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is in agreement with the consensus sequence for N-linked glycosylation Asn-Xaa-Thr/Ser.

Several lines of evidence indicate that this extra N-glycosylation site, created by the mutation, is utilized. A population of profibrillin-1 molecules migrating more slowly on SDS-PAGE than the control's sample was observed in the patient's sample. Immunohistochemical and ultrastructural analyses revealed that the microfibril formation was severely affected in the patient's fibroblast culture. In the presence of tunicamycin, an inhibitor of N-glycosylation, the patient's cell culture was capable of producing a better organized microfibril network. The creation of a neonatal cDNA construct consisting of exons 24–37 of the FBN1 gene was also proven to be a powerful tool in the analyses of the consequences of this mutation. The polypeptide translated from the minigene construct carrying the analogous I1048T mutation migrated more slowly on SDS-PAGE than the corresponding wild type polypeptide. Treatment with either tunicamycin, endoglycosidase H or N-glycosidase F abolished the migration difference indicating that the difference was originally related to the over-N-glycosylation of the mutant polypeptide.

We conclude that excessive N-glycosylation due to a newly formed N-glycosylation site represents an interesting novel pathogenic mechanism for Marfan syndrome and should stimulate further studies.

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NEONATAL MARFAN SYNDROME AND RESPIRATORY DISEASE

J. C. S. Dean¹, D. J. Lloyd², G. F. Cole³

¹ Department of Medical Genetics; ² Neonatal Unit;

³ Department of Medical Paediatrics, Aberdeen Royal Hospitals NHS Trust, Foresterhill, Aberdeen, Scotland, UK

Neonatal Marfan syndrome is a severe form of the disease usually associated with cardiac valvular regurgitation and aortic dilatation resulting in death in the first year of life. In addition to the usual skeletal and ocular features, flexion contractures, crumpled ears, redundant skin and a progeroid facial appearance are not uncommon. The disease may be associated with mutations in exons 23–32 of the fibrillin gene and with deficient decorin production.

We describe a patient with neonatal Marfan syndrome presenting as a newborn with arachnodactyly, joint laxity, abdominal wall laxity, sunken eyes giving a progeroid facial appearance and blue sclerae. Iridodonesis was noted at 2 months of age. There were initial feeding difficulties and later concerns about poor muscle tone. At 6 months of age, she developed a respiratory infection complicated by recurrent pneumothorax. Emphysematous bullae were seen on chest X-ray. The aortic root was dilated. She died from respiratory failure 7 days from the onset of symptoms.

In neonatal Marfan syndrome, attention is usually focused on the cardiovascular abnormalities which often lead to death. However, in one literature series, 7/22 patients had respiratory disease. Review of the Aberdeen Marfan Clinic revealed a point prevalence of Marfan syndrome in North East of Scotland (population 0.5 million) of 1/14 000, suggesting reasonably complete ascertainment. 10 patients were defined as "childhood" cases, being diagnosed at under 18 years of age, 2 of 3 sporadic cases in this group (including the subject of this report) suffered recurrent pneumothorax with emphysema but none of the 7 familial cases were so affected. 3/33 adult patients have suffered pneumothorax and a further adult has emphysema and pulmonary fibrosis without pneumothorax – none are sporadic cases. Serious lung disease such as pneumothorax and emphysema appears to be more common in sporadic childhood-diagnosed Marfan syndrome (including neonatal Marfan

syndrome), than in familial adult cases. This may reflect biased ascertainment of severe cases in the sporadic childhood group, but could also reflect altered interactions between certain mutated fibrillins and other matrix proteins affecting lung integrity. In view of the morbidity and potential mortality from lung disease in Marfan patients, this area warrants further study.

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IN VITRO EXPRESSION OF THE NEONATAL MARFAN MUTATIONS

T. Rantamäki¹, L. Karttunen¹, C. M. Kielty², L. Peltonen¹

¹ Department of Human Molecular Genetics, National Public Health Institute, Helsinki, Finland;

² School of Biological Sciences, University of Manchester, UK

Neonatal Marfan syndrome (nMFS) represents the most severe, neonatally lethal form of different Marfan syndrome (MFS) phenotypes. Several mutations in nMFS have been detected in the fibrillin-1 gene (FBN1). These mutations appear to have clustered in a distinct region of FBN1, exons 24–32. These exons code for a part of the longest stretch of consecutive EGF-like (epidermal growth factor like) motifs in fibrillin polypeptide.

