

SIALIDOSIS (MUCOLIPIDOSIS I)

Sialidosis, also known as mucopolipidosis I, is a neurosomatic lysosomal storage disease belonging to the group of the glycoproteinoses. The disease is inherited as an autosomal recessive trait and is caused by genetic lesions at the *NEU1* gene encoding the lysosomal sialidase, neuraminidase 1 or NEU1. This enzyme is essential for initiating the degradation of glycoproteins containing the sugar sialic acid (SIA) at the end of their glycan chains. Thus, defective or deficient activity of NEU1 leads to the buildup of sialylated glycoproteins in lysosomes and other subcellular sites. The excessive and progressive accumulation of non-digested or only partially digested SIA-containing metabolites in many cells of the body is pathognomonic of the disease, and at the basis of the disease pathogenesis.

Sialidosis affects primarily cells of the reticuloendothelial system, so tissues and organs of epithelial origin show the first and most acute signs of the disease.

The patients present with two distinct clinical conditions:

Sialidosis type I (normomorphous) is also referred to as "cherry red spot - myoclonus syndrome" because gradually reduced visual acuity and twitching of the muscles are often the only discernible symptoms at least initially. Normal intellectual capacity, late presentation and long survival are characteristics of these forms and explain why patients with type I sialidosis go undiagnosed for many years or are misdiagnosed. However, the occurrence of the aforementioned features combined with abnormal secretion of high molecular weight sialyloligosaccharides in urine and low NEU1 activity in white blood cells or fibroblast could help in making the diagnosis of most of these cases. Thus, this type of sialidosis patients might be more numerous than previously thought.

Sialidosis type II (dysmorphic) is the severe, neuropathic form of the disease. Three subtypes are recognized within this group, depending on the time of onset and severity of the symptoms: congenital or hydropic with onset in utero, infantile with onset between birth and 12 months of age, juvenile with onset past 2 years of age. The clinical presentation includes coarse face, enlargement of spleen and liver, vertebral deformity, dysostosis multiplex, neurological involvement.

In general, the severity and penetrance of specific phenotypic abnormalities correlate with the type and combination of *NEU1* mutations and the levels of residual enzyme activity. More than 40 *NEU1* disease-causing mutations have been identified in type I and type II sialidosis patients. They are mostly missense mutations leading to single amino acid substitutions.

(Seyrantepi et al 2003 <https://www.ncbi.nlm.nih.gov/pubmed/14517945>;

Caciotti et al 2009 <https://www.ncbi.nlm.nih.gov/pubmed/19568825>;

d'Azzo et al 2015 <http://www.ncbi.nlm.nih.gov/pubmed/26949572>;

Franceschetti et al 2016 <https://www.ncbi.nlm.nih.gov/pubmed/27621198>)

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Lysosomal NEU1 and its chaperone PPCA

NEU1 is ubiquitously but differentially expressed in different tissues and cell types. The enzyme is catalytically active and stable in lysosomes when in complex with two other enzymes: the protective protein/cathepsin A (PPCA) and β -galactosidase. NEU1 interaction with PPCA is essential for maintaining its activity; hence, PPCA functions as a canonical chaperone/auxiliary protein for NEU1. Extensive biochemical studies have been done on complex formation and mode of assembly of NEU1 and PPCA; although a full understanding of the way these proteins interact awaits the resolution of the 3 dimensional structure of the complex. Detailed information about these studies can be found in the following recent papers:

(Bonten et al 2009 <http://www.ncbi.nlm.nih.gov/pubmed/1966647>;

Bonten et al 2014 <http://www.ncbi.nlm.nih.gov/pubmed/24337808>)

Mouse models and the identification of pathogenic processes caused by Neu1 deficiency

A *Neu1* KO mouse was generated and characterized in early 2000. Total loss of Neu1 activity in these mice is compatible with life, but they develop a severe and progressive condition closely similar to type II sialidosis, and have a short lifespan (5-7 months) compared to wild type mice. KO mice present with hallmarks of the disease already at birth, including oligosacchariduria and extensive lysosomal vacuolation of cells in virtually all systemic organs and the nervous system. Epithelia, reticulo-endothelia and histiocytes/macrophages are the first cell types to get affected. The main pathologic features that recapitulate those seen in patients are growth retardation, time-dependent enlargement of the spleen, diffuse edema (buildup of fluid), spine abnormalities and neurodegeneration. The sialidosis mouse model has proven valuable to study the basis of disease pathogenesis downstream of Neu1 deficiency and consequent impaired processing or degradation of substrates that are target of the enzyme in vivo.

