ELECTRONIC LETTER

FBN2 mutation associated with manifestations of Marfan syndrome and congenital contractural arachnodactyly

P A Gupta, D D Wallis, T O Chin, H Northrup, V T Tran-Fadulu, J A Towbin, D M Milewicz

J Med Genet 2004;**41**:e56 (http://www.jmedgenet.com/cgi/content/full/41/5/e56). doi: 10.1136/jmg.2003.012880

Congenital contractural arachnodactyly (CCA; OMIM #121050) and Marfan syndrome (MFS; OMIM #154700]) are autosomal dominant disorders of connective tissue that are often difficult to differentiate clinically because of phenotypic similarities. Both syndromes have skeletal complications including arachnodactyly, dolichostenomelia, pectus deformities, and kyphoscoliosis.[1–5] Congenital contractures involving multiple joints and a crumpled appearance of the helix of the ear are more common in CCA than MFS. Ectopia lentis is a complication present in approximately half of patients with MFS. The most common cardiovascular complication in patients with MFS is progressive dilatation of the ascending aorta, initially involving the sinuses of Valsalva.[6] Although patients affected with CCA were initially felt not to have aortic involvement, three children under 6 years of age with CCA have had dilated aortic roots, further blurring the clinical distinction between the two syndromes.[5] The overlap in the clinical features has a molecular basis; CCA and MFS result from mutations in two homologous genes, *FBN2* and *FBN1*, respectively.[5 7–9] These genes encode the large, cysteine rich, extracellular matrix (ECM) glycoproteins, fibrillin-2 and fibrillin-1, which are major components of microfibrils. Although the mutations in *FBN1* causing MFS can occur over the length of the gene, *FBN2* mutations causing CCA cluster in a central region of the gene bracketed by exons 22 and 36.[10 11] Most mutations in both *FBN1* and *FBN2* are missense mutations that disrupt one of the numerous domains of the proteins with homology to epidermal growth factor. Exon splicing errors occur in both genes but are more common in *FBN2* than *FBN1*.[5]

We describe a family from Mexico with three members affected with CCA, in whom a genomic *FBN2* mutation causing missplicing of exon 32 was identified. Serial echocardiograms of the affected children demonstrated aortic dilatation at the level of the sinuses of Valsalva that persisted over 5 years as the children grew. In contrast, the affected mother had a normal echocardiogram. These results indicate that the aortic dilatation can persist over time, and emphasises the need for aortic imaging of patients with CCA. The affected children met the diagnostic criteria for MFS, further blurring the clinical distinction between the syndromes.[12]

Key points

- Patients with *FBN2* mutations causing congenital contractural arachnodactyly (CCA) are expected to present with scoliosis, arachnodactyly, contractures, and abnormal ears, whereas patients with *FBN1* mutations causing Marfan syndrome (MFS) have skeletal and ocular complications, and develop aortic aneurysms over time. Cardiovascular anomalies have not been considered to be a part of the disease course of CCA.
- We describe a family in which the *FBN2* mutation that the children were followed led to progressive dilatation of the aorta at the sinuses of Valsalva in two children. This is the first report of a progressive dilatation over 5 years in a CCA patient.
- We identified a mother and her two children who presented with skeletal features of MFS, and contractures, scoliosis, and a crumpled appearance to the helices of the ear, suggesting a diagnosis of CCA. The children also had aortic root dilatation on echocardiograms, which progressed over the 5 years that the children were followed. Intron based, exon specific primers were used to sequence *FBN2* to identify a mutation.
- A heterozygous G→A transversion at the +5 bp position in intron 32 of *FBN2* was identified, resulting in the mis-splicing of exon 32. The mutation was present in DNA from all three affected family members.
- Current clinical practice differentiates CCA from MFS based on the absence of the cardiovascular and ocular complications that characterise MFS. The findings presented here further blur the distinction between MFS and CCA and suggest that CCA patients should also be evaluated for aortic disease and screened regularly for cardiovascular complications.