We have constructed a FBN1 minigene to study the consequences of different nMFS mutations by *in vitro* expression. This construct contains exons 24–32 of FBN1 cDNA inserted into a SV-Poly expression vector together with a signal sequence derived from a lysosomal enzyme, aspartylglucosaminidase. Several nMFS as well as a couple of classical MFS mutations were introduced into this minigene using an *in vitro* mutagenesis kit. For transient transfection COS-1 cells are transfected with different minigenes, then pulse-labeled, and medium, cells and ECM are harvested at different time-points. Polypeptides are immunoprecipitated with a polyclonal antibody and then analyzed on SDS-PAGE and fluorography. So far, all the minigenes have been shown to be expressed and the polypeptides secreted into the medium. Some variation in the processing of different polypeptides is seen. In some cases the polypeptides have also been detected in ECM. We have also set up a stable cell line in CHO cells that expresses the wild type minigene. By rotary shadowing electron microscopy we could demonstrate that these cells produce fibrillin 'minifibers' that are seen as short linear fibrillar structures in cell layers.

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NEONATAL MARFAN SYNDROME: A CASE REPORT

S. de Knecht¹, J. L. Yntema², B. Hamel³, J. R. M. Cruysberg⁴

¹ Paediatric Heart Center and

² Paediatric Pulmonology, Dept. of Paediatrics;

³ Dept. of Human Genetics;

⁴ Dept. of Ophthalmology, University of Nijmegen, The Netherlands

Aim of the case report: The Marfan syndrome has a wide variability in expression. Symptoms in adults and older children are well known but may differ from the neonatal Marfan syndrome in which serious problems lead to early disability and death, especially cardiac valve insufficiency and pulmonary emphysema.

A full-term newborn girl of a mother with classical Marfan syndrome and a father with skeletal findings of Marfan syndrome, was admitted one day after birth because of a large diaphragmatic

hernia in the right thorax. She had the typical marfanoid habitus; mitral valve insufficiency (MI); mitral- and tricuspid valve prolapse and aortic diameter > P95.

Bilateral blepharophimosis of the eyelids was an unusual finding. After lateral canthotomy normal eyes were seen. The hernia was operated on. At the age of two months a second admission was necessary because of severe dyspnea, possibly due to severe MI. However, no signs of pulmonary hypertension at heart catheterization, no high pCO₂ level and no pulmonary engorgement on X-ray were found. On echo/Doppler cardiography a large left atrium with severe MI was seen. Afterload reduction did not change the clinical condition and at last mitral valve replacement by a St. Jude prosthesis was performed. At the time of surgery diffuse pulmonary emphysema was noted. A short period after surgery the patient's condition improved, but after a respiratory syncytial viral infection she died at the age of four months. Post mortem investigations confirmed severe heart disease and pulmonary emphysema.

Conclusions: be aware of fatal pulmonary emphysema in the neonatal Marfan syndrome.

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MICROFIBRILLAR PROTEINS: THE LONG AND THE SHORT OF IT

J. Rosenbloom

Department of Anatomy and Histology,
University of Pennsylvania School of Dental Medicine,
Philadelphia, PA 19104, USA

