およびクモ状指(arachnodactyly)と称される．その他，叢生歯を伴う高口蓋，特徴的顔貌(長頭，眼球陥凹，頬骨低形成，下顎後退)もしばしば認める所見である．

（2）心血管系

大動脈弁輪拡張および大動脈瘤を約70％の患者で認める．大動脈瘤はいずれの部位にも起こりうるが，特に大動脈基部(Valsalva洞)および遠位弓部大動脈に好発する．大動脈基部の拡張傾向は学童期頃から徐々に認められるようになり，青年期以降に大動脈瘤に至るというケースが多い．Marfan症候群では，拡張がそれほど高度でなくても大動脈解離に至る場合も多く，また，いったん解離すると広範になりやすいため，早期診断と適切なフォローが重要である．また，弁の脆弱化や弁輪部拡張による大動脈弁閉鎖不全症や僧帽弁逸脱を認めることも多く，特に小児期においては診断上重要な所見である．病理学的には嚢胞性中膜壊死(cystic medial necrosis)が特徴的といわれているが，Marfan症候群以外の遺伝性大動脈疾患でもしばしば認める所見である．

（3）眼系

特徴的所見は水晶体偏位(水晶体亜脱臼・脱臼・振盪)であり，水晶体を支えるチン小帯の異常による．Marfan症候群では耳側にずれるのが特徴的で，しばしば幼少期にMarfan症候群を疑われるきっかけとなる．その他，近視・乱視・斜視を認めることも多く，網膜剥離・緑内障の合併もしばしば認める．

（4）硬膜

CTまたはMRIの所見として腰仙部の硬膜拡張を認めることがあり，単独で大基準となっている．

（5）その他

肺症状として，自然気胸や胸部X線写真による肺尖部のブレブを認めることがある．また，皮膚症状として，線条皮膚萎縮(肩甲部や大腿部に認めることが多い)やヘルニアなどを認めることがあり，これらは，Ghent基準では小基準に分類されている．

3.　診　　断

臨床的診断は，罹患器官ごとに定められたGhent

診断基準による[4]．

異なる2器官において大基準を満たし，ほかの1器官での罹患を認める場合に，臨床的にMarfan症候群と認められる．一方，FBN1遺伝子の明らかな変異などの遺伝学的基準も大基準の一つと同等の扱いとなるため，臨床症状だけではMarfan症候群と診断できない症例においては，遺伝学的検査が，診断上重要になってくる．実際，遺伝項目を除いた臨床所見のみでこの診断基準を厳密に満たしている症例の割合を調べたところ，自験例では，成人例でもFBN1遺伝子変異を検出した症例の約4割にすぎなかった．

一方で，後述するように，しばしばMarfan症候群との鑑別が必要となるLoeys-Dietz症候群でも，Ghent基準を満たす症例も珍しくなく，この場合は，鑑別のためには遺伝学的検査が必須となる．

4.　遺伝学的解析

血管平滑筋において，細胞表面に分泌されたフィブリリン分子は互いに重合し，その他の構成成分とともにmicrofibrilを形成し，tropoelastinがこれに結合することにより成熟した弾性線維となる．したがって，FBN1遺伝子に異常があると弾性線維の成熟および維持に問題をきたし，結果として大動脈の病変をひき起こすとされている．フィブリリン1分子(分子量350kDa)は高度の反復構造を持つ線維状の糖蛋白であり，それをコードするFBN1遺伝子も第15番染色体長腕上の約235kbの領域を占め，65個のエクソンからなる巨大遺伝子である．FBN1の遺伝子解析は白血球から抽出したゲノムDNAを用い，これを鋳型として，全65エクソンを1エクソンごとにエクソン・イントロン境界領域も含めてPCR法で増幅し，直接シークエンス法あるいはDHPLC法で解析するというのが一般的である．しかし，イントロン部の変異によりエクソンの欠失を生じる場合や，稀ではあるがPCR primer領域にSNPが存在し一方のアレルのみしか増幅されない場合などは，この方法では解析不可能である．最近では，遺伝子領域をまとめて増幅したあとに断片をre-sequencing DNAチップにハイブリダイズさせて解析するという方法も開発されている．しかし，大きな欠失や重複などの変異の検出は，
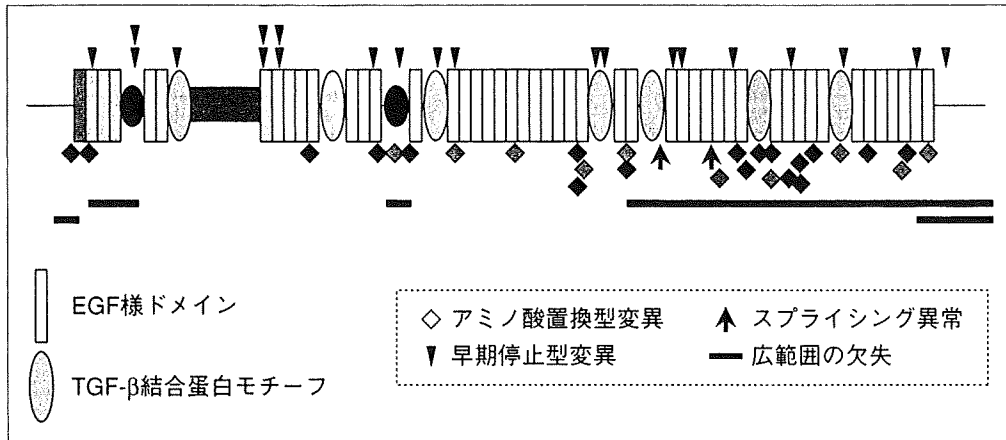
図1　フィブリリン1蛋白質の構造と検出された*FBN1*遺伝子変異（自験例）
▽：早期停止型変異（ナンセンス変異，フレームシフト変異），◇：アミノ酸置換型変異（ミセンス変異），↑：スプライシング異常変異，太線：領域欠損．フィブリリン1蛋白質は，47個のEGF様モチーフと7個のTGF-β結合蛋白（LTBP）モチーフからなる高度繰り返し配列を持つ線維状蛋白である．変異はほぼ全域にわたって検出されている．

これらの方法では難しく，その場合には，MLPA法（multiplex ligation-dependent probe amplification）を用いて，対応するゲノムDNA量を定量することにより，はじめて検出が可能となる．一方，イントロン部の変異により，最終的に蛋白合成の鋳型となるmRNAの構造や量に変化が生じる場合は，血管あるいは皮膚組織，あるいはこれらの組織に由来する培養細胞から抽出したmRNAでの解析が必要であり，これらの解析方法を併用すると変異の検出効率は高くなる．自験例では，臨床的にGhentの診断基準を満たす症例の約70%は，最初の方法により*FBN1*遺伝子変異が検出されるが，その他の方法を併用することにより，最終的には90%以上で*FBN1*遺伝子の変異が検出されている．

