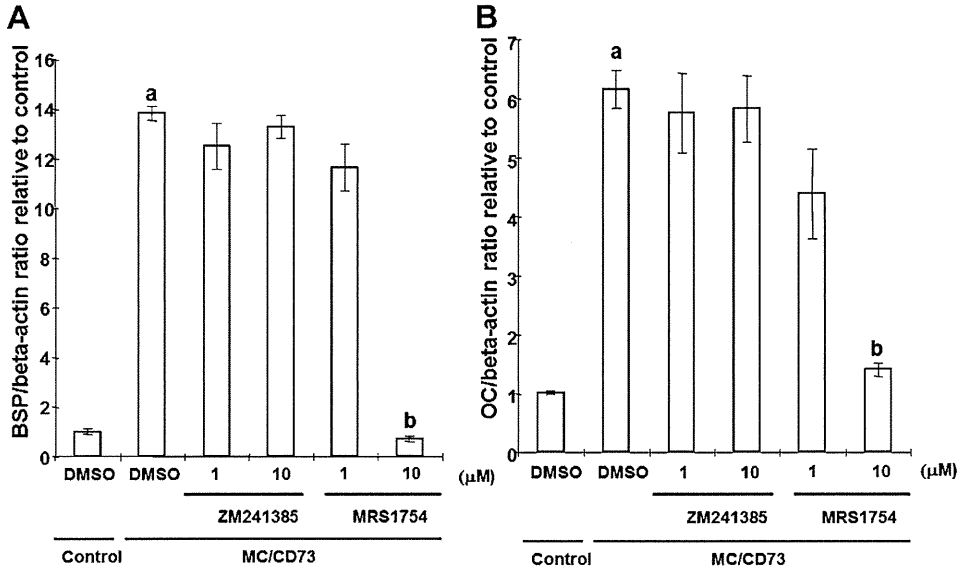

Fig. 6. Involvement of A2BAR signaling in osteoblast differentiation. Suppressive effect of an A_{2B}AR antagonist on (A) BSP and (B) OC mRNA expression in MC/CD73 cells. MC/CD73 cells were cultured for 3 days in α-MEM supplemented with 10% FBS in the presence of the indicated concentration of ZM241385 (A_{2A}AR antagonist) or MRS1754 (A_{2B}AR antagonist) or DMSO only. The DMSO concentration in each well was 0.1%. The expression of BSP and OC were determined by real-time RT-PCR. [a]$P < 0.05$ compared with DMSO-treated control transfectants. [b]$P < 0.05$ compared with DMSO-treated MC/CD73 cells. Representative results from more than three experiments are shown.

(trabecular vs. cortical regions) requirements for CD73. Additionally, whole mount staining with alcian blue and alizarin red of E18.5 fetal skeletions, revealed no significant abnormalities between *cd73*$^{-/-}$ and wild-type embryos (data not shown). These data reveal that CD73 is not required for embryonic bone patterning or initial bone formation but is most likely required for bone remodeling that occurs with age. In this study, we showed that CD73 deficiency resulted in osteopenia in male mice but not in female mice at 13 weeks of age. Mature male and female mice are known to show different bone status and remodeling rates. Thus, there may be an interaction between CD73-generated adenosine and one or more age-dependent factors such as sex hormones. Exploring this interaction will be a topic of future work.

In this study, a series of in vitro studies revealed that CD73 promoted osteoblast differentiation, consistent with earlier reports indicating that AR activation regulated proliferation and differentiation of osteoblasts in vitro (Shimegi, 1998; Costa et al., 2011). The relatively modest bone phenotype of *cd73*$^{-/-}$ mice may be due to redundant pathways of adenosine production such as via cytoplasmic nucleotidases or S-adenosyl homocysteine hydrolase. As it is possible that some of these pathways could be up regulated as a consequence of life-long CD73 deficiency, it would be interest to compare the bone phenotype in mice with conditional CD73 deficiency when they become available.

Unlike previous reports suggesting that adenosine supports osteoclast formation and bone resorption (Evans et al., 2006; Kara et al., 2010a,b), we found osteoclast markers were normal in *cd73*$^{-/-}$ mice in the steady state (Fig. 2A) and TRAP staining of tibia showed comparable osteoclast numbers in wild-type and *cd73*$^{-/-}$ mice (data not shown). However, CD73-generated adenosine may modulate osteoclast formation and function during inflammatory bone diseases such as rheumatoid arthritis and periodontitis, because inflammatory cytokines are capable of inducing CD73 expression (Kalsi et al., 2002; Niemelä et al., 2004) and adenosine is a well-known anti-inflammatory mediator (Haskó et al., 2008; Blackburn et al., 2009). Future studies utilizing *cd73*$^{-/-}$ mice in experimental bone disease models will give us more insight into the role of CD73 and endogenous adenosine in the pathogenesis of these diseases.

Elevation of A_{2A}AR and A_{2B}AR expression was observed during osteogenic differentiation (Fig. 5). These subtypes of AR are coupled with Gs proteins that can initiate signaling to stimulate bone formation (Sakamoto et al., 2005; Hsiao et al., 2008). Interestingly the positive role of CD73 on osteoblast differentiation in vitro was mediated by the A_{2B}AR but not the A_{2A}AR (Fig. 6A,B). Our experiments do not rule out the possibility that the A_{2A}AR functions in osteoblast differentiation in vivo independently of CD73; additional experiments with gene-targeted mice will be necessary to address this issue. Based on our data, we hypothesize that CD73-generated adenosine stimulates the A_{2B}AR but not the A_{2A}AR or that A_{2A}AR signaling is not coupled to osteogenic pathways. This idea is supported by reduced bone volume in A_{2B}AR deficient mice (data not shown) and previous studies that demonstrated a tight relationship between CD73 and the A_{2B}AR in endothelial and epithelial cell function (Strohmeier et al., 1997; Lennon et al., 1998; Eltzschig et al., 2003; Eckle et al., 2007; Takedachi et al., 2008). Although the mechanism by which CD73-generated adenosine activates the A_{2B}AR is not known yet, we speculate that the proximity between the A_{2B}AR and CD73 on microdomains of the plasma membrane may lead to efficient activation of the A_{2B}AR by CD73-generated adenosine.

In conclusion, we propose that endogenous adenosine generated by CD73 promotes osteoblast differentiation via A_{2B}AR signaling. The A_{2B}AR is a seven-transmembrane—spanning G protein—coupled receptor that is coupled to Gs and uses cAMP as a second messenger. It has been reported that cAMP promotes osteoblast function and the anabolic action of bone formation by enhancement of bone morphogenetic protein signaling (Nakao et al., 2009). Experiments are now

ongoing to further define the role of the $A_{2B}AR$ in osteoblast differentiation. Together with our findings in this study, such information may lead to the development of new anabolic therapeutic targets for bone diseases.

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歯周組織再生療法の最前線 ～FGF-2とテリパラチド～

竹立 匡秀*1) 村上 伸也*2)

歯周病の進行により失われた歯周組織を元通りに再生させることは，歯ひいては口の機能を維持・増進する上で重要である。我々は新規歯周組織再生療法として，歯周組織欠損部への塩基性線維芽細胞増殖因子(FGF-2)の局所投与による歯周組織再生誘導効果を報告した。一方，米国では，骨粗鬆症に対する骨形成促進薬である副甲状腺ホルモン遺伝子組み換え製剤を皮下投与することにより，歯周組織再生誘導効果を発揮することが報告されている。これら両療法は次世代を担う歯周組織再生療法として期待されている。

Calcium metabolism associated with oral diseases.

