The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/22432

Please be advised that this information was generated on 2019-04-16 and may be subject to change.
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 II048T 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.

Grants. The National Marfan Foundation, US

NEONATAL MARFAN SYNDROME AND RESPIRATORY DISEASE

J. C. S. Dean1, D. J. Lloyd2, G. F. Cole
1 Department of Medical Genetics; 2 Neonatal Unit;
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, 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.

49 P

IN VITRO EXPRESSION OF THE NEONATAL MARFAN MUTATIONS

T. Rantamäki1, L. Karttunen1, C. M. Kielly2, 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 ‘mini-fibers’ that are seen as short linear fibrillar structures in cell layers.

Grants. The National Marfan Foundation, US

50 P

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
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 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.

Grant: NIH grants AR20553 & AR41474.