MATERIALS AND METHODS

Dermal fibroblasts were explanted from skin biopsies, and genomic DNA was isolated from white blood cells, fibroblasts, and buccal cells. PCR with intron based, exon specific primers for *FBN2* to amplify exons 22−36 from genomic DNA were used as reported previously.[5] The fragments were sequenced in both the sense and antisense directions using an ABI Prism 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA). Total RNA was isolated from dermal fibroblasts using TRIzol reagents (Life Technologies, Grand Island, NY, USA). Two *FBN2* specific primers recognising the cDNA sequence between base pairs 4065 and 4372 were designed for reverse transcription PCR (RT-PCR). The amplified cDNA fragments were gel purified by the GFX PCR DNA and Gel Band Purification kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) and sequenced. Dermal fibroblasts were explanted, and metabolic labelling and analysis of fibrillin was performed as previously described.[13]

Abbreviations: CCA, congenital contractural arachnodactyly; ECM, extracellular matrix; MFS, Marfan syndrome; RT-PCR, reverse transcription PCR

Restriction digest analysis was performed using the *Apo*I enzyme (New England Biolabs, Beverly, MA, USA). The samples were PCR amplified and then quantified using Pico Green reagents (Molecular Probes, Eugene, OR, USA) and imaged on a Storm Imager (Molecular Dynamics, San Francisco, CA, USA). A 1% agarose gel (Sigma) was prepared, and 200 ng of each sample loaded and run on it.

Patients

The proband (patient #2560) was a 12 year old girl from Mexico who presented with bilateral crumpled ears, severe scoliosis, kyphosis, pectus carinatum, a highly arched palate, contractures of all digits, bilateral pes planus, and arachnodactyly with a positive thumb sign and a negative wrist sign. She was born full term without any prenatal complications. Contractures were noted at birth, and she was initially diagnosed with MFS at the age of 1 year. At 7 years of age she had an echocardiogram, which revealed a dilated aorta (sinuses of Valsalva 2.8 cm, BSA 0.9 m^2), and a small perimembranous ventricular septal defect (3.5–4.5 mm) with restrictive velocity. Repeat echocardiograms over the next 5 years indicated that her aorta at the sinuses remained markedly dilated (fig 1). Atenolol was prescribed but the patient did not take the medication. Ophthalmological evaluation was normal and she did not have any motor or language deficits.

The proband's brother (#2561) had a highly arched palate, pectus carinatum, bilateral contractures of the elbows and all the digits, arachnodactyly, scoliosis, lower abdominal striae, and bilateral pes planus. At the age of 10.6 years, his aortal root was dilated, measuring 2.95 cm (Z score 3.11, BSA 1.23 m^2), and remained enlarged as he grew over the next 4 years (fig 1). At the age of 14.5 years, his sinuses of Valsalva measured 3.48 cm with a BSA of 1.88 m^2. The proband's mother (#2562) was a 34 year old woman with marked crumpled ears bilaterally, a highly arched palate with crowding of the frontal teeth, arachnodactyly, bilateral contractures of all digits except the thumbs, slight contractures of the elbows, and bilateral flat feet. Her echocardiogram revealed an aortic root measuring 2.4 cm with a BSA of 1.67 m^2. The father was not available for evaluation. The proband's mother was the middle of eight children. Her parents and siblings were healthy and did not have contractures or skeletal abnormalities.