　1991年にMarfan症候群の原因遺伝子として15番染色体のfibrillin 1（*FBN1*）遺伝子が同定されて以来，これまでに全世界で500種類以上の*FBN1*遺伝子変異が原因変異として報告されている[5)~10)]．一部の例外を除いて，ほとんどの変異は個々の家系に特異的であり，遺伝子領域のほぼ全域に分布しており，好発部位は認められない（図1）．重症型の新生児Marfan症候群は第24～32エクソンに変異を認めることが多く，この領域の変異は予後不良であるといわれてきたが，最近の1,013例の検討でも同様の傾向は認められるものの，成人期にはじめて診断される例も少なくない．

また，システインを含む変異では水晶体亜脱臼を合併する症例がやや多いという傾向はあるが，明らかな遺伝子型・表現型の相関は認められておらず，遺伝子変異から表現型を予測することはできない．また，同一家系で同一変異を有する場合であっても，臨床症状や重症度などの表現型は一致しない場合がほとんどであり，*FBN1*遺伝子変異以外の要因が表現型を修飾していると考えられている．変異の種類には，①アミノ酸が変わるミスセンス変異，②蛋白合成を停止させる停止コドンに変化するナンセンス変異，③アミノ酸の読み枠を変えるフレームシフト変異，④エクソンの接続を変えるスプライシング異常変異，⑤複数のエクソン領域の欠失あるいは重複によりペプチド構造を大きく変える構造異常，などがあるが，このうち①が最も多く，報告された変異の60%近くを占め，前述のEGF様モチーフあるいはTGF-β結合モチーフ内の保存されたアミノ酸がほかのアミノ酸に置換される場合が多い．従来は，こうしたアミノ酸置換型による異常フィブリリン蛋白が正常な蛋白の機能を阻害する，いわゆるdominant negative阻害が病態の本質であると考えられていたが，患者の解析が進むにつれて，②や③により早期停止コドン（premature stop codon：PTC）をきたす変異を有する症例も多いことがわかり，最近では，正常なフィブリリン1蛋白質量の減少によるハ

プロ不全(haploinsufficiency)がより重要であると考えられている．実際，2007年の欧米での解析例をまとめた報告でも，早期停止型変異は31％を占めており，著者の研究室での解析でも39％を占めている．こうしたハプロ不全によるMarfan症候群発症の機序としては，最近TGF-βシグナル制御におけるフィブリリンの働きが注目されている．次述するTGF-β受容体遺伝子変異によるLoeys-Dietz症候群がMarfan症候群に酷似した症状を呈する場合があることからもわかるとおり，Marfan症候群の臨床所見の多くがフィブリリンの構造異常に由来するのではなく，むしろTGF-βを介した機能異常に由来する可能性を示唆しており，Marfan症候群のみならず，Loeys-Dietz症候群も含めたMarfan症候群類縁疾患全般におけるパラダイム・シフトを生じている[11]．

Loeys-Dietz症候群

1. 疾患概念および臨床像

Loeys-Dietz症候群は，2005年にLoeysらにより新たに提唱された疾患概念である．Marfan症候群に酷似した臨床像を呈するも，水晶体亜脱臼が認められないとされていた従来の2型Marfan症候群を含む一群の遺伝性疾患で，トランスフォーミング増殖因子βサブタイプ(TGF-β)の2種類の受容体をコードするTGFBR1遺伝子，およびTGFBR2遺伝子の変異で発症する[12]~[14]．Marfan症候群同様，常染色体優性遺伝性の全身性結合織疾患であり，類似の血管系および骨格系病変を主症状とするが，一般的に，Marfan症候群に比して心血管系病変がより若年化かつ重症化しやすく，また大動脈のみならず中小動脈にも罹患が及ぶ傾向があり，鑑別を要する．2005年の最初の報告では，口蓋裂・二分口蓋垂，眼間解離，全身血管の蛇行が特徴的所見としてあげられており，その他，頭蓋骨早期癒合，先天性心疾患，精神運動発達遅滞などの合併をしばしば認めるとされていた．その後，患者における遺伝子解析が進むにつれ，TGFBR1およびTGFBR2の遺伝子変異を有する患者の中には，特徴的な顔貌はあまり認められず，むしろ，薄く透過性の皮膚や易出血性など，血管型Ehlers-Danlos症候群類似の臨床症状を呈する症例も多いことが

わかり，従来の患者群をLDS1型，後者の患者群をLDS2型と分類することが提唱された．いずれにおいても，血管病変はMarfan症候群に比べ広範であり，脳動脈・頸部動脈・上腸管膜動脈などの血管蛇行や瘤形成をしばしば認めるため，Loeys-Dietz症候群と診断された場合は頭部MRAなどによる頭頸部動脈系の精査を含め，全身の血管病変の評価をするよう勧められている[14][15]．

Loeys-Dietz症候群の典型的症例では，臨床所見よりFBN1遺伝子変異によるMarfan症候群との鑑別診断は可能であるが，全体としてはMarfan症候群と臨床的にオーバーラップする点もかなり多く，鑑別のために遺伝子検査が必要となる症例も少なくない．一方で，血管系以外にはLoeys-Dietz症候群に特徴的といわれる所見が認められず，家族性大動脈瘤と考えられていた症例もあり，Loeys-Dietz症候群の臨床像はかなり幅広い．他方，Marfan症候群の臨床的診断基準であるGhent基準を満たしていてもTGFBR1やTGFBR2の変異によると考えられる症例も少なくなく，実際，これまで当院で遺伝子解析により診断したLoeys-Dietz症候群の多くの症例は，初期診断ではMarfan症候群と診断されている．こうした症例については，これらの2疾患を鑑別できるような新たな診断基準の作成が求められている．

2. 遺伝学的解析

TGFBR1遺伝子はTGF-βのI型受容体，TGFBR2遺伝子はII型受容体をコードしている．TGFBR1遺伝子は9エクソン，TGFBR2遺伝子は7エクソンよりなり，FBN1遺伝子と同様，ゲノムDNAを用いてエクソンごとにPCR法で増幅し，直接シークエンス法で解析するのが一般的であり，これにMLPA解析やmRNA解析を併用する．その他の注意点もFBN1遺伝子の解析と同様である．

これまでに同定された遺伝子変異のほとんどはC側のキナーゼ領域に集中しており，その他も高度に保存されたアミノ酸の変異である．また，変異の種類としても，ほとんどがアミノ酸置換型のmissense変異である点が特徴的である．同じ変異でもLDS1型，LDS2型の両方の症状を呈する場合があることが知られ，これまでのところ，明らかな遺伝子型・表現型の相関は認められていない．

前述したとおり，Marfan症候群とLoeys-Dietz症候群は臨床的にオーバーラップする点もかなり多く，実際，Marfan症候群と診断されている症例もかなり多い．しかし，Loeys-Dietz症候群ではより若年で大動脈瘤や大動脈解離を発症する傾向があり，また，脳動脈瘤の合併にも注意する必要があるなど，経過観察上の留意点がやや異なるため，遺伝子検査による鑑別が重要になる．自験例では，Marfan症候群と診断されている症例の約10％がLoeys-Dietz症候群であった．

