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 profilin-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 pathogenetic 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.

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.

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IN VITRO EXPRESSION OF THE NEONATAL MARFAN MUTATIONS

T. Rantamäki1, L. Karttunen1, C. M. Kiely2, 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 variants 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 'mini fibrils' 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...
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 significance or is merely coincidental. Several other glycoproteins including emilin and a 36 kDa protein have been localized to the 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 11092T to 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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