RESULTS

We screened exons 22−36 of *FBN2* for mutations by sequencing the amino acid coding regions and splice junctions using DNA from the proband (#2560), and a heterozygous G→A transversion was found at the +5 bp position in intron 32 (fig 2A). The mutation was present in DNA from all three affected family members, and the mutation was not present in 70 unrelated Hispanic chromosomes. RNA isolated from control fibroblasts and from fibroblasts explanted from the mother's cells (#2562) was used for RT-PCR of an *FBN2* cDNA fragment from base pairs 4065−4372. RT-PCR of the control fibroblast RNA demonstrated one band of the correct size, whereas the mother's cells demonstrated two bands, one 120 bps smaller than the normal (fig 2B). Sequencing of the smaller band confirmed the deletion of exon 32 (bp 4097−4220). Genomic DNA was obtained from the mother's fibroblasts, blood, and buccal cells to determine if the mother was a somatic mosaic for the *FBN2* mutation. The genomic *FBN2* mutation introduced a new restriction site, *Apo*I, which was used to confirm that the mutant and normal alleles were present in equal abundance in the mother's genomic DNA in all the tissues studied (fig 2C).

To determine if the mutated fibrillin-2 disrupted fibrillin incorporation into the matrix, pulse chase analysis of fibrillin synthesis, secretion, proteolytic processing, and matrix accumulation was performed using fibroblasts explanted from the mother. Analysis of cellular metabolism of fibrillin-1 indicated that the *FBN2* mutation present in the mother's fibroblast did not disrupt fibrillin-1 cellular metabolism, including fibrillin incorporation into the extracellular matrix (fig 3).

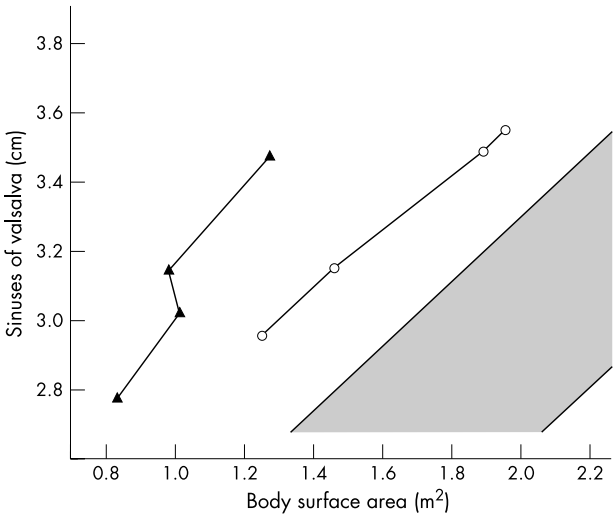

Figure 1 Aortic root measurements at the sinuses of Valsalva for 2560 and 2561. The shaded area shows the normal range for aortic measurements at the sinuses of Valsalva based on the chart published by Roman *et al*.[17] ▲ represents the proband (2560), ○ represents the proband's brother (2561). The graph incorporates results from serial echocardiograms taken over 6 years; the aortic dilatation has persisted over that time.

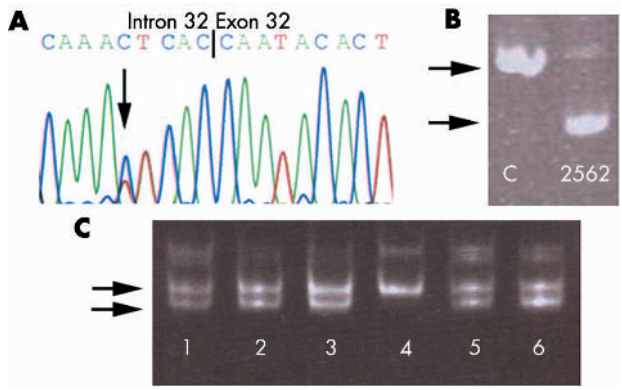

Figure 2 Genomic *FBN2* mutation in intron 32 results in mis-splicing of exon 32. (A) Partial antisense sequencing of the intron32−exon32 boundary using genomic DNA from patient 2562. The line indicates the intron−exon boundary. The mutation is a G→A transversion +5 bp into intron 32. (B) Reverse transcription and PCR amplification of an *FBN2* cDNA fragment from bp 4097−4220 using control fibroblast RNA (C) and RNA from the cells of patient 2562. The patient's RNA demonstrates two bands, the wild type fragment and a fragment that is 120 bp smaller. (C) The mutation inserts an *Apo*I site in the genomic DNA. Genomic DNA was isolated from blood, fibroblasts, and buccal cells and digested with *Apo*I for patient 2562 (lanes 1, 2, and 3, respectively) to assess if this patient's mild phenotype resulted from mosaicism. Genomic DNA from the proband (2560; lane 5) and her brother (2561; lane 6) was also digested with *Apo*I, along with control DNA (lane 4). The doublet bands in the gel indicate the presence of the mutation.