血管型Ehlers-Danlos症候群

1．疾患概念および臨床像

Ehlers-Danlos症候群は，種々のコラーゲンの生成や代謝にかかわる遺伝子の変異により発症する全身性結合織疾患で，異常をきたすコラーゲンの種類により，古典型，関節可動性亢進型，血管型，後側彎型，多関節弛緩型，皮膚脆弱型などに分類されるが，なかでも，3型コラーゲンをコードするCOL3A1遺伝子の変異による常染色体優性遺伝性の血管型Ehlers-Danlos症候群は全身血管の脆弱性が特徴で，大動脈および全身の中小動脈の動脈破裂，動脈瘤，動脈解離などの血管病変のほか，腸管・子宮の脆弱性による消化管穿孔，妊娠中の子宮破裂などのリスクが高く，特に注意を要する[16]．臨床症状としては，このほか，特徴的顔貌，薄く透過性の皮膚と易出血性，小関節の過可動性を認める．組織の脆弱性は加齢とともに進行し，20歳までに4分の1の患者が，40歳までに5分の4の患者がなんらかの明らかな医学的問題を経験する．全身の血管系の評価は大切であるが，スクリーニングとしての血管造影はカテーテル挿入部位で動脈破裂や解離を起こす可能性があるため，一般的には勧められない．

2．遺伝学的解析

COL3A1遺伝子は51エクソンからなる巨大遺伝子であり，一般的には培養皮膚線維芽細胞などから抽出したmRNAを用いて，まずcDNAの変異解析を行い，ゲノムDNAで確認する．トリプルヘリカルドメインに存在する[Gly-X-Y]配列のグリシン残基の変異が3分の2を占め，3分の1がスプライス変異やその他の構造変異による．

この方法では，mRNAを得るために皮膚生検や大動脈摘出組織などが必要であったが，最近ではFBN1遺伝子の解析と同様，末梢血リンパ球から抽出したゲノムDNAを用いたre-sequencing DNAチップでの解析もすすめられている．

おわりに

Marfan症候群の病因遺伝子としてFBN1遺伝子が単離同定されてから18年経つ．当初は単なる結合織異常と考えられていたMarfan症候群であるが，その発症におけるTGF-βシグナル異常が近年明らかとなり，Marfan症候群およびその類縁疾患に伴う血管系合併症の根底には，共通してTGF-βシグナルの制御異常があると考えられるようになってきている．さらに，TGF-β作用の阻害効果のあるロサルタンに大動脈瘤発症予防効果があることが動物実験で示された[17]．分子遺伝学，分子病態学の着実な進歩により，「遺伝子の異常は治せない」から「遺伝子病の発症を防ぐ」への可能性が現実化されつつある．

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Progressive Aortic Root and Pulmonary Artery Aneurysms in a Neonate With Loeys–Dietz Syndrome Type 1B

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Loeys–Dietz Syndrome (LDS) is an autosomal dominant aortic aneurysm syndrome with multisystem involvement, caused by heterozygous mutations of transforming growth factor β receptor type 1 (*TGFBR1*) or type 2 (*TGFBR2*) genes. We report on a neonate with the disorder caused by a known *TGFBR2* mutation, who developed neonatal-onset progressive dilation of the aortic valve and aneurysms of the aortic root and main pulmonary artery (PA) associated with a large left-to-right shunt via a ventricular septal defect (VSD) and an atrial septal defect (ASD). He also had skeletal features (flexion contractures of the fingers, talipes equinovarus, a cleft palate, and joint laxity), mild facial dysmorphisms, and developmental delay. The dilation and aneurysms progressed after PA banding at age 12 days; and the patient received an intracardiac repair of the defects and PA plasty at age 42 days, followed by no further progression of the dilation and the aneurysms. Neonates with generalized hypotonia, a cleft palate, inguinal herniae, musculoskeletal features such as camptodactyly and talipes equinovarus, and a cardiac murmur should be suspected to have LDS, and extensive cardiovascular evaluation and testing of *TGFBR1* and *TGFBR2* are recommended. LDS patients with cardiac defects that lead to a large left-to-right shunt and congestive heart failure such as VSD should be considered for intracardiac repair even in early infancy. © 2010 Wiley-Liss, Inc.

Key words: Loeys–Dietz Syndrome; type 1B; *TGFBR2*; neonate; aortic valve dilation; aortic root aneurysm; pulmonary artery aneurysm; pulmonary artery banding; intracardiac repair

Loeys–Dietz Syndrome (LDS) is a recently recognized autosomal dominant aortic aneurysm syndrome with multisystem involve-

ment, caused by heterozygous mutations of transforming growth factor β receptor type 1 (*TGFBR1*) or type 2 (*TGFBR2*) genes [Loeys et al., 2005, 2006]. The disorder is typically characterized by the triad of arterial tortuosity and aneurysms, hypertelorism, and bifid uvula or cleft palate. Other manifestations include craniofacial features (craniosynostosis, malar hypoplasia, retrognathia, blue sclera, and ectopia lentis), skeletal features (dolichostenomelia, arachnodactyly, pectus deformity, scoliosis, talipes equinovarus, camptodactyly, joint laxity, and cervical spine instability), cutaneous changes (velvety skin and translucent skin), congenital heart defects (patent ductus arteriosus, PDA; atrial septal defect, ASD),

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and developmental retardation [Loeys et al., 2006]. LDS patients without typical craniofacial features but with at least two signs of the vascular type Ehlers-Danlos syndrome (OMIM130050) (translucent and/or velvety skin, wide atrophic scars, bruisability, joint laxity, and visceral rupture) have been subtyped as LDS type 2 with distinction from those with typical craniofacial presentations, subtyped as LDS type 1 [Loeys et al., 2006; Aalberts et al., 2008; Adès, 2008]. LDS type 1 is classified into 1A (OMIM 609192) and 1B (OMIM 610168), caused by mutations of TGFBR1 and TGFBR2, respectively. Similarly, LDS type 2 is classified into 2A (OMIM 608967) and 2B (OMIM 610380).

To date, roughly 160 LDS patients from 90 families have been reported. However, there has been limited information about clinical variations and management of the disorder especially in the neonatal period or early infancy [Yetman et al., 2007]. Here, we report on a neonate with LDS type 1B (LDS1B), who showed progressive dilation of the aortic valve and aneurysms of the aortic root and main pulmonary artery (PA) associated with a large left-to-right shunt via a VSD and an ASD, in addition to skeletal features (flexion contractures of the fingers, talipes equinovarus, a cleft palate, and joint laxity), mild facial dysmorphisms, and developmental retardation. Detailed description of the patient, representing the severest course of LDS, would be helpful to discuss appropriate management of early-onset progressive cardiovascular complications in the disorder.