Present status of periodontal regeneration － FGF-2 and Teriparatide －.

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Periodontal regenerative therapy is required to regain the tooth function and oral health for patients with periodontal diseases. We reported that topical application of basic fibroblast growth factor (FGF-2) into alveolar bone defects stimulated significant periodontal regeneration in periodontitis patients. On the other hand, subcutaneous administration of Teriparatide, a drug composed of the first 34 amino acids of parathyroid hormone that has anabolic effects for osteoporosis, has been reported to induce periodontal regeneration in the clinical trial. Both of the therapies have been expected to be the periodontal regeneration therapy of the next generation. In this review, we introduce the present status of these new periodontal regenerative therapies.

はじめに

歯周組織再生療法とは，歯周炎によって失われた歯槽骨・セメント質・歯根膜を再生することを目的として行われる歯周外科処置である。1990年代から現在まで日常の臨床で用いられている歯周組織再生療法に加え，近年，塩基性線維芽細胞増

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殖因子（basic fibroblast growth factor：bFGF；FGF-2）の局所投与，さらには骨形成促進作用を有する骨粗鬆症治療薬であるヒト副甲状腺ホルモンの遺伝子組み換えタンパク質（テリパラチド）の投与による歯周組織再生誘導効果が明らかとなっている。

本稿では，これら次世代型歯周組織再生療法の現状について概説する。

歯周組織再生療法について

歯周炎に対する治療は，まずその原因となるデンタルプラークを歯根表面の壊死セメント質とともに機械的に除去し，加えて歯根周囲の炎症肉芽組織を掻爬除去することにより歯周組織の炎症消退を図る。しかしながら，その際におこる歯周組織の治癒形態は一般的に歯肉上皮の歯根側への下方増殖を伴い，歯が本来持ち合せる支持組織である歯槽骨やセメント質，歯根膜組織の修復・再生は限定的である。それでは，歯周組織を歯周病罹患前の状態に再生させることは可能なのであろうか。近年，歯根膜組織の中には，骨芽細胞やセメント芽細胞へ分化し得る間葉系幹細胞が成人になっても存在することが報告されており[1]，このような歯周組織幹細胞のポテンシャルを引き出すことにより，歯槽骨やセメント質，歯根膜組織の再生を誘導することが理論上可能であると考えられている。実際に，現在の臨床で用いられているGTR（Guided tissue regeneration）法やブタ由来のアメロジェニン[脚注]を主体としたエナメルマトリックスタンパクの局所投与などの歯周組織再生療法も，歯根膜組織中の幹細胞なしには効果を発揮することはなく，歯周組織幹細胞による再生誘導過程を一部活性化することにより歯周組織再生に一定の効果を発揮しているといえる。

しかしながら，現行の歯周組織再生療法では，①部分的な再生しか期待できない，②術式が困難，③適応症の限定，④十分な予知性に欠ける――などの克服すべき問題点が残されている。そこで，より効率よく，また容易な術式で，科学的根拠に裏付けられたメカニズムにより歯周炎罹患前の歯周組織に再生することができる治療法の開発が望まれている。

FGF-2による歯周組織再生

1．FGF-2とは

線維芽細胞増殖因子（FGF）は，線維芽細胞の増殖促進活性を有するタンパク質として，脳および下垂体組織から単離・同定された[2]。現在では22種類のサブタイプからなるファミリーを形成していることが判明している。なかでもFGF-2は，線維芽細胞のみならず血管内皮細胞，神経外胚葉系細胞，骨芽細胞，軟骨細胞，血管平滑筋細胞，上皮細胞などに対しても増殖活性を示すことが知られている。そしてその強力な血管新生促進作用と，未分化間葉系細胞の多分化能を保持させたままの状態でその増殖を促進する作用は，創傷治癒促進や組織再生誘導を目的とした治療薬としてこのサイトカインが注目されてきた一因である。

既に，FGF-2は褥瘡性潰瘍や熱傷潰瘍などの難治性皮膚潰瘍に対する治療薬（フィブラスト®スプレー）として製剤化されている。さらに近年，脛骨骨幹部新鮮骨折患者に対する患部への局所投与により骨治癒に要する日数が短縮されることが示され[3]，硬組織の治癒過程においても促進的な

bFGF：basic fibroblast growth factor（塩基性線維芽細胞増殖因子）
GTR：Guided tissue regeneration（歯周組織再生誘導法のこと。わが国では1990年代より臨床応用が始まった。2008年からは保険適用となっている。）
アメロジェニン：エナメル質が発生当初に多く含む疎水性蛋白質。amelogenin。エナメル芽細胞（ameloblast）から分泌される基質蛋白質でもある。

働きを有することも明らかとなった。

2．FGF-2の歯周組織再生誘導効果の検討

我々は，FGF-2を歯周炎により失われた歯槽骨欠損部に局所投与することにより，歯周組織の再生を人為的に誘導・促進するか否かを動物実験により検証した。ビーグル犬の下顎臼歯部複根歯に実験的2級根分岐部病変を作製し，炎症性肉芽組織を掻爬，除去した後にFGF-2を局所投与し，歯周組織再生効果を解析した[4]。その結果，FGF-2の局所投与により，統計学的に有意な新生骨量，新生セメント質量を伴った歯周組織再生が誘導されることが明らかとなった。このようなFGF-2による歯周組織再生効果は，ビーグル犬の歯槽骨に人工的に作製した垂直性骨欠損モデルや自然発症歯周炎における根分岐部病変，霊長類モデルとしてカニクイザルを用いた実験においても確認された[5][6]。また，いずれの実験においてもFGF-2投与群において上皮の下方増殖や骨性癒着，歯根吸収等の異常な治癒所見は観察されなかった。

動物実験により得られた研究成果を受け，2001年には全国13施設において臨床試験「KCB-1D (FGF-2) 歯周組織再生試験（第Ⅱ相）」が実施された[7]。合計79名の患者がエントリーされ，ハイドロキシプロピルセルロース製剤を基材として0.03%，0.1%，0.3%の各濃度FGF-2を歯周外科処置時に歯槽骨欠損部局所に添加し，その歯周組織再生効果が検討された。その結果，0.3% FGF-2の局所投与は基材のみを添加した群に比べ，約2倍の歯槽骨増加率を示した。次いで2005年から24施設247名の患者を対象として行われた並行群間比較用量反応試験では，

表1　垂直性骨欠損に対する0.3% FGF-2の新生骨形成率

「KCB-1D (FGF-2) 歯周組織再生試験（後期第Ⅱ相）」で得られたFGF-2の新生骨形成率。12週目から有意な骨形成がみられた。

	control	0.3% FGF-2
12週	4.65±13.93	15.89±23.96 *
24週	12.06±19.65	31.73±24.30 *
36週	15.11±21.90	50.58±31.46 *

