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 pro-fibrillin-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 anachondroplasty, 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 1/14000, 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

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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, aspartyl glucosaminidase. 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 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
homology among them. MFAP1 is a 439 amino acid, highly acidic and as a group they have been designated microfibril associ­
tative proteins (MFAPs). Sequence analysis has not revealed any
suggestions that these two gene families have evolved from a com­
gruous for the mutation. Analysis of the parents’ DNA revealed that
hair bulb and buccal cells, but not in white blood cells; presum­
tings confirmed severe heart disease and pulmonary emphy­
Conclusions: be aware of fatal pulmonary emphysema in the

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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 incom­
plete, 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,
we introduce a novel glycosylation site into the domain, suggesting a
unique pathogenesis due to this particular mutation. A third puta­
tive mutation in exon 24 (G1056D) was identified in a CCA fam­
ting that occurs 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
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Congenital Contractural Arachnodactyly (CCA) is an autosomal dominant disorder that is phenotypically similar to, but genetically distinct from Marfan syndrome. Genetic linkage analysis implic­
ated 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 mu­
tant allele with the disease phenotype has not been established. We
have investigated the primary cause of CCA in a large, well char­
acterized kindred with four generations comprising 18 affected in­
dividuals. Previous studies showed linkage of the CCA phenotype