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The patient, a boy, was the second child of healthy nonconsanguineous Japanese parents. The mother was age 30 and the father age 31 at the time of his birth. His brother was healthy. He was born at 41 weeks and 3 days of gestation by spontaneous vaginal delivery after an uncomplicated pregnancy. Birth weight was 3,336 g (−0.1 SD), length 52.5 cm (+1.2 SD), and OFC 35.5 cm (+0.9 SD). APGAR score was 9 at 1 min, and 10 at 5 min. He sucked poorly, and was transferred to our neonatal intensive care unit. He had long palpebral fissures, a cleft palate, and a mild retrognathia (Fig. 1A). He had slender fingers with flexion contractures (Fig. 1B) and talipes equinovarus (Fig. 1C), bilaterally. He also had a left inguinal hernia. Echocardiography showed a ventricular septal defect (VSD), an ASD, dilation of the aortic valve, and aneurysms of the aortic root and main PA. The size of the VSD was 8 mm with a large left-to-right shunt. The diameter of the aortic valve was 11.1 mm (Z-score was 5.88), the aortic root (sinuses of Valsalva) 19.0 mm (Z-score 9.42), the ascending aorta (sinotubular junction) 7.78 mm (Z-score 0.45), the pulmonary valve 8.11 mm (Z-score −0.74), the main PA 19.2 mm (Z-score 5.55), left PA 5.4 mm (Z-score 0.66), and right PA 6.4 mm (Z-score 1.90). Z-scores represent the standard deviation from the mean diameter normalized for the patient's body-surface area and age, calculated according to the equations shown by Daubeney et al. [1999].

He showed congestive heart failure due to an increased left-to-right shunt via a large VSD and an ASD. PA banding was performed on day 12. A large aneurysm was observed at the root of the PA (Fig. 2A). After the procedure, he showed further progression of aortic valve dilation (14.3 mm by echocardiography), aortic root (20.9 mm) and main PA (24.8 mm) aneurysms, and congestive
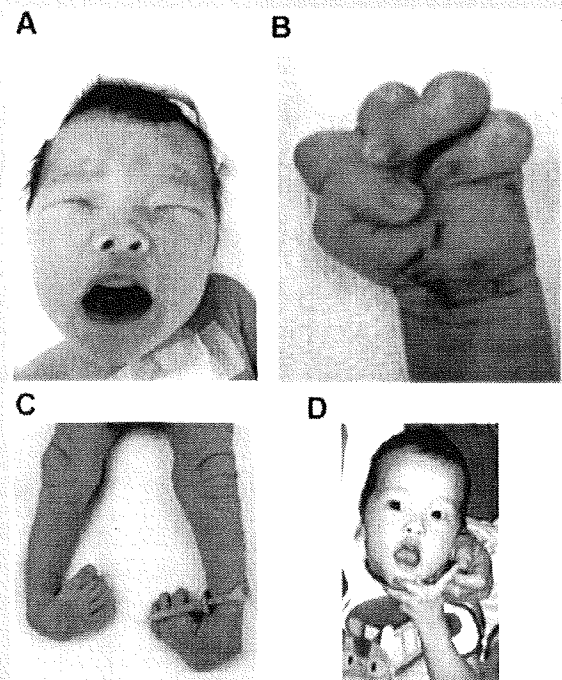

FIG. 1. A–C: Patient in a neonatal period. D: Patient at age 12 months. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

heart failure; as well as dilation of the ascending aorta (11.2 mm), the pulmonary valve (14.8 mm), and bilateral PA (left, 8.6 mm; right, 15.9 mm; Fig. 2B–D). On day 42, patch closure of the VSD and ASD accompanied by PA plasty was performed. The aneurysmal wall was thin, and bloody pericardial effusion was accumulated around it. Congestive heart failure improved and further progression of aortic valve dilation and aneurysms of the aortic root and main PA ceased. However, he suffered from tension pneumothorax, treated by continuous chest tube drainage. He subsequently showed respiratory failure due to bronchomalacia, necessitating positive airway pressure by a ventilator. He had tracheostomy on day 140, and was discharged to home on day 200 with a home ventilatory care and gavage feeding. Enalapril, an angiotensin-converting enzyme (ACE) inhibitor, was started to prevent further progression of aortic valve dilation and aneurysms of the aortic root and main PA.

At age 1 year, his weight was 8,320 g (−1.3 SD), length 81.0 cm (+1.9 SD), and OFC 48.0 cm (+1.2 SD). He had dolichocephaly with frontal bossing, downslanting palpebral fissures, strabismus, and a retrognathia (Fig. 1D). He showed hyperextensibility and instability of the elbows, shoulders, and knees, which had not been seen in the neonatal period. He could lift his head and rolled over freely, and could express various emotions. He still needed positive airway pressure by a ventilator. An MR angiography showed tortuosity of bilateral internal carotid arteries and vertebral arteries (Fig. 2E). An enhanced 3D-CT excluded tortuosity of major branches of thoracic and abdominal aorta.

Isolation System (Kurabo Industries Ltd, Osaka, Japan), according to the manufacturer's protocol.

The coding and flanking regions of all exons of *FBN1* (65 exons), *TGFBR1* (9 exons), and *TGFBR2* (7 exons), as well as exons 22–36 of *FBN2*, were amplified by polymerase chain reaction (PCR). The nucleotide sequence of each set of primers was described previously [Matsukawa et al., 2001; Mizuguchi et al., 2004; Loeys et al., 2005]. After purification on ExoSAP-IT (GE Healthcare Life Sciences, Buckinghamshire, UK), the PCR products were directly sequenced using the ABI Big Dye terminator mix (Applied Biosystems, Foster City, CA) and forward or reverse primers used for amplification. Reactions were run on an ABI 3730 automated sequencing analyzer (Applied Biosystems).

A heterozygous T → A transition was identified at nucleotide position 1370 in exon 5 of *TGFBR2* (c.1370T>A; Fig. 3), which resulted in a methionine to lysine substitution at amino acid position 457 (p.Met457Lys). It was previously identified in a patient with LDS1B [Loeys et al., 2006]. Neither of the parents had the mutation. No mutation was detected in the other genes.

The patient we have described had skeletal features (flexion contractures of the fingers, talipes equinovarus, a cleft palate, and joint laxity), facial changes (long palpebral fissures and a retrognathia), developmental delay, and neonatal-onset progressive cardiovascular complications (dilation of the aortic valve and aneurysms of the aortic root and main PA) associated with a large left-to-right shunt via a VSD and an ASD. He had a heterozygous missense mutation of *TGFBR2* (c.1370T>A, p.Met457Lys), reported in a patient with LDS1B [Loeys et al., 2006] and was diagnosed as LDS1B, though hypertelorism was not apparent.

