Reference		Reference	
Location of *FBN1* variants		0.03*		0.02*
Neonatal region (exon 25–33)	2.52 [1.11–5.51]		2.84 [1.22–6.44]	
Other regions	Reference		Reference	

OR, odds ratio; CI, confidence interval; PTV, protein-truncating variant. *: $p < 0.05$.

Supplementary Table 4. Demographic data of the severe scoliosis and control cases with Cobb angle ≥ 10 º

		N	Severe group		Control group		*p*
Number of patients		220	57		163		
Sex (male:female)		103:117	20:37		83:80		0.045*
Type of *FBN1* variants							0.06
PTVs	(%)	110	35	(31.8)	75	(68.2)	
Non-PTVs	(%)	110	22	(20.0)	88	(80.0)	
Location of *FBN1* variants							0.04*
Neonatal region (exon 25–33)	(%)	31	13	(41.9)	18	(58.1)	
Other regions	(%)	189	44	(23.3)	145	(76.7)	

PTV, protein-truncating variant. *: $p < 0.05$

Supplementary Table 5. Univariate and multivariate logistic regression analysis limited to patients with Cobb angle ≥ 10 º

	Univariate analysis		Multivariate analysis	
	OR 95% [CI]	*p*	OR 95% [CI]	*p*
Sex		0.04*		0.01*
Female	1.92 [1.04–3.63]		2.22 [1.17–4.32]	
Male	Reference		Reference	
Type of *FBN1* variants		0.04*		0.01*
PTVs	1.87 [1.01–3.49]		2.20 [1.17–4.25]	
Non-PTVs	Reference		Reference	
Location of *FBN1* variants		0.04*		0.02*
Neonatal region (exon 25–33)	2.38 [1.06–5.22]		2.82 [1.22–6.46]	
Other regions	Reference		Reference	

OR, odds ratio; CI, confidence interval; PTV, protein-truncating variant. *: $p < 0.05$.