値は平均値 ± 標準偏差
* $P < 0.05$ vs control

（文献8より改変）

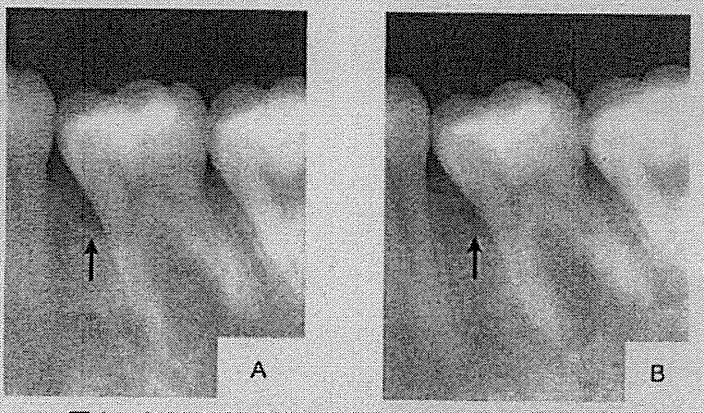

図1　0.3%FGF-2投与による歯周組織再生の一例

左側第一大臼歯近心における3壁性骨欠損に0.3% FGF-2を投与した一例。
投与前（A），投与36週後（B）のレントゲン写真を示す。
矢印部に変化のあることがわかる。

（文献9より改変）

0.3% FGF-2 でその効果が既に最大限に到達していることが統計学的に示された[8)9)]（表1，図1）。なお，両臨床治験において問題となる有害事象や副作用は治験期間を通じて認められなかった。

テリパラチドによる歯周組織再生

1．テリパラチドとは

テリパラチドはヒト副甲状腺ホルモン(PTH)の活性部分であるN端側34個のアミノ酸から構

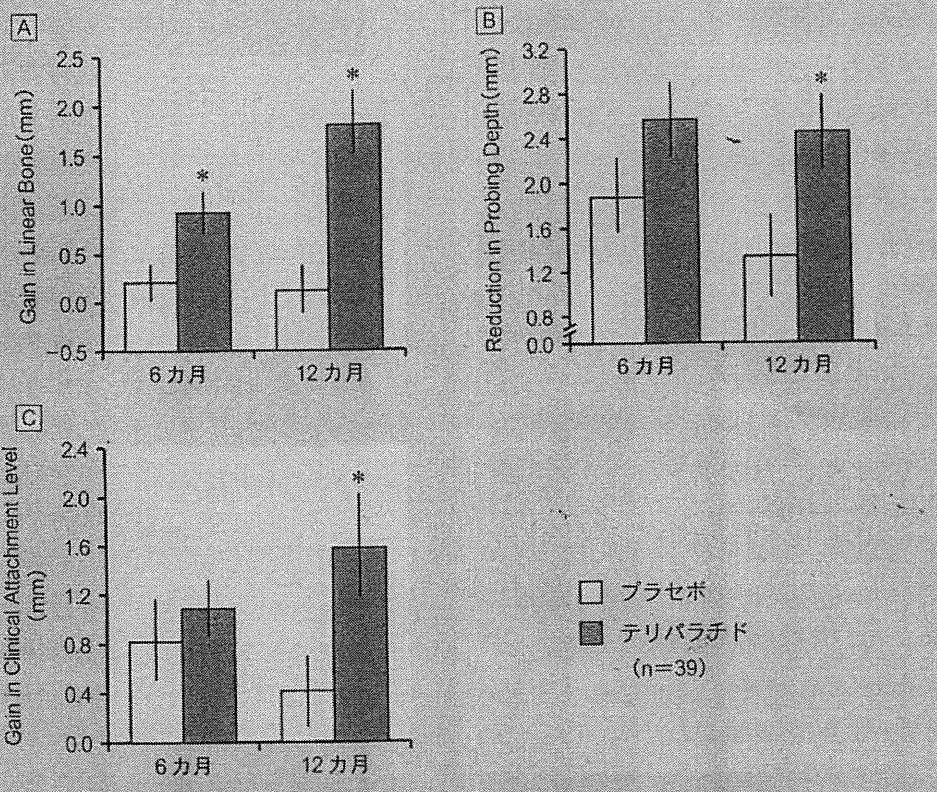

図2　テリパラチドによる歯周組織再生効果（術後6カ月，12カ月）

テリパラチドが歯周組織の再生を誘導するか否かをみた米国における臨床治験。39名の患者を対象として，歯周外科処置を行う3日前から6週間，1日1回のテリパラチド皮下投与（20μg，20名）またはプラセボの皮下投与（19名）を行い，その歯周組織に及ぼす影響が検討された。すべての患者においてテリパラチドあるいはプラセボ皮下投与期間中，カルシウム(1,000mg)，ビタミンD (800IU) の経口投与が行われた。

その結果，テリパラチド投与群ではコントロール群に比べ，統計学的に有意な歯槽骨新生と歯周ポケットの減少，臨床的新付着量の増加が認められた。

（A）レントゲン写真から解析した歯根面に沿った歯槽骨の回復量
（B）ポケット深さの減少量
（C）新付着量
＊ $P < 0.05$ vs プラセボ

（文献13より改変）

PTH：parathyroid hormone（ヒト副甲状腺ホルモン）
RANKL：receptor activator of nuclear factor κB ligand（破骨細胞分化因子。TNFαファミリーのひとつ。RANKはRANKLの受容体）

成された遺伝子組み換えタンパク質製剤である。テリパラチドを持続的に投与すると，重度の甲状腺機能亢進症と同様に，破骨細胞の分化を促進するRANKLの発現が増加し，骨吸収亢進により骨量が減少する。一方で，テリパラチドを1日1回20μg皮下投与するという方法で間歇的に投与すると，前駆細胞から骨芽細胞への分化が促進され，さらに骨芽細胞の細胞死が抑制されることにより骨形成が促進される。結果として骨量の増加，骨微細構造の改善を誘導し，骨粗鬆症患者の骨折のリスクを軽減する[10][11]。骨粗鬆症に対する薬剤の多くが，破骨細胞による骨吸収を抑制することにより効果を発揮しているのに対し，テリパラチドは唯一骨芽細胞による骨形成を促進する薬剤として製剤化された。2002年に米国にて骨粗鬆治療薬として承認され，2010年時点で日本を含む84カ国にて承認され，臨床応用されている。

2．テリパラチドの歯周組織再生誘導効果の検討

テリパラチドの皮下投与は，骨粗鬆症に対する効果だけでなく，骨折の治癒過程においても促進的な効果を発揮することが臨床治験の結果として報告されている[12]。このことは同薬剤の効果が局所的な骨の治癒にも積極的に作用することを示している。Bashutskiらは，このようなテリパラチドの効果が歯周組織の再生を誘導するか否かを臨床治験にて検証した[13]。2005年から2009年にかけて米国にて行われた同治験では，39名の患者を対象として，歯周外科処置を行う3日前から6週間，1日1回のテリパラチド皮下投与とカルシウム，ビタミンDの経口投与を行い，その歯周組織に及ぼす影響が検討された。コントロール群では，歯周外科処置とカルシウム，ビタミンDの経口投与のみが行われた。その結果，テリパラチド投与群ではコントロール群に比べ，統計学的に有意な歯槽骨新生と歯周ポケットの減少，臨床的新付着量の増加が認められた(図2)。テリパラチドによる歯周組織再生誘導は，これまで歯周外科部位に対する局所的なアプローチが主であった歯周組織再生療法とは一線を画すといえるが，1日1回の皮下投与が患者に強いる負担は少ないとはいえない。