DISCUSSION

We identified an *FBN2* mutation in a family from Mexico with features of both CCA and MFS including crumpled appearance to the helix of the ear, contractures of multiple joints, dolichostonomelia, scoliosis, pectus carinatum, striae, and a highly arched palate. Both affected children had dilatation of their aortas at the level of the sinuses of Valsalva on initial echocardiographic evaluation, and the aortic root dilatation persisted over 5 years as the children grew. These echocardiographic findings support the conclusion that *FBN2* mutations can lead to aortic root dilatation in a subset of patients, and that the dilatation can persist for years. The outcome of aortic involvement in CCA has not been determined but it is interesting to note that the affected mother did not have an aortic root dilatation when we examined her at the age of 34 years. We studied three tissues from the mother (blood, skin, and buccal cells) and did not find evidence that she was mosaic for the *FBN2* mutation. It is possible that the mutation may lead to various levels of mutant and normal transcript from the mutant allele, which could affect the clinical phenotype, but this could not be tested because we were unable to obtain skin biopsies from the children. Finally, the lack of cardiovascular involvement in the mother raises the question on whether the aortic dilatation resolves rather than progresses and results in aortic dissection. Further studies are needed to verify the natural history of the aortic disease in patients with *FBN2* mutations. Although we were not able to determine conclusively if the children acquired different *FBN1* alleles from the parents by conducting studies using four *FBN1* markers, we believe that the likelihood of an independent mutation in *FBN1* is too low to merit sequencing *FBN1*. In addition, the normal fibrillin deposition in the matrix by the mother's cells suggests the lack of an *FBN1* mutation (fig 3). A reduction of fibrillin deposition in the matrix using dermal fibroblasts is a sensitive assay for the presence of a *FBN1* mutation.[14]

The affected children met the diagnostic criteria of MFS including the major criteria in both the skeletal and cardiovascular systems, and involvement in another system (integument, striae).[12] The children also had features that are found in MFS patients but are more common in CCA, including contractures of the fingers and a crumpled appearance to the helix of the ears. There have been two reports of CCA patients with aortic root dilatation and one report of a patient with mitral valve prolapse and a dilated aortic root.[5] In all three cases, the patients were diagnosed with CCA before 6 months of age. The patients in this report are the first to establish that the aortic root dilatation in CCA can persist and progress over many years, indicating that these patients need to have their aortas imaged routinely, similar to MFS patients.

The major cardiovascular abnormalities associated with *FBN1* mutations are aortic root dilatation progressing to aneurysm formation and aortic dissection, and mitral valve prolapse and regurgitation.[1 6] This report establishes that *FBN2* mutations can also lead to aortic root dilatation, but other cardiovascular abnormalities associated with *FBN2* mutations are less clearly established. None of the three affected members of this family had mitral valve prolapse or regurgitation, and only 2 of the previous 21 individuals with previously reported *FBN2* mutations had mitral valve prolapse, suggesting that this is not a common feature of CCA. It has been suggested in the literature that CCA is associated with congenital heart defects, but only 2/22 CCA patients with documented *FBN2* mutations have had congenital heart disease, including one of the affected patients in this report. Therefore, congenital heart disease is also not a common finding in these patients.

