

## **MUCOLIPIDOSIS II, II/III and III**

Mucopolipidosis II, II/III and III (more formally classified as MLII alpha/beta, MLII/III alpha/beta and MLIII alpha/beta) belong to a group of lysosomal storage disorders known as the glycoproteinoses because they impact the turnover of glycoproteins in the lysosome. There is also a very rare variant of ML III known as ML III gamma. These diseases are inherited and caused by genetic lesions in the GNPTAB and GNPTG genes that encode subunits of the GlcNAc-1-phosphotransferase enzyme. This enzyme is essential for initiating the formation of the mannose 6-phosphate recognition tag on soluble lysosomal hydrolases that directs these hydrolases to lysosomes. Defective or deficient activity of GlcNAc-1-phosphotransferase leads loss of these hydrolases from the lysosomes and the buildup of many different types of molecules, including glycoproteins, glycolipids and mucopolysaccharides. Importantly, the untagged hydrolases are secreted in abundance outside cells where they accumulate in the blood and in the extracellular space.

Mucopolipidosis II, II/III and III primarily affect cells within the skeletal system but many other tissues and organs including the heart and lungs can be impacted. There are notable differences between mucopolipidosis II alpha/beta and III alpha/beta, with regard to onset and severity. In general, the severity and penetrance of specific phenotypic abnormalities correlate with the type and combination of GNPTAB mutations and the levels of residual enzyme activity. More recently, analysis of the missense mutations found in ML patients has uncovered new insights into the manner in which these mutations alter GNPTAB function. MLII/III is often described as an intermediate form of ML as the mutations in the GNPTAB gene produce a phenotype that sits between the severe to attenuated forms of MLII and MLIII.

## **Understanding the maturation and function of the GlcNAc-1-phosphotransferase Enzyme**

Our understanding of how GlcNAc-1-phosphotransferase is made in the cell and the significance of conserved domains with this enzyme has greatly expanded over the last ten years. Cell-based studies have identified the key protease (site 1 protease) responsible for cleaving the GNPTAB gene product into active alpha and beta subunits and revealed how different domains and subunits of the phosphotransferase enzyme function to add mannose 6-phosphate residues to lysosomal enzymes. From this work we now understand which domains of the GNPTAB gene product are important for catalysis and which are needed to specifically recognize lysosomal hydrolases. Defining the function of these domains and how the subunits of the phosphotransferase enzyme interact with each other provides the opportunity to study how missense or point mutations within the alpha/beta (encoded by GNPTAB) and gamma (encoded by GNPTG) subunits affect the biosynthesis, transport and activity of the enzyme. Collectively, this information has helped explain how different patient mutations (i.e. genotype) correlate with unique disease symptoms and severities. In some cases, specific mutations (e.g. K4Q) have been linked to clinically distinct forms of the disease. Significant new insight into the function of the gamma subunit has also been gained over the last five years. Studies have demonstrated that the trafficking and activity of this subunit can be impacted by its proteolytic processing in certain cell types as well as

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its post-translational modification. Still others studies have demonstrated that some missense mutations in GNPTG can cause misfolding of this subunit. Lastly, attempts to engineer the phosphotransferase enzyme have successfully demonstrated that certain variants can actually increase the mannose phosphorylation of both lysosomal and non-lysosomal substrates. More detailed information can be found in the following papers:

Liu L et al. Mol Ther Methods Clin Dev. 201729;5:59-65 (<https://www.ncbi.nlm.nih.gov/pubmed/28480305>)  
van Meel et al. J Biol Chem 2016 Apr 8;291(15):8295-307 (<https://www.ncbi.nlm.nih.gov/pubmed/26833567>)  
Qian et al. J Biol Chem 2015 Jan 30;290(5):3045-56 (<https://www.ncbi.nlm.nih.gov/pubmed/25505245>)  
De Pace et al. Hum Mutat 2014 Mar;35(3):368-76 (<https://www.ncbi.nlm.nih.gov/pubmed/24375680>)  
Qian et al. Proc Natl Acad Sci 2013 Jun 18;110:10246-51 (<https://www.ncbi.nlm.nih.gov/pubmed/23733939>)  
Velho et al. Hum Mol Genet 2015 Jun 15;24(12):3497-505 (<https://www.ncbi.nlm.nih.gov/pubmed/25788519>)  
Marschner et al. Science 2011 Jul 1;333(6038):87-90 (<https://www.ncbi.nlm.nih.gov/pubmed/21719679>)  
van Meel et al. Proc Natl Acad Sci 2014 Mar 11;111:3532-7 (<https://www.ncbi.nlm.nih.gov/pubmed/24550498>)  
Leroy et al. Eur J Hum Genet 2014 May;22(5):594-601. (<https://www.ncbi.nlm.nih.gov/pubmed/24045841>)  
Pohl et al. J Biol Chem 2010 Jul 30;285(31):23936-44 (<https://www.ncbi.nlm.nih.gov/pubmed/20489197>)  
Encarnacao et al. J Biol Chem 2011 Feb;286(7):5311-8 (<https://www.ncbi.nlm.nih.gov/pubmed/21173149>)  
Van Meel et al. Hum Mutat Jul;37(7):623-6 (<https://www.ncbi.nlm.nih.gov/pubmed/27038293>)