おわりに

FGF-2，テリパラチドは共に臨床治験にて一定の効果が確認され，今後歯周組織再生療法として日常の臨床で用いられるようになることが期待されている。しかしながら，現時点では新規の治療法であり，その有効性と安全性を真摯に評価し，適正な適応症を見極めることで，次世代を担う歯周組織再生療法として正しく育成してもらいたいと期待してやまない。

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Brief Report

Prenatal complex congenital heart disease with Loeys–Dietz syndrome

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Abstract We report an infantile case of Loeys–Dietz syndrome prenatally diagnosed with congenital complex heart disease – double outlet right ventricle and interruption of the aortic arch. The patient also showed prominent dilatation of the main pulmonary artery. Emergency bilateral pulmonary artery banding was performed on the 9th day. However, on the 21st day, the patient died of massive bleeding due to rupture of the right pulmonary artery. Subsequently, a mutation of the TGFBR1 gene was detected. As cardiovascular lesions of Loeys–Dietz syndrome appear early and progress rapidly, the prognosis is generally poor. Patients require periodic examination and early intervention with medical therapy such as Losartan administration and surgical therapy. Early genetic screening is thought to be useful for the prediction of complications as well as vascular disease.

Keywords: Prenatal diagnosis; aneurysm; chromosomal anomaly; connective tissue disorder

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Loeys–Dietz syndrome is a newly recognised, rare autosomal dominantly inherited connective tissue disorder caused by heterogeneous mutations in the genes encoding the transforming growth factor beta receptor one or two.[1] This syndrome is characterised by the triad of arterial tortuosity, aneurysm or dissections, hypertelorism, and bifid uvula or cleft palate.[2] Here, we present a patient prenatally diagnosed with complex congenital heart disease and confirmed with Loeys–Dietz syndrome after birth.

Case report

A 31-year-old pregnant woman was referred to our paediatric cardiology unit at the 36th week of gestation because of foetal congenital heart disease and dilatation of the pulmonary artery.

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The first foetal echocardiography revealed a huge aneurysm of the main pulmonary artery and complex congenital heart disease – double-outlet right ventricle and interruption of the aortic arch (Fig 1). Detailed multi-planar scanning showed that there was no pulmonary valve stenosis, because of no acceleration in pulmonic flow, and no absent pulmonary valve. Therefore, we suspected a connective tissue disorder, such as Marfan syndrome. The foetus was followed up weekly for foetal decompensation and signs of hydrops until the 39th week of gestation, and an elective caesarean section was then performed. The male infant weighed 2834 grams at birth. After delivery, the infant developed dyspnoea and was intubated for artificial ventilation. Subsequently, a cleft of the soft palate and bifid uvula were noted. To treat the interruption of the aortic arch, we started him on a prostaglandin infusion to maintain patent ductus arteriosus and on nitrogen inhalation to prevent pulmonary blood flow increase. Computed tomography and angiocardiography confirmed the heart

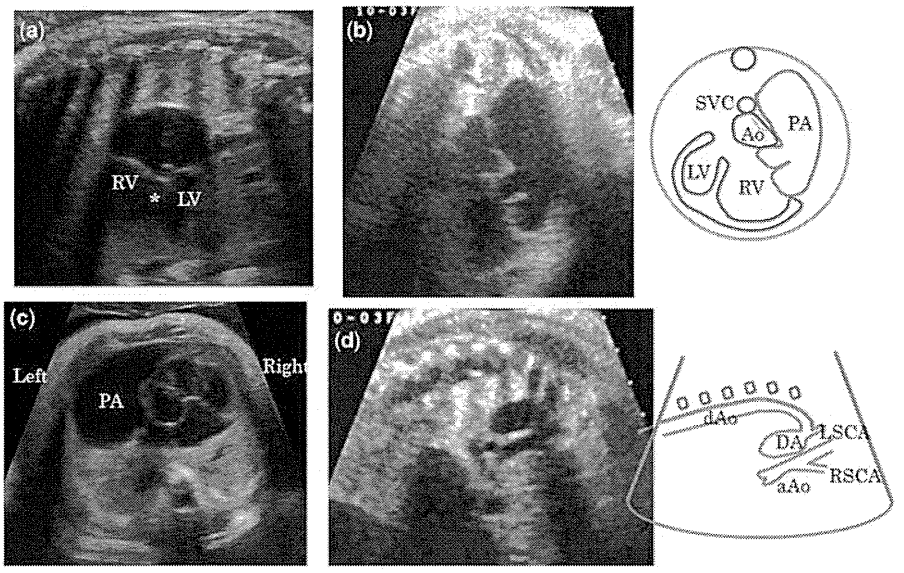

Figure 1.
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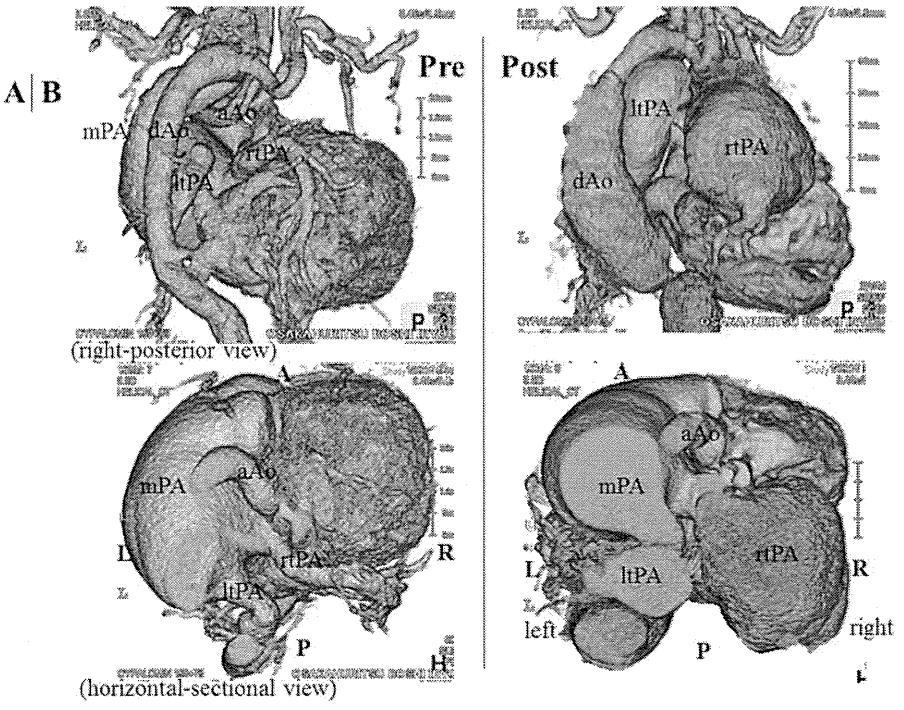

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Computed tomography (day 0) shows the interruption of the aortic arch and aneurysmal main pulmonary artery before operation (a). Computed tomography (day 18) shows progress of the significant expansion of the right and left pulmonary arteries and descending aorta after operation (b). A = anterior; aAo = ascending aorta; dAo = descending aorta; L = left; ltpa = left pulmonary artery; P = posterior; mPA = main pulmonary artery; rtPA = right pulmonary artery; R = right.