Generalized skeletal features and vascular fragility in this patient raised a possibility of having a Marfan-related connective tissue disorder in the neonatal period, resulting in early diagnosis of LDS1B through genetic analyses of *FBN1*, *FBN2*, *TGFBR1*, and *TGFBR2*. Information is limited on clinical manifestations in neonatal periods of patients with LDS. In the original report by Loeys et al. [2005] and the first large series by Loeys et al. [2006], clinical characteristics that could call medical attention in neonatal periods included cleft palate, camptodactyly, talipes equinovarus, and PDA. Hypertelorism, bifid or broad uvula, craniosynostosis, malar hypoplasia, retrognathia, blue sclera, dolichostenomelia, arachnodactyly, pectus deformity, scoliosis, joint laxity, and velvety or translucent skin were also included in characteristic external findings of LDS [Loeys et al., 2005, 2006], but these features could be too subtle to be identified in a nursery for healthy neonates or might develop after neonatal periods. Aortic root aneurysms, aneurysms of other vessels, arterial tortuosity, ASD, and cervical spine instability were usually identified through appropriate screening examinations.

Additional neonatal findings of LDS have been collected: bilateral inguinal herniae were noted in a patient with a *TGFBR1* mutation reported by Adès et al. [2006]; and prominent ears, asymmetric ptosis, micrognathia, glossoptosis with sucking and feeding difficulties, bilateral inguinal and femoral herniae, and a right hydrocele were observed in an unrelated patient with the same
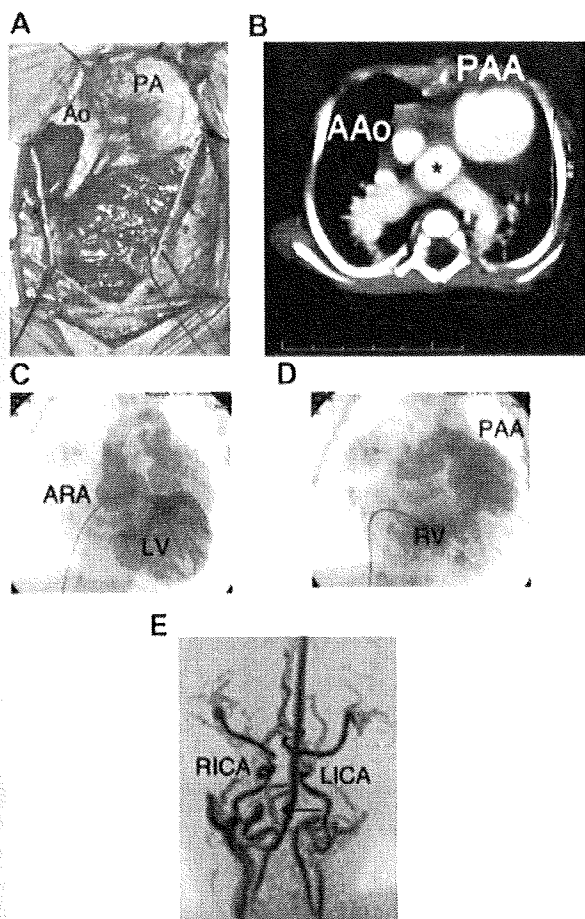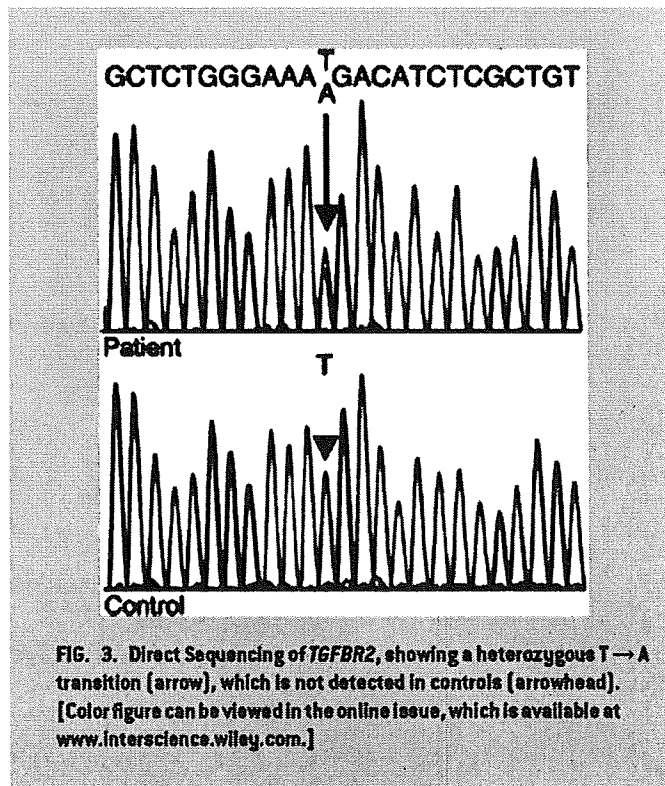

FIG. 2. A: An intraoperative view at pulmonary artery banding on day 12. B: An enhanced CT on day 34. AAo, ascending aorta; PAA, pulmonary artery aneurysm; * denotes the main pulmonary artery distal to the aneurysm. C: A left ventriculography on day 41. ARA, aortic root aneurysm; LV, left ventricle. D: A right ventriculography on day 41. PAA, pulmonary artery aneurysm; RV, right ventricle. E: An MR angiography. Bilateral internal carotid arteries and vertebral arteries (arrow) are tortuous. RICA, right internal carotid artery; LICA, left internal carotid artery. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

When last seen by us at age 22 months, his weight was 9,431 g (−1.7 SD), length 90.5 cm (+2.2 SD), and OFC 48.5 cm (+0.4 SD). He could sit unsupported and played with blocks. He was sometimes independent of positive airway pressure. No progression of aortic valve dilation and aneurysms of the aortic root and main PA was observed.

After receipt of informed consent, blood samples were obtained from the patient and his parents. Genomic DNA was extracted from the peripheral blood leukocytes using an NA-3000 Nucleic Acid

TGFBR1 mutation in the same report. Dislocation of knee joints was noted in a patient with a *TGFBR2* mutation reported by Kosaki et al. [2006]. Viassolo et al. [2006] described a patient with a *TGFBR2* mutation who presented fetal aortic root dilation or an aortic aneurysm at 19 weeks of gestation. The lesion did not progress during pregnancy, and the ascending aorta dimension and appearance were normal after 32 weeks of gestation. The patient had sinus sacralis at birth in addition to cardinal features of LDS (cleft of the soft palate, a bifid uvula, and bilateral talipes equinovarus). Yetman et al. [2007] described five patients with LDS and *TGFBR2* mutations who presented for medical attention in the neonatal period. The patients were referred to a tertiary pediatric care center for evaluation of genetic syndromes and orthopedic problems, in the setting of diffuse hypotonia, macro-crania, musculoskeletal abnormalities with or without a cardiac murmur. Craniofacial features included hypertelorism in five, a high-arched palate in five, a bifid or broad uvula in five, macro-crania in four, strabismus in four, and blue sclerae in three. Musculoskeletal features of upper extremities included campto-dactyly in five, metacarpophalangeal dislocations in three, and radial-ulnar dislocation in one. Those of lower extremities included talipes equinovarus in five, metatarsus adductus in two, knee dislocations in two, hip dislocation in two, and calcaneal-cuboid dislocation in two. Inguinal herniae were observed in four, and umbilical hernia in one. Three had cardiac murmurs of PDA. All had dilated aortic roots (diagnosed within 1 month in three of them), and three had dilated pulmonary arteries. Thus, neonates who present with generalized hypotonia, cleft palate, inguinal herniae, and musculoskeletal features such as camptodactyly and talipes equinovarus should undergo genetic analyses of *TGFBR1* and *TGFBR2* as well as appropriate screening and intervention of cardiovascular complications, as proposed by Yetman et al. [2007].