The expression pattern of fibrillin-1 and -2 has been extensively studied in mouse cardiac development development.[15] Fibrillin-1 and -2 expression is detected early in murine cardiovascular systems (10.5 days post-conception) when the heart is still a single tube undergoing active morphogenesis. Fibrillin-1 is expressed at a higher level than fibrillin-2, and both genes were expressed in the endocardial cushion tissue associated with the wall of the atrioventricular canal. Recent studies in human embryos suggest that both fibrillin-1 and -2 are expressed early in cardiac and aortic anlage, similar to findings in the mouse.[16] The expression of both genes in the developing human cardiovascular system is consistent with the findings of aortic involvement in both MFS and CCA.

Establishing that the children meet the diagnostic criteria for MFS but have an *FBN2* mutation raises some issues. Should the diagnosis be based on the mutation or clinical features? Should this be considered genetic heterogeneity for MFS? Does this case represent a failure of the diagnostic criteria for MFS? The conclusion drawn is that there is significant overlap in the clinical features in patients with mutations in *FBN1* and *FBN2*, blurring lines of distinction between MFS and CCA.

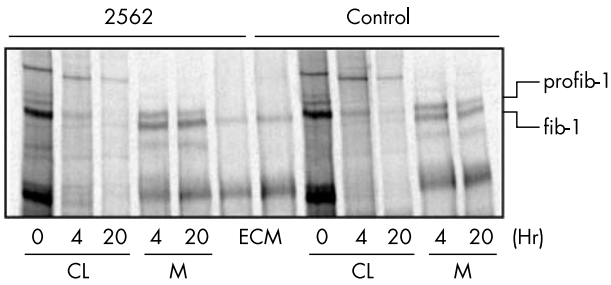

Figure 3 Pulse chase analysis of the synthesis, secretion, proteolytic processing, and extracellular matrix (ECM) incorporation of fibrillin by 2562 and an age matched control cell strain. Fibroblasts were metabolically labelled using ^{35}S-cysteine for 30 minutes (time 0), and then chased with cold cysteine for 4 and 20 hours. At 0, 4, and 20 hours, the cellular lysate (CL) was collected to follow the intracellular fate of profibrillin (profib). Both cell strains made similar amounts of profibrillin, and secreted the protein by 20 hours. The media (M) was collected at 4 and 20 hours, and shows that the secreted profibrillin was proteolytically processed into fibrillin, approximately 20 kDa smaller (fib-1). The ECM was scraped and pelleted, and showed that the amount of fibrillin in the ECM was similar for both the patient (2562) and the control. Samples were fractionated by 4% SDS-PAGE. Arrows indicate the migration of profibrillin/fibrillin.

ACKNOWLEDGEMENTS

We would like to acknowledge the assistance of M Moreno-Smith, L Xue, J Cretiou, and R Keasler. This work was supported by a March of Dimes Clinical Scientist Award (D M Milewicz), NIH HL62594 (D M Milewicz) and NIH NCRR funding of the University of Texas-Houston Medical School Clinical Research Center (M01-RR-02558). D M Milewicz is a Doris Duke Distinguished Clinical Scientist.

Authors' affiliations

P A Gupta, D D Wallis, T O Chin, V T Tran-Fadulu, D M Milewicz, Department of Internal Medicine, University of Texas Medical School at Houston, Houston, TX, USA
H Northrup, Department of Pediatrics, University of Texas Medical School at Houston, Houston, TX, USA
J A Towbin, Department of Pediatric Cardiology, Texas Children's Hospital and Baylor College of Medicine, Houston, TX, USA

Conflicts of interest: none declared.