disease diagnosed prenatally (Fig 2a). Loeys–Dietz syndrome was strongly suspected because of the presence of cardiovascular lesions, thin skin, and facial appearance. On the 9th day, as the patient had suffered a pulmonary haemorrhage due to pulmonary blood flow increase, emergency bilateral

pulmonary artery banding was performed. However, during surgery, it became apparent that application of normal pulmonary artery banding was impossible because of the very thin condition of the pulmonary artery wall. Therefore, the surgeon performed bilateral banding with the clip, not the usual tape, but the banding was insufficient. This may be a reason why his haemodynamics and respiratory status were not subsequently stable. We again performed computed tomography, which showed a further significant expansion of the right pulmonary artery and descending aorta caused by the pressure of the expanded artery (Fig 2b). Therefore, we started internal use of Losartan. On the 21st day, he developed sudden hypotension and massive bleeding from the thoracic cavity, thought to be caused by right pulmonary rupture, and he died the same day. Subsequently, as the genetic analysis showed p.Thr200Pro (c.598A > C) mutation of the transforming growth factor beta receptor one, he was definitively diagnosed with Loeys–Dietz syndrome. The mutation was *de novo*.

Discussion

Loeys–Dietz syndrome is a recently described connective tissue disorder characterised by aggressive ascending aortic aneurysm and dissection. The clinical features are similar to Marfan syndrome,[3] but this is a more severe syndrome because life-threatening aortic dissection may occur even in early childhood.[4,5] Most patients have the triad of vascular aneurysms, hypertelorism, and bifid or broad uvula/cleft palate associated with variable features. Heterogeneous mutations in the genes encoding for transforming growth factor beta receptors one and two are a consistent finding among affected patients.

In addition, this syndrome shows various cardiovascular manifestations involving not only aortic lesions – such as distortion, aneurysm, and dissections – but also congenital heart diseases.[6] The case described in this report was also complicated with congenital heart disease. The patient's pulmonary artery showed an abnormal expansion because of his heart defect. That is, because he had an interruption of the aortic arch, much more blood than normal flowed through the pulmonary artery and the artery was stressed by "volume overload". Furthermore, the pulmonary artery was stressed by high "pressure overload" because the patient had double-outlet right ventricle and a large ventricular septal defect. It is thought that a pulmonary artery spread for both reasons from the foetal period.

Muramatsu et al[6] reported a case that was complicated with a ventricular septal defect and showed aortic and pulmonary expansion. It is thought that, in the Muramatsu case, the mechanism producing pulmonary artery dilatation was similar to that in the case reported herein. After birth, the patient's pulmonary blood flow increased due to the ventricular septal defect, which led to acute heart failure. He then underwent pulmonary artery banding on the 12th day. After surgery, however, the root of the main pulmonary artery, which was stressed by pressure, had spread in the shape of an aneurysm and intracardiac surgical repair, that is, closure of ventricular septal defect, was performed on the 42nd day. After the operation, the vascular expansion stopped worsening, and in conclusion they recommended early radical operation. However, because our case was a Fontan candidate, he required gradual surgery and radical operation was impossible in early infancy. Therefore, we performed bilateral pulmonary artery banding as a life-saving procedure, but, owing to mural abnormal thinning, the banding was insufficient, and his vascular expansion and thinning progressed, which finally led to explosion and bleeding to death.

In the case reported herein, significant pulmonary expansion from the foetal period led us to suspect a connective tissue disorder such as Marfan syndrome. Viassolo et al[7] reported a similar case in a female patient with Loeys–Dietz syndrome, who showed dilated aortic root from the foetal period. Only aortic dilatation was noted in screening foetal echocardiography at 19 gestational weeks and a connective tissue disease was suspected. She underwent genetic analysis and Loeys–Dietz syndrome was confirmed after birth. At present, the Viassolo case and the one we report herein are the only two cases showing a manifestation of Loeys–Dietz syndrome from the foetal period.

Some cases of Loeys–Dietz syndrome are complicated with congenital heart diseases.[2,6,8] However, those reported hitherto are associated with "simple" congenital heart diseases such as ventricular septal defect, atrial septal defect, patent ductus arteriosus, and aortic bicuspid valve. There is no previous report of Loeys–Dietz syndrome combined with complex congenital heart disease, such as double-outlet right ventricle and interruption of the aortic arch. In such a case, the cardiovascular lesion as an expansion of the great vessels, that is, the aorta or pulmonary artery, may be aggravated during the foetal period. Consequently, the foetus may die in utero. Even if they can be born, their great vessels are continuously or more strongly stressed after birth. Therefore, their arteries expand and finally explode, leading to an early death without undergoing any surgery.

This may be the reason why this is the first reported case of complex heart disease with Loeys–Dietz syndrome.

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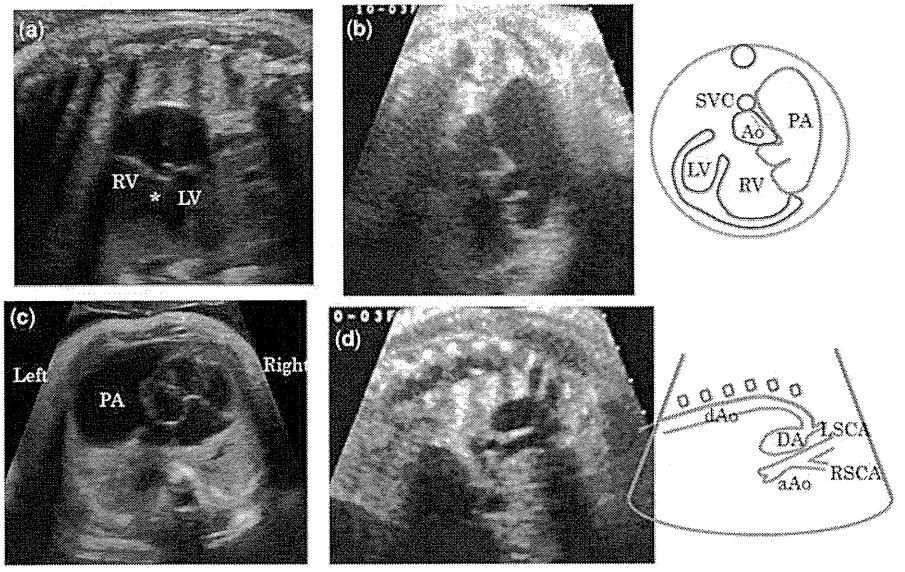