To treat congestive heart failure due to an increased left-to-right shunt via a large VSD and an ASD in this patient, we did not choose intracardiac repair but PA banding, considering possible connec-tive tissue fragility, which might lead to surgery-related rupture of cardiovascular system. Our choice resulted in deterioration of these lesions. Infants having a VSD with a large left-to-right shunt and significant congestive heart failure, spontaneous closure of which is unlikely, are candidates for early surgical closure, regardless of the patient's size; and if medical therapy (such as diuretics, digoxin, and ACE inhibitors) fails, surgical repair should be undertaken prompt-ly and can be done safely even in neonates [Redmond and Lodge, 2006]. Recently, Watanabe et al. [2008] described a milder case having a VSD, bicuspid aortic valve, and progressive dilation of the aortic root and main PA. The boy underwent a surgical correction of the VSD at age 50 days, which resulted in mild dilation of ascending aorta on MR angiography at age 5 years. Thus, cardiac defects with a large left-to-right shunt, accompanied by aortic root and/or PA dilation, in LDS patients might have to be repaired surgically in a standard manner in neonatal periods or early infancy, though such cases are supposed to be rare. In a large series by Loeys et al. [2006], patients with LDS type 1 had PDA in 35% (14/40) and ASD in 22% (9/40). Patients with PDA and a large left-to-right shunt should undergo surgical ligation, as described in a series by Yetman et al. [2007], and those with only ASD are unlikely to develop a large left-to-right shunt in early infancy.

FIG. 3. Direct Sequencing of *TGFBR2*, showing a heterozygous T → A transition (arrow), which is not detected in controls (arrowhead). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

In conclusion, we have described a patient with LDS1B, showing neonatal-onset progressive dilation of the aortic valve and aneur-ysms of the aortic root and PA associated with a large left-to-right shunt via a VSD and an ASD, in addition to typical skeletal features and mild facial dysmorphisms. Neonates with generalized hypoto-nia, a cleft palate, inguinal herniae, musculoskeletal features such as camptodactyly and talipes equinovarus, and/or a cardiac murmur should be suspected to have LDS; and cardiovascular screening and genetic testing of *TGFBR1* and *TGFBR2* are recommended. LDS patients with cardiac defects that lead to a large left-to-right shunt and congestive heart failure should be considered for intracardiac repair even in early infancy.

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ORIGINAL ARTICLE

Molecular Cardiology

Genetic Analysis of Young Adult Patients With Aortic Disease Not Fulfilling the Diagnostic Criteria for Marfan Syndrome

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Background: Although the existence of the young patients with aortic disease not fulfilling the diagnostic criteria for Marfan syndrome (MFS) has been known, the etiology of their disease has not yet been elucidated. The purpose of the present study was to elucidate the genetic and clinical features of the young patients with aortic disease not having MFS.

Methods and Results: Eighty young adult patients with aortic disease were examined. They were divided into a definite MFS (n=51) and a non-definite MFS group (n=29) according to the Ghent nosology. Clinical and genetic characteristics were compared between the 2 groups. Among 29 non-definite MFS probands, 1 (3%) *FBN1*, 2 (7%) *TGFBR1*, and 3 (10%) *TGFBR2* mutations were found, and 4 *ACTA2* mutations were found in the 23 probands examined without *FBN1*, *TGFBR1*, or *TGFBR2* mutations. In total, more than 10 out of 29 (34%) probands in the non-definite MFS group were associated with genetic mutations. Skeletal involvement was less frequent in the non-definite than in the definite MFS group (7% vs 82%, P<0.01).

Conclusions: In the probands with aortic diseases in young who cannot be diagnosed with MFS, mutations other than *FBN1* mutations accounted for at least one-third of all causes of aortic disease. (*Circ J* 2010; **74:** 990–997)

Key Words: *ACTA2*; Aortic disease; Marfan syndrome; *TGFBR1*; *TGFBR2*

Aortic dissection or annulo-aortic ectasia (AAE) often develops in young patients with Marfan syndrome (MFS), which is caused by mutations in the *FBN1*.[1] Recently, progress in genetic analysis has revealed genetic disorders other than *FBN1* mutations, such as mutations of *TGFBR1* or *TGFBR2*, *ACTA2*, *MYH11*, and *SLC2A10*, which also cause aortic disease in young patients.[2–7] It is often believed that the cause of aortic disease in young patients is MFS. However, these patients cannot always be diagnosed as MFS.

Although the existence of the young patients with aortic disease not fulfilling the diagnostic criteria for MFS has been known, the details of their disease have not yet been elucidated. The purpose of the present study was to elucidate the genetic and clinical features of young patients with aortic disease not fulfilling the diagnostic criteria for MFS.

