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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 11048T 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

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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.

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 minigene sequences. 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

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Aim of the case report: The Marfan syndrome has a wide variabil­
ity in expression. Symptoms in adults and old children are well known but may differ from the neonatal Marfan syndrome in which serious problems lead to early disability and death, espe­
cially 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
Microfibrillar proteins: the long and the short of it

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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 transforming growth factor (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 one 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 whose human gene is located near the FBN1 locus, 15q15-q21. MFAP3 is a 362 amino acid protein whose human gene is located 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 microfibrillar 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

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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 both parents carried the mutation in a somatic mosaic state. Analysis of the DNA from a third affected sibling revealed that the mutation occurred in a de novo manner.

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Aberrant Splicing of Fibrillin-2 in a Family with Congenital Contractural Arachnodactyly

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Congenital Contractural Arachnodactyly (CCA) is a congenital, 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...