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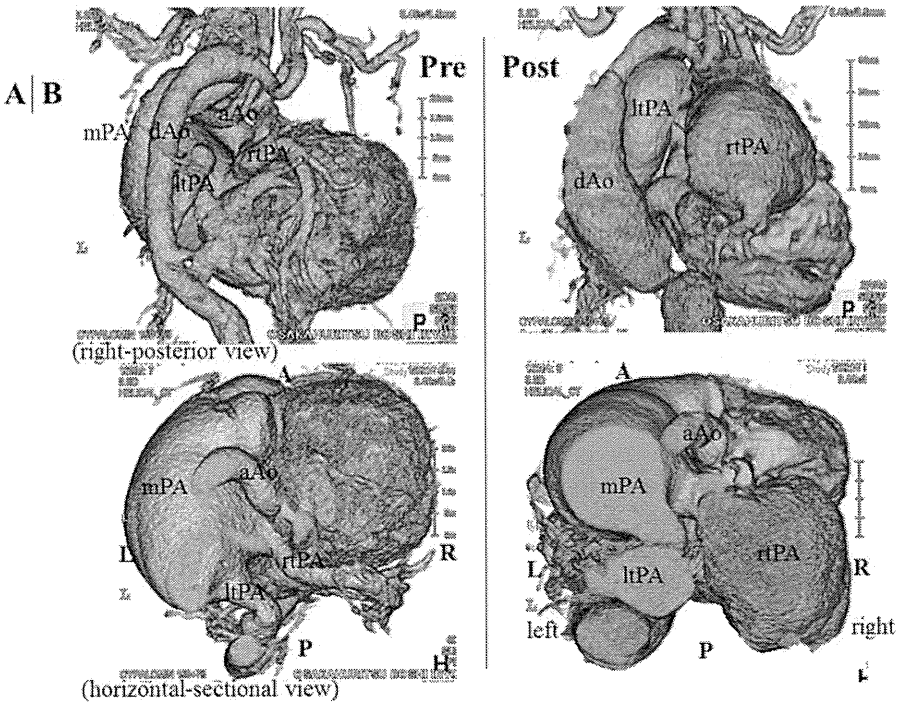

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Muramatsu et al[6] reported a case that was complicated with a ventricular septal defect and showed aortic and pulmonary expansion. It is thought that, in the Muramatsu case, the mechanism producing pulmonary artery dilatation was similar to that in the case reported herein. After birth, the patient's pulmonary blood flow increased due to the ventricular septal defect, which led to acute heart failure. He then underwent pulmonary artery banding on the 12th day. After surgery, however, the root of the main pulmonary artery, which was stressed by pressure, had spread in the shape of an aneurysm and intracardiac surgical repair, that is, closure of ventricular septal defect, was performed on the 42nd day. After the operation, the vascular expansion stopped worsening, and in conclusion they recommended early radical operation. However, because our case was a Fontan candidate, he required gradual surgery and radical operation was impossible in early infancy. Therefore, we performed bilateral pulmonary artery banding as a life-saving procedure, but, owing to mural abnormal thinning, the banding was insufficient, and his vascular expansion and thinning progressed, which finally led to explosion and bleeding to death.

In the case reported herein, significant pulmonary expansion from the foetal period led us to suspect a connective tissue disorder such as Marfan syndrome. Viassolo et al[7] reported a similar case in a female patient with Loeys–Dietz syndrome, who showed dilated aortic root from the foetal period. Only aortic dilatation was noted in screening foetal echocardiography at 19 gestational weeks and a connective tissue disease was suspected. She underwent genetic analysis and Loeys–Dietz syndrome was confirmed after birth. At present, the Viassolo case and the one we report herein are the only two cases showing a manifestation of Loeys–Dietz syndrome from the foetal period.

Some cases of Loeys–Dietz syndrome are complicated with congenital heart diseases.[2,6,8] However, those reported hitherto are associated with "simple" congenital heart diseases such as ventricular septal defect, atrial septal defect, patent ductus arteriosus, and aortic bicuspid valve. There is no previous report of Loeys–Dietz syndrome combined with complex congenital heart disease, such as double-outlet right ventricle and interruption of the aortic arch. In such a case, the cardiovascular lesion as an expansion of the great vessels, that is, the aorta or pulmonary artery, may be aggravated during the foetal period. Consequently, the foetus may die in utero. Even if they can be born, their great vessels are continuously or more strongly stressed after birth. Therefore, their arteries expand and finally explode, leading to an early death without undergoing any surgery.

This may be the reason why this is the first reported case of complex heart disease with Loeys–Dietz syndrome.

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Review

新生児マルファン症候群と国内12症例のまとめ

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Key words:
neonatal Marfan syndrome, fibrillin, *FBN1*

Neonatal Marfan Syndrome and Review of 12 Cases in Japan

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Neonatal Marfan syndrome (nMFS) is the severest form of classical Marfan syndrome (cMFS). Although both nMFS and cMFS are caused by defects in fibrillin (mutation of the *FBN1* gene), and the skeletal anomalies are similar, nMFS patients show severe cardiopulmonary failure from birth or early infancy and a poor prognosis compared to cMFS patients. Effective treatment of nMFS has not been established and almost all patients died in early infancy. In Japan, only 12 cases of nMFS have been reported and their clinical courses and cardiopulmonary findings are similar to those of foreign cases. Because 2 cases with mitral valvuloplasty or mitral valve replacement were reported to be alive for more than 1 year after surgery among Japanese cases, intervention for mitral regurgitation may improve the prognosis. LA-PCR/MLPA methods enable us to diagnose nMFS with large deletion of *FBN1*.

要　旨

新生児マルファン症候群(neonatal Marfan syndrome：nMFS)は，一般的によく知られたマルファン症候群(classical Marfan syndrome：cMFS)と責任遺伝子である*FBN1*は共通で，クモ状指等の外表奇形も似通っているものの，出生直後ないし乳児期早期より重篤な心肺機能不全を呈し予後不良である．MFSの中でも最重症型に位置しており，臨床像は大きく異なる．*FBN1*遺伝子の中でも心血管系のホットスポットとも言うべきexon 23-32の欠失が特徴とされ，遺伝子診断が望ましい．外表奇形などから本疾患を類推することは比較的容易であるが，診断に至っても現在のところ有効な治療は報告されておらず，多くが乳児期早期に死亡している．希少な疾患であり，本邦においての報告症例はわずかに12例に留まる．海外報告と臨床像・検査所見は同様であったが，僧帽弁形成術ないし僧帽弁置換術を行った2例は生存しており，僧帽弁への介入が予後改善をもたらす可能性が示唆された．近年LA-PCR法・MLPA法などの導入で以前は解析困難であった遺伝子診断が可能となっており，今後の症例蓄積が望まれる．

はじめに

新生児マルファン症候群(nMFS)は，思春期～成人にかけて大動脈弁閉鎖不全・水晶体脱臼などが顕在化して診断される，いわゆるマルファン症候群(cMFS)と同じく，*FBN1*遺伝子の変異で生じる先天異常症候群である[1)]．新生児期から特徴的な外表奇形と重篤な心肺機能不全を呈し予後不良であり，cMFSに比較し重症である．2009年時点では本邦で12例，世界でも100例程度の報告のみの[2)]希少な疾患である．今回自検例を元に本疾患を概説し，また本邦での報告例を概評した．本邦の症例でも75%が死亡する予後不良の疾患であったが，僧帽弁への手術介入が行われた例では生存していた．遺伝子診断がなされていない症例が

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多く，今後は遺伝子診断を併用した正確な診断と症例の蓄積が必要であろうと思われる．

自検例の紹介[3]

生後0日の男児で，Fig. 1に示すような外表奇形と四肢関節の拘縮を有した．出生時より重篤な僧帽弁閉鎖不全，軽度の大動脈弁・肺動脈弁閉鎖不全，心拡大(LVDd 28.0 mm, 150% of normal)，大動脈洞・肺動脈洞の拡大を認め(Fig. 2)，自発呼吸が弱く生後すぐに人工換気を要した．しかし各種治療に抵抗性であり，僧帽弁以外の弁逆流も次第に増悪し日齢20には全心臓弁で重度の逆流を呈するに至った．また日齢11頃から両側の肺の気腫性病変が増悪し換気条件が悪化，呼吸が保てず日齢27に永眠した．ご両親の承諾と当院倫理委員会の承認を経て遺伝子解析を実施した．