Methods

Patients who were suspected of connective tissue disorders and who consented to undergo genetic analysis (n=129) were initially enrolled for the present study to investigate the characteristics of young patients with aortic disease, such as, aortic dissection, AAE and other forms of aortic aneurysm. Then, patients with the following characteristics were excluded: age <15 years (n=5), patients with relatives diagnosed with MFS (n=21), patients who did not have aortic disease (n=11), patients whose aortic dissection developed at age ≥50 years or whose aneurysms were found at age ≥50 years (n=9), and patients with aortitis that was regarded as having other etiologies (n=3). In total, 80 young adult patients (probands) with aortic disease who were suspected of connective tissue disorders were included in the present study.
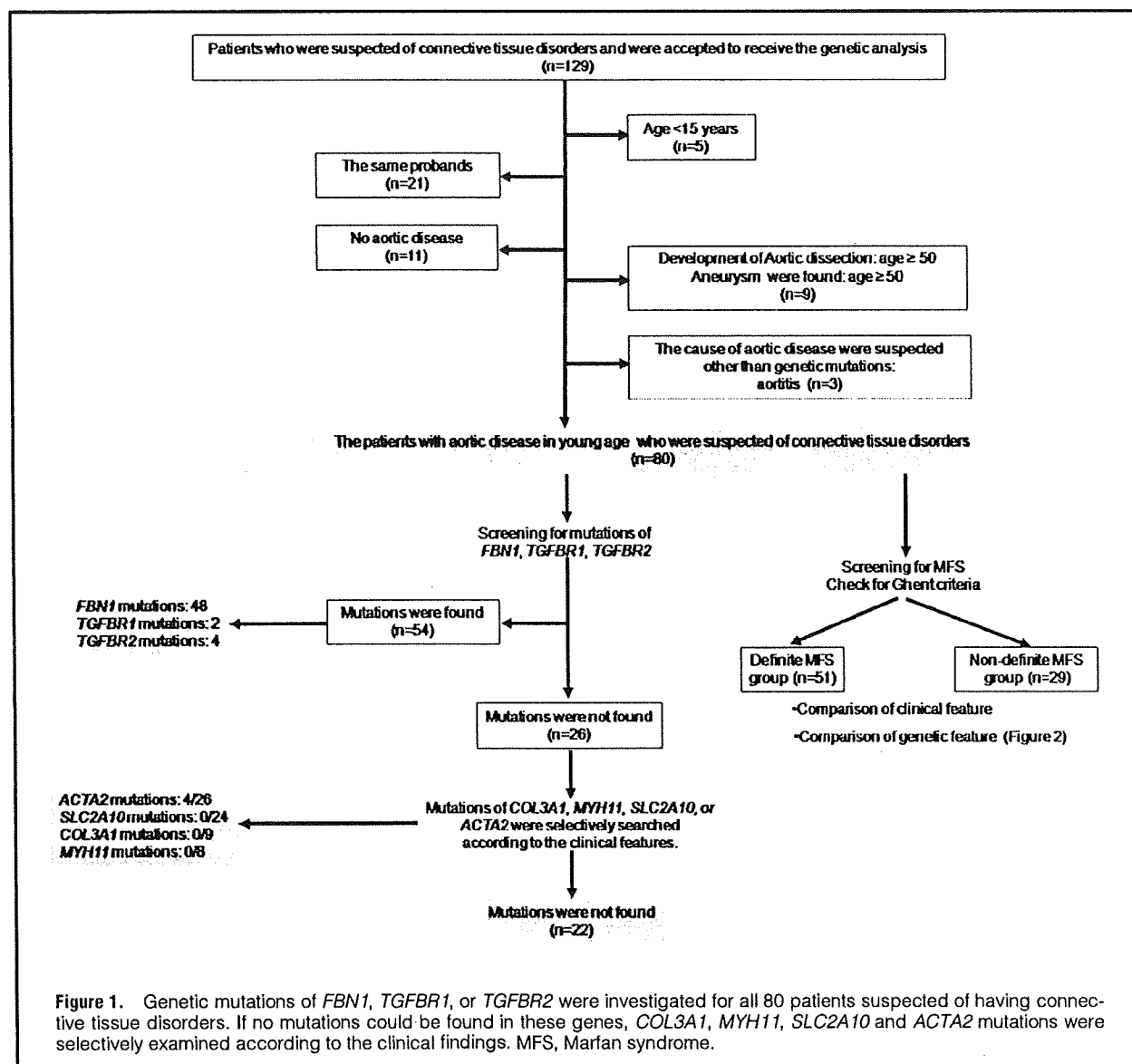
Figure 1. Genetic mutations of *FBN1*, *TGFBR1*, or *TGFBR2* were investigated for all 80 patients suspected of having connective tissue disorders. If no mutations could be found in these genes, *COL3A1*, *MYH11*, *SLC2A10* and *ACTA2* mutations were selectively examined according to the clinical findings. MFS, Marfan syndrome.

Genetic Features

The probands of all 80 probands were investigated for *FBN1*, *TGFBR1*, or *TGFBR2* mutations. If we could find no mutations in these genes, *COL3A1*, *MYH11*, *SLC2A10* and *ACTA2* mutations were selectively examined according to the clinical findings of each patient. A flow diagram of the investigations is shown in Figure 1.

FBN1, *TGFBR1*, *TGFBR2*, *ACTA2*, and *SLC2A10* mutations were examined using genomic DNA, which was isolated from the peripheral blood leukocytes of patients and amplified using polymerase chain reaction as described previously.[5] Genetic variants were screened with a denaturing high performance liquid chromatography method and the detected variations were further confirmed using direct sequencing as described previously.[1,2,5,8] *COL3A1* and *MYH11* mutations were examined using mRNA, which was obtained from surgical tissue specimens. Therefore, we could not determine the existence of *COL3A1* and *MYH11* mutations if the surgical specimen could not be obtained.

Clinical Features Related to the Ghent Nosology

In order to determine whether the patients fulfilled the diagnostic criteria of MFS using the Ghent nosology, all patients received careful assessments, including physical examination, computed tomography scanning or magnetic resonance imaging, echocardiography, and slit-lamp examination for ocular lesion, which covered all criteria listed in the Ghent nosology.[8] We defined the patients who fulfilled the Ghent nosology as the "definite MFS group", and the rest as "non-definite MFS group". According to the results of these examinations, all 80 probands were divided into definite MFS group (n=51) and non-definite MFS group (n=29).

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

First, clinical features were compared between the definite and non-definite MFS groups with respect to: (1) age, gender, height; (2) family history of aortic dissection or sudden death at age <50 years, or family history of suspected MFS; (3)

The patients with aortic disease in young age who were suspected of connective tissue disorders
(n=80)

Screening for MFS
Check for Ghent criteria

Definite MFS group (n=51)   Non-definite MFS group (n=29)

Screening for mutations of FBN1, TGFBR1, TGFBR2

FBN1 mutations: 47   Mutations were found   Mutations were found   FBN1 mutations: 1
TGFBR1 mutations: 0   (n=48)   (n=6)   TGFBR1 mutations: 2
TGFBR2 mutations: 1   TGFBR2 mutations: 3

                            Mutations were not found   Mutations were not found
                                  (n=3)   (n=23)

ACTA2 mutations: 0/3   ACTA2 mutations: 4/23
SLC2A10 mutations: 0/3   Mutations of COL3A1, MYH11, SLC2A10, or ACTA2 were selectively searched   SLC2A10 mutations: 0/21
COL3A1 mutations: 0/3   according to the clinical features.   COL3A1 mutations: 0/0
MYH11 mutations: 0/2   MYH11 mutations: 0/6

                            Mutations were not found   Mutations were not found
                                  (n=3)   (n=19)

Figure 2. Genetic mutations of FBN1, TGFBR1, or TGFBR2 were investigated for each of the 51 probands with definite MFS and 29 probands with non-definite MFS. If no mutations could be found in those genes, COL3A1, MYH11, SLC2A10, and ACTA2 mutations were selectively examined according to the clinical findings. MFS, Marfan syndrome.

Ghent nosology. Ghent nosology included: involvement of skeletal system such as arm-span-to-height ratio, thumb and wrist signs, and joint hypermobility; involvements of the cardiovascular system, such as AAE, aortic dissection or mitral valve prolapse; ectopia lentis; dural ectasia; involvement of the pulmonary system, such as pneumothorax and apical blebs in the apex; and involvement of skin system, such as atrophic striae and recurrent hernia. Second, the genetic features were compared between the 2 groups (Figure 2).