(de Geest et al 2002 <http://www.ncbi.nlm.nih.gov/pubmed/12023988>)

These studies have uncovered new functions of lysosomal NEU1 in basic physiological processes that go beyond its degradative capacity, and, when deregulated, contribute to the expression and severity of specific phenotypic alterations known to occur in sialidosis. For examples, we now can explain, at least in part, the mechanism, controlled by Neu1, that underlie progressive spleen enlargement, hearing loss, muscle atrophy and neurodegeneration in the Neu1 KO mouse and likely in patients. Some of the cellular and extracellular changes have been attributed to the capacity, and loss thereof, of NEU1 to keep in check the process called lysosomal exocytosis. The way NEU1 does it is through the processing of the SIAs on one of the enzyme' substrates, the lysosomal membrane protein LAMP1. When NEU1 is deficient, LAMP1 remains unprocessed and this causes an abnormal number of lysosomes to move to and dock at the membrane surrounding the cells and eventually to fuse with such membrane and discharge their contents extracellularly. This continuous, excessive discharge of potentially harmful products from the lysosomes eventually affects the balance between the intracellular and extracellular composite of molecules that maintain tissue

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and organ physiology. Detailed information about this research and its implication for sialidosis can be found in:

(Yogalingam et al 2008 <http://www.ncbi.nlm.nih.gov/pubmed/18606142>;

Zanoteli et al 2010 <http://www.ncbi.nlm.nih.gov/pubmed/20388541>;

Wu et al 2010 <http://www.ncbi.nlm.nih.gov/pubmed/19857571>;

Annunziata et al 2013 <http://www.ncbi.nlm.nih.gov/pubmed/24225533>)

These findings were also presented at several conferences during the last years including the ISMRD family conferences in July 2015 and November 2017.

Therapy for sialidosis

The nature and biochemical characteristics of NEU1 have hindered the development of suitable therapies for sialidosis. The enzyme by itself tends to aggregate, it is highly immunogenic and it is catalytically inactive unless bound to its auxiliary chaperone PPCA. Nonetheless, the strict dependence of NEU1 on PPCA for catalytic activation and stability may turn advantageous for therapeutic purposes. This is because NEU1 residual activity in fibroblasts from several type I patients could be increased by increasing the levels of available PPCA. This indirect therapeutic approach may be conceivable for patients carrying *NEU1* missense mutations that permit its interaction with PPCA. In this regard, both AAV-PPCA-mediated gene therapy and PPCA-ERT that hopefully will become available for the treatment of patients with galactosialidosis in the near future, could in principle be extended to the treatment of at least some patients with type I sialidosis. If this will become an optional therapy for these patients, its successful implementation and the identification of eligible patients will require the coordinated effort of expert clinicians, basic scientists, pharmaceutical companies, and most importantly family organizations like ISMRD and patient advocacy groups. (Hu et al 2012 <http://www.ncbi.nlm.nih.gov/pubmed/22008912> and Bonten et al 2013 <http://www.ncbi.nlm.nih.gov/pubmed/23770387>)

In conclusion, despite the rarity and complexity of sialidosis, remarkable progress has been made over the last decade towards the understanding of the underlying pathogenesis in this disease. If the bench work will continue at this pace, I am inclined to predict that additional new functions for NEU1 will be uncovered that might open new possibilities for alternative therapies. In this respect I believe that the most helpful hints on the roads to such discoveries often come from the sialidosis patients themselves and their families. Their insightful and courageous drive is a daily encouragement for us scientists to move ahead and keep looking forward.