MLPA(Multiplex ligation-dependent probe amplification)法での解析で*FBN1*遺伝子の片アレルでexon 28の欠失が認められた．またLA-PCR(Long and Accurate Polymerase Chain Reaction)法を用いた解析ではexon 26の下流164 bpからexon 29の上流18 bpまでが広範囲に欠失していた(Fig. 3)．*TGFBR1*・*TGFBR2*遺伝子・*FBN2*遺伝子には変異を認めず，広範な*FBN1*遺伝子の欠損により生じた新生児マルファン症候群との診断に至った．

新生児マルファン症候群の病態

nMFSの責任遺伝子は染色体15q21.1に存在する，弾性線維の構成成分であるfibrillinをコードする*FBN1*遺伝子である．63のexonを持つ遺伝子で，cb-EGFドメイン(calcium-binding-EGFドメイン)を多数持つ．nMFSの病態はcMFSと同様に*FBN1*遺伝子の欠損・障害により弾性線維中のmicrofibrilの主要構成成分であるfibrillinが十分な量・質が産生されず，弾性線維の形成に障害を来すと考えられている[4-9]．

nMFS患者由来の線維芽細胞はfibrillinでの染色が薄く，またmicrofibrilの突起が少なく異常な丸い形態を示す[7]．病理学的な検討によると，マクロでは心臓においては大動脈・肺動脈弁輪の拡大や動脈瘤形成を，

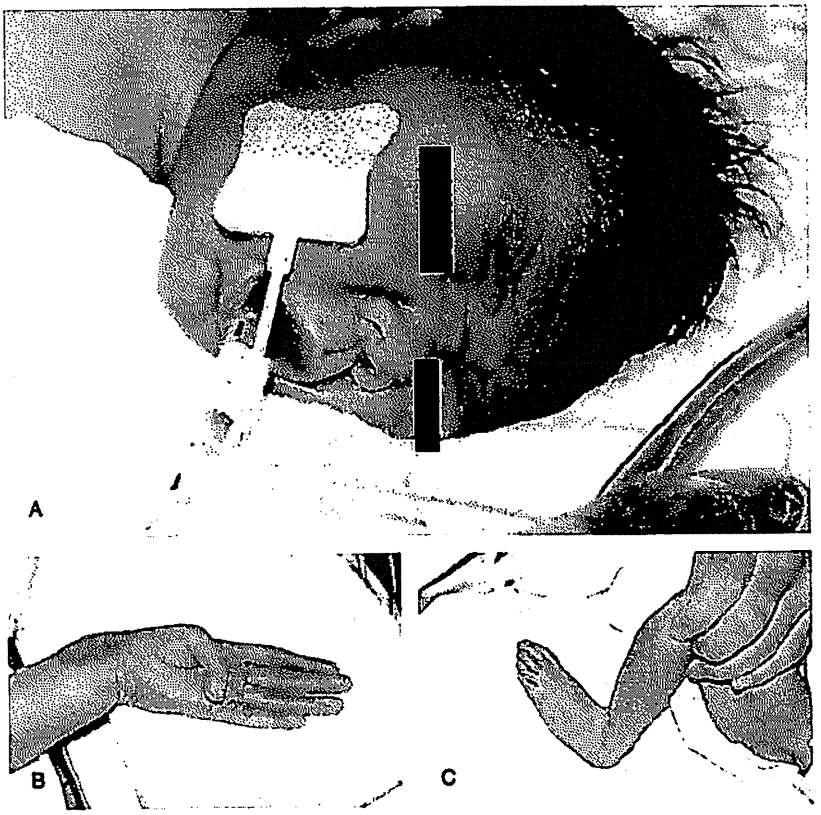

Fig. 1 General appearance
A: Senile appearance
B, C: Long extremities with arachynodactyly

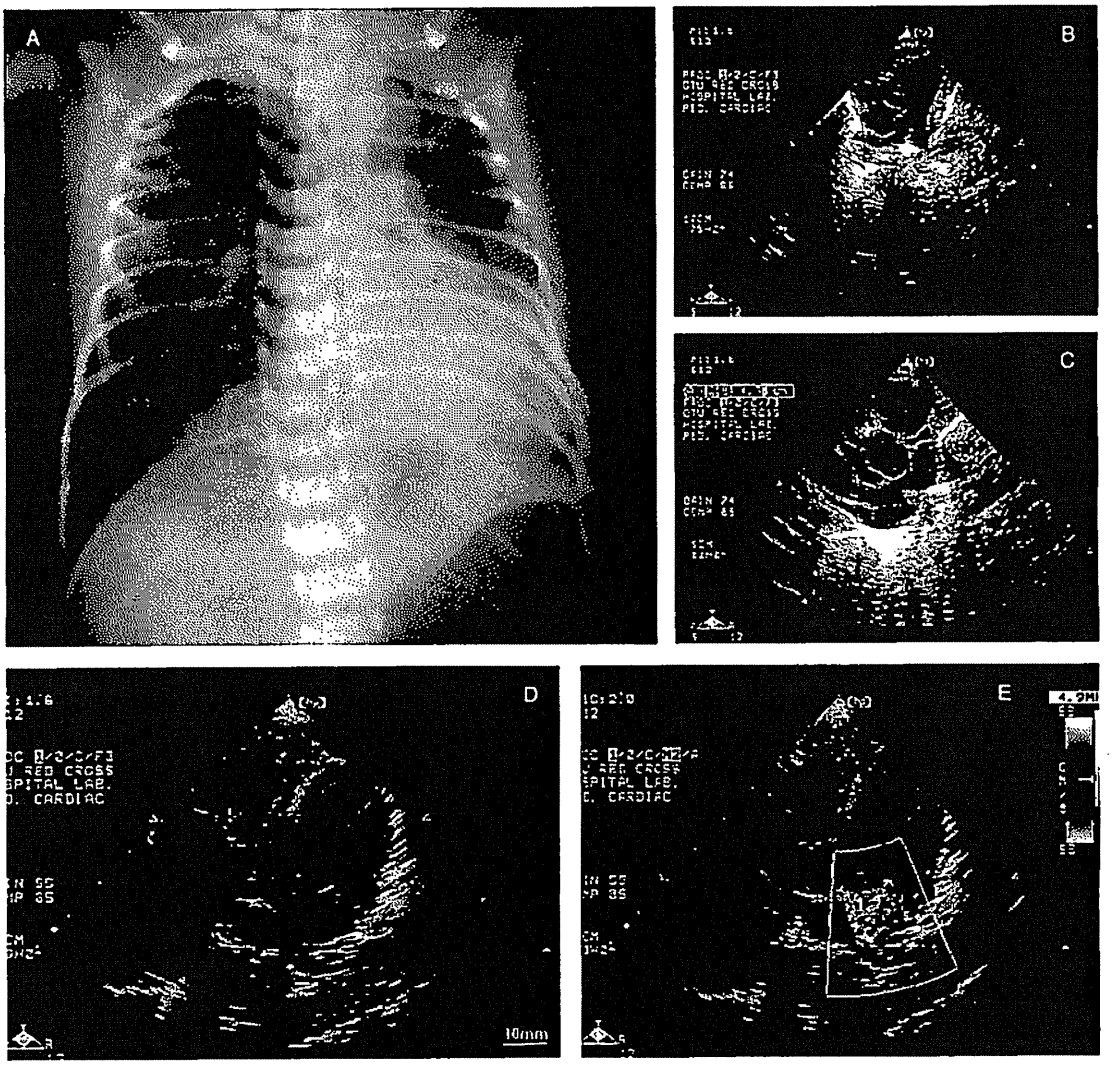

Fig. 2 Chest X-ray and echocardiographic images
A: Bilateral pulmonary emphysema on day 11
B: Dilated pulmonary root
C: Aortic root was dilated to 18.8mm in diameter with a clover leaf like shape
D: All four (atrial and ventricular) chambers were dilated and LA was compressed by the descending aorta from the backside
E: Severe mitral regurgitation flow toward the pulmonary veins