Correspondence to: Dr D M Milewicz, University of Texas Medical School at Houston, 6431 Fannin, MSB 1.614, Houston, TX 77030, USA; dianna.m.milewicz@uth.tmc.edu

Received 30 September 2003
Accepted for publication 2 October 2003

REFERENCES

1 **Pyeritz R**. The Marfan syndrome. *Annu Rev Med* 2000;**51**:481–510.
2 **Ramos Arroyo M**, Weaver D, Beals R. Congenital contractural arachnodactyly. Report of four additional families and review of literature. *Clin Genet* 1985;**27**:570–81.
3 **Viljoen D**. Congenital contractural arachnodactyly (Beals syndrome). *J Med Genet* 1994;**31**:640–643.
4 **Epstein C**, Graham C, Hodgkin W, Hecht F, Motulsky A. Hereditary dysplasia of bone with kyphoscoliosis, contractures, and abnormally shaped ears. *J Pediatr* 1968;**73**:379–86.
5 **Gupta P**, Putnam E, Carmical S, Kaitila I, Steinmann B, Child A, Danesino C, Metcalfe K, Berry S, Chen E, Delorme C, Thong M, Ades L, Milewicz D. Ten novel FBN2 mutations in congenital contractural arachnodactyly: delineation of the molecular pathogenesis and clinical phenotype. *Hum Mutat* 2002;**19**:39–48.
6 **Roman M**, Rosen S, Kramer-Fox R, Devereux R. Prognostic significance of the pattern of aortic root dilation in the Marfan syndrome. *J Am Coll Cardiol* 1993;**22**:1470–6.
7 **Dietz H**, Cutting G, Pyeritz R, Maslen C, Sakai L, Corson G, Puffenberger E, Hamosh A, Nanthakumar E, Curristin S. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. *Nature* 1991;**352**:337–9.
8 **Lee B**, Godfrey M, Vitale E, Hori H, Mattei M, Sarfarazi M, Tsipouras P, Ramirez F, Hollister D. Linkage of Marfan syndrome and a phenotypically related disorder to two different fibrillin genes. *Nature* 1991;**352**:330–4.
9 **Putnam E**, Zhang H, Ramirez F, Milewicz D. Fibrillin-2 (FBN2) mutations result in the Marfan-like disorder, congenital contractural arachnodactyly. *Nat Genet* 1995;**11**:456–8.
10 **Putnam E**, Cho M, Zinn A, Towbin J, Byers P, Milewicz D. Delineation of the Marfan phenotype associated with mutations in exons 23-32 of the FBN1 gene. *Am J Med Genet* 1996;**62**:233–42.
11 **Nijbroek G**, Sood S, McIntosh I, Francomano C, Bull E, Pereira L, Ramirez F, Pyeritz R, Dietz H. Fifteen novel FBN1 mutations causing Marfan syndrome detected by heteroduplex analysis of genomic amplicons. *Am J Hum Genet* 1995;**57**:8–21.
12 **De Paepe A**, Devereux R, Dietz H, Hennekam R, Pyeritz R. Revised diagnostic criteria for the Marfan syndrome. *Am J Med Genet* 1996;**62**:417–26.
13 **Wallis D**, Tan F, Kielty C, Kimball M, Arnett F, Milewicz D. Abnormalities in fibrillin 1-containing microfibrils in dermal fibroblast cultures from patients with systemic sclerosis (scleroderma). *Arthritis Rheum* 2001;**44**:1855–64.
14 **Schrijver I**, Liu W, Brenn T, Furthmayr H, Francke U. Cysteine substitutions in epidermal growth factor-like domains of fibrillin-1: distinct effects on biochemical and clinical phenotypes. *Am J Hum Genet* 1999;**65**:1007–20.
15 **Zhang H**, Hu W, Ramirez F. Developmental expression of fibrillin genes suggests heterogeneity of extracellular matrix. *J Cell Biol* 1995;**129**:1165–76.
16 **Quondamatteo F**, Reinhardt D, Charbonneau N, Pophal G, Sakai L, Herken R. Fibrillin-1 and fibrillin-2 in human embryonic and early fetal development. *Matrix Biol* 2002;**21**:637–46.
17 **Roman M**, Devereux R, Kramer-Fox R, O'Loughlin J. Two-dimensional echocardiographic aortic root dimensions in normal children and adults. *Am J Cardiol* 1989;**64**:507–12.