Specific Clinical Features in the Non-Definite MFS Group

We divided the non-definite MFS group into 2 groups, the patients with some mutations in FBN1, TGFBR1 or TGFBR2, ACTA2, MYH11, SLC2A10, or COL3A1 (Mutation (+) group) and the patients without these mutations (Mutation (−) group). Then, we investigated specific clinical features in each group, which were the characteristics of connective tissue disorders other than MFS, such as Loeys-Dietz syndrome (LDS), Ehlers-Danlos syndrome type IV, arterial tortuosity syndrome (ATS), and thoracic aortic aneurysm and/or aortic dissection (TAAD). The following specific features of each disease were examined: hyperterolism; bifid uvula; aortic branch aneurysms; squint (which were often seen in LDS); easy bruising; thin and visible veins, (which were often seen in Ehelers-Danlos syndrome type IV); arterial tortuosity (which were often seen in LDS or ATS); livedo reticularis; iris flocculi (which were often seen in patients with ACTA2 mutation); and patent ductus arteriosus (LDS and patients with MYH11 mutations).[4,6,9–12] In addition to comparing the phenotypes of patients with non-definite MFS with the definite

MFS group, we examined how the patients in non-definite MFS group fulfilled each feature of Ghent criteria.

Ethical Considerations

The present study was conducted according to the articles of the Declaration of Helsinki regarding the participation of human subjects in clinical studies and was approved by the Ethics Committee of the National Cardiovascular Center (Suita, Japan). All patients gave written informed consent to participate in the present study.

Statistical Analysis

Continuous variables were expressed as mean±standard deviation (SD). The Student t-test was used to analyze significant differences in factors between the 2 groups. Differences in percentages between the 2 groups were evaluated using Fisher's exact test. SPSS (11.0) software (SPSS Inc, Chicago, IL, USA) was used for all statistical analyses. A P value<0.05 was considered statistically significant.
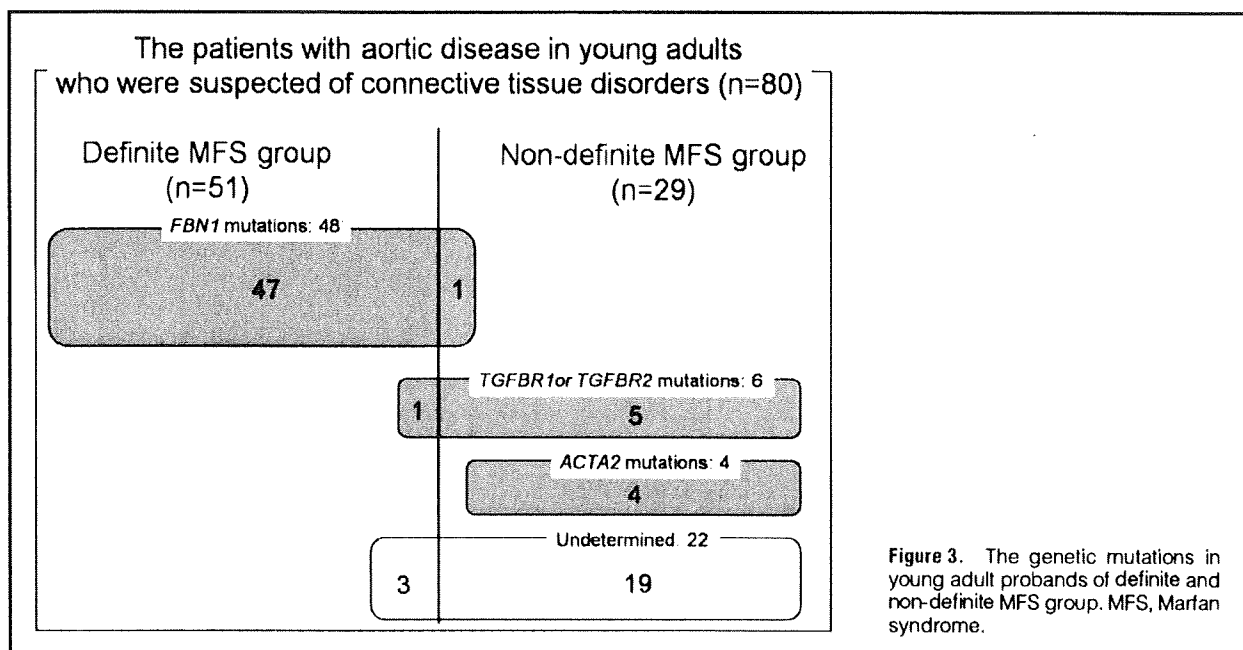
Results

Genetic Features of Patients

For all 80 probands, mutations of FBN1, TGFBR1, and TGFBR2 were investigated. Mutations of FBN1, TGFBR1, and TGFBR2 were found in 48 (60%), 2 (3%), and 4 (5%) of the probands, respectively. At the next step, COL3A1, MYH11, SLC2A10, and ACTA2 mutations were selectively examined according to the clinical features among the 26 probands who did not have any mutations in FBN1, TGFBR1,

Table 1. Genotypic Manifestations

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

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

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

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

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

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

Genotypic Manifestations Genotypic manifestations in each group are shown in Table 1. Among 51 probands in the definite MFS group, 47 (92%) *FBN1* mutations and 1 (2%)

TGFBR2 mutations were found. *ACTA2* and *SLC2A10* mutations were investigated in the remaining 3 probands in the definite MFS group and no mutations were found.

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

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

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

Table 2. Baseline Clinical Features

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

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

Table 3. Clinical Features Related to Ghent Nosology

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

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

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

Clinical features related to Ghent nosology are shown in Table 3. The following manifestations of Ghent nosology

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

Clinical Features in Non-Definite MFS Group
The specific clinical features of the patients in the non-defi-

Table 4. Clinical Feature of Non-Definite MFS Group

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

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

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

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

Discussion

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

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

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

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

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

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

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

Some limitations of the present study must be taken into account. First, *COL3A1*, *MYH11*, *SLC2A10*, and *ACTA2* mutations were not examined for all 80 probands. We studied these mutations only in a maximum of 26 probands without *FBN1*, *TGFBR1*, and *TGFBR2* mutations. We have identified simultaneous two-gene mutations of *FBN1* and *TGFBR2* in one proband, although such double mutations seem to be rather rare. Therefore, we suspect the incidence of *ACTA2* mutations may be close to 4 out of 80 in the present study. Although we could not determine the exact incidence of

the mutations, it is important to note that some patients with *ACTA2* mutations can be found in the patient population with aortic disease in young age. Second, we only showed the general characteristics in young patients with aortic disease without MFS. The presentation of the non-definite MFS could be heterogeneous. They may consist of various patients including patients with unknown genetic mutations, those with unknown acquired factors, etc. However, the present study showed that the non-definite MFS patients with aortic disease at a young age possess only a few obvious characteristic features, and it is difficult for us to discriminate them from normal individuals. Third, the method used to search genetic mutations in the present study might not capture all the causative mutations.

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

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