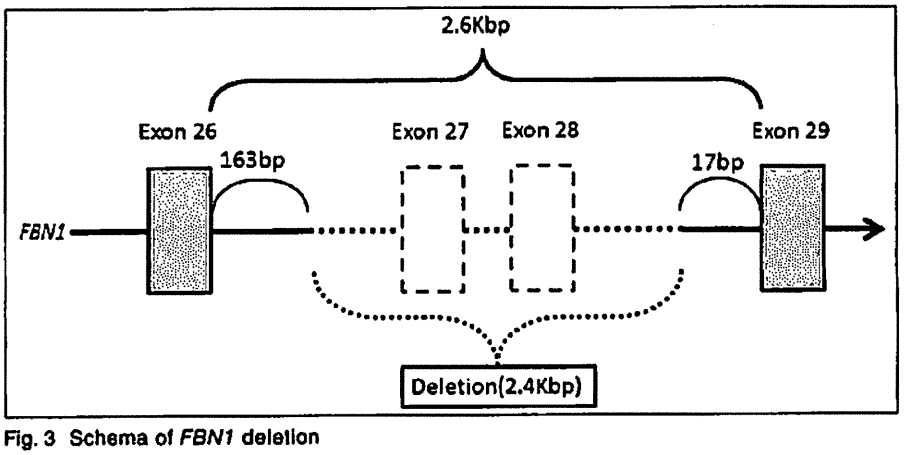

Fig. 3 Schema of *FBN1* deletion

肺においては肺尖部のブラや肺全体の嚢胞性の気腫を伴う．ミクロでは皮膚において疎で錯綜構造を呈する皮下結合組織を，心血管においては房室弁の粘液変性を認めるとされ，また大動脈・肺動脈においては中膜の嚢状変性や動脈壁解離と，弾性線維の断裂・錯綜配列などが報告されている．肺においては肺胞中隔が非薄かつ短縮し，特にブラ・ブレブの部分では細葉の破壊変形と拡大を認める[10-13]．線維芽細胞が弾性線維を産生できず，皮膚・血管・心臓弁・肺胞の脆弱性を生み出しているものと推測される．実際に fibrillin-1 ノックアウトマウスの肺病理組織では，加齢に伴い肺胞中隔が傷害を受け，破壊性気腫の形成が認められる[14]．また生後5日の剖検児の所見[12]では既に解離性大動脈瘤と大動脈弾性線維の離断が確認されており，血管壁が非常に脆弱であることが示唆される．

nMFS の遺伝形式

cMFS は約25%が孤発，75%が常染色体優性遺伝であるが，nMFS では国内の報告の全例，また海外報告の大半が孤発例である．しかし cMFS 患者を多数擁する家系での同族結婚での nMFS の兄弟例の報告[15]や，同胞が続けて nMFS に罹患する報告[16]（性腺細胞の mosaicism による）もあり，遺伝性が全くないというわけではない．

小児期の Marfan 症候群 320 例の調査において nMFS を 14%で認めた報告[17]もあるが，nMFS に限った一般的な疾患頻度の報告はなされておらず，不明である．

nMFS の国内外での臨床症状（Table 1）

nMFS 症例をまとめた報告では表のような所見を認めている．表のように，国内外での症例で大きな差はない．いずれも家族歴は乏しく生後すぐに診断されており，また1歳未満での死亡が多い[18-21]．

外表奇形ではクモ状指，扁平足，老人用顔貌，耳介変形，関節拘縮の頻度が高い．cMFS で特徴とされる水晶体偏位は3割程度で多くはない．心血管合併症では三尖弁・僧帽弁閉鎖不全及び大動脈弁輪拡大の頻度が高く，また肺気腫も多くの症例で見られているが，大動脈弁閉鎖不全は多くはない．

nMFS の診断・鑑別（Table 2）

nMFS の診断基準としては現時点では定まったものは存在せず，また neonatal / infantile Marfan syndrome など文献により呼称も分かれている[22]．新生児期から Marfan 症候群の外表奇形を有し，弁逆流を中心とした重篤な心肺機能不全を伴うもの，との判断が多い．MFS は診断基準である Ghent 基準が存在するが，以前は小児・新生児例への適応は困難であった[2]．2010年に改訂された Ghent 基準では，孤発例でも *FBN1* 遺伝子変異が重視され，加えて水晶体偏位と大動脈弁輪の拡張のみで診断が可能となっており，孤発例が大半の新生児でも適応しやすくなった[23]．

新生児・乳児期より nMFS と類似の外表奇形や心機能障害を示す疾患には Loeys-Dietz syndrome（LDS）[24,25]，先天性拘縮性クモ状指症候群（congenital contractural arachynodactyly：CCA，Beals 症候群）などが存在する．これらの疾患の中では nMFS が最も心肺合併症が重篤であること，家族性がないこと等が鑑別点ではあるが，責任遺伝子が明確に異なる．治療方針を決定し鑑別疾患を除外し，Ghent 基準に沿った診断を行う意味でも遺伝子検査は今後必須となろう．

nMFS の経過・予後

海外報告での平均寿命は 14 ～ 16.3 カ月[18]であり，生後数日での死亡例も散見される．内科的治療・管理に抵抗性の大動脈弁・僧帽弁などの左心系の弁機能不全の進行から1歳未満では心不全，それ以降では肺の気腫性病変の進行を死亡原因と挙げている報告が多く，2歳以上まで生存している例は稀である．海外を含め治療は僧帽弁に手術介入を行ったものや，ACE 阻害剤・ARB を投与した症例の報告はあるものの，散発的であり，まとまった治療成績の報告はない．これらの報告症例も最終的には死亡，病理解剖となったものなどの報告が大半である．

nMFS の遺伝子変異

cMFS の遺伝子変異の報告が *FBN1* 遺伝子のほぼ全体に分布しているのとは対照的に，nMFS の遺伝子変異は海外・国内報告の全例で *FBN1* 遺伝子の exon 23 ～ 32 の neonatal region と呼ばれる部分に集中しており，これが nMFS の遺伝子変異の大きな特徴と考えられている[4,5]．cMFS においても，一概には言えないものの，この領域の変異を持つ症例では心血管系の病変が重篤になりやすく予後不良が多いとの報告もされている[26,27]．

これまでの報告例では点突然変異，exon skipping な