Microfibrils having a diameter of 10–12 nm are widely distributed in many tissues of the body, often but not always in association with elastin. Characterization of the microfibrils remains incomplete, but recent findings have suggested that the proteins composing the microfibrils can be grouped into two classes: (1) large ones > 150 kDa and (2) small ones < 50 kDa. The large class contains two closely related gene families, the fibrillins (FBNs) and latent TGF- β -binding proteins (LTBPs), which share structural domains, including epidermal growth factor and 8-cysteine motifs. Presently, two distinct FBNs and three LTBPs are known. Phylogenetic analysis suggests that these two gene families have evolved from a common ancestral gene. The small class of proteins is more heterogeneous and as a group they have been designated microfibril associated proteins (MFAPs). Sequence analysis has not revealed any homology among them. MFAP1 is a 439 amino acid, highly acidic protein whose human gene is located near the FBN1 locus, 15q15-q21. MFAP2, previously designated MAGP, is a 183 amino acid protein with a wide tissue distribution, whose human gene locus is 1p36.1-p35. MFAP3 is a 362 amino acid protein whose human gene is near the FBN2 locus, 5q21-q31. It is not known whether the linkage of these two MFAPs near FBN loci has any functional significance or is merely coincidental. Several other glycoproteins including emilin and a 36 kDa protein have been localized to the microfibrils. While it is likely that the FBNs provide the basic scaffolding of the microfibrils, the function of the other proteins is unclear. They may stabilize the microfibril structure, interact with other matrix components as has been demonstrated for MFAP2 and elastin and thereby act as nucleation sites in fiber formation, or serve as cytokine storage depots as has been suggested for the LTBPs.

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FBN2 MUTATIONS IN PATIENTS WITH CONGENITAL CONTRACTURAL ARACHNODACTYLY AND RELATED PHENOTYPES

D. M. Milewicz¹, E. S. Park¹, C. M. Aalfs², R. C. M. Hennekam²,
H. Zhang³, F. Ramirez³, E. A. Putnam¹

¹ Univ. of Texas Houston Medical School; ² Univ. of Amsterdam;
³ Mount Sinai School of Medicine New York, New York, USA

Congenital contractural arachnodactyly (CCA) or Beals syndrome is an autosomal dominant condition phenotypically related to the Marfan syndrome (MFS). We have recently established that CCA results from mutations in FBN2, a gene that encodes fibrillin-2 found in 10–12 nm microfibrils. The previously characterized mutations in unrelated patients were C1252Y in exon 29 and C1433S in exon 33. We have subsequently characterized novel FBN2 mutations in three unrelated CCA patients. We identified an exon 29 splicing error in two affected siblings with unaffected parents. The splicing defect is due to an A to G transition at the -15 position in the 3' splice site of intron 28. The affected siblings were heterozygous for the mutation. Analysis of the parents' DNA revealed that the father was a somatic mosaic with the mutation present in his hair bulb and buccal cells, but not in white blood cells; presumably, the father was also a germline mosaic. Another unrelated CCA patient was heterozygous for a mutation resulting in I1092T in exon 25. This mutation is not predicted to alter the secondary structure of the EGF-like domain encoded by exon 25, but does introduce a novel glycosylation site into the domain, suggesting a unique pathogenesis due to this particular mutation. A third putative mutation in exon 24 (G1056D) was identified in a CCA family. We have also identified a family with features of CCA, as well as a characteristic facies with hypertelorism, a broad forehead and flat facial profile. The phenotype segregates with FBN2 (LOD score > 3). These results indicate that FBN2 mutations producing CCA are private. The predicted effects of many of the FBN2 mutations on fibrillin-2 are similar to those of FBN1 mutations. All the currently characterized FBN2 mutations occur in a region of the gene equivalent to the location of FBN1 mutations that produce the severe, neonatal MFS phenotype. Finally, our data suggest that FBN2 mutations result in conditions related to CCA.

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ABERRANT SPLICING OF FIBRILLIN-2 IN A FAMILY WITH CONGENITAL CONTRACTURAL ARACHNODACTYLY

C. L. Maslen¹, D. Babcock¹, M. Raghunath², B. Steinmann³

¹ Oregon Health Sciences University, Portland, Oregon, USA;

² University of Münster, D-48149 Münster, Germany;

³ University Children's Hospital, CH-8032 Zürich, Switzerland

Congenital Contractural Arachnodactyly (CCA) is an autosomal dominant disorder that is phenotypically similar to, but genetically distinct from Marfan syndrome. Genetic linkage analysis implicated the fibrillin-2 gene (FBN2) as the CCA locus. Mutation analysis of single CCA patients indicate that defects in FBN2 may be responsible for that disorder. However, co-segregation of a mutant allele with the disease phenotype has not been established. We have investigated the primary cause of CCA in a large, well characterized kindred with four generations comprising 18 affected individuals. Previous studies showed linkage of the CCA phenotype