### **Animal models and the identification of pathogenic processes in ML disease**

Multiple animal models were developed and characterized for MLII in recent years including three unique mouse models and two zebrafish models. Mice that either lack GNPTAB or express a mutant form share much of the same pathology seen in patients, including neurodegeneration and abnormal bone development. Progressive bone loss in MLII mice was shown to arise from a combination of dysfunctional bone forming cells (osteoblasts) and excessive formation of bone degrading cells (osteoclasts). This work has also demonstrated cell type-specific effects upon loss of mannose phosphorylation. For example, the finding that B cells but not T cells are affected in ML mice suggests that impaired lysosomal targeting affects cell types in different ways. Parallel investigation of MLII zebrafish has highlighted a novel role for secreted lysosomal enzymes in cartilage and heart pathogenesis. This work demonstrates that secreted cathepsins underlie abnormal development of cartilage and cardiac tissues by altering the growth factors that regulate their development. Suppressing this excessive cathepsin activity improves these phenotypes. Collectively, the studies in mice and zebrafish are beginning to suggest new avenues for disease treatment, such as the use of drugs that boost bone-forming cells (e.g. bisphosphonates), reduce bone-degrading cells and suppress the damaging activity of cathepsin proteases secreted outside the cell.

More detailed information can be found in the following papers:

Boonen et al. Mol Biol Cell 2011 Apr 15;22(8):1135-47. (<https://www.ncbi.nlm.nih.gov/pubmed/21325625>)  
Vogel et al. Vet Pathol 2009 Mar;46(2):313-24. (<https://www.ncbi.nlm.nih.gov/pubmed/19261645>)  
Kollmann et al. Brain 2012 Sep;135(Pt 9):2661-75. (<https://www.ncbi.nlm.nih.gov/pubmed/22961545>)  
Paton et al. J Biol Chem 2014 Sep 26;289(39):26709-21. (<https://www.ncbi.nlm.nih.gov/pubmed/25107912>)  
Otomo et al. J Cell Biol 2015 Jan 19;208(2):171-80. (<https://www.ncbi.nlm.nih.gov/pubmed/25601403>)  
Kollmann et al. EMBO Mol Med 2013 Dec;5(12):1871-86. (<https://www.ncbi.nlm.nih.gov/pubmed/24127423>)  
Flanagan-Steet et al. J Bone Miner Res 2016 31(3):535. (<https://www.ncbi.nlm.nih.gov/pubmed/26404503>)  
Petrey et al. Dis Model Mech 2012 Mar;5(2):177-90. (<https://www.ncbi.nlm.nih.gov/pubmed/22046029>)  
Flanagan-Steet et al. Am J Pathol 2009 175(5):2063-75. (<https://www.ncbi.nlm.nih.gov/pubmed/19834066>)

### **Therapy for ML disease**

The recent research on MLII, MLII/III and MLIII has broadened our understanding of the

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disease process and identified several secondary biochemical pathways altered by loss of mannose-6-phosphate biosynthesis. This information points to the potential for new modes of therapy that differ from the conventional treatments utilized in other LSDs. This is particularly important for MLII in light of the challenges associated with the standard therapeutic strategies. The failure of hematopoietic stem cell transplantation to improve MLII outcomes highlights these challenges and may indicate that enzyme replacement therapies (ERT) and gene therapies may face roadblocks in MLII. For ERT, the task of replacing all the necessary hydrolases lost from MLII cells is a daunting and costly one. For gene therapy, challenges may arise from the fact that the phosphotransferase is a large membrane-bound enzyme with multiple subunits and thus its expression in all affected tissues may prove difficult. Nonetheless, the availability of well characterized mouse and feline MLII models provide an encouraging platform to test the efficacy of gene therapy approaches. Exploring the use of pharmacological agents (e.g. bisphosphonates, cathepsin inhibitors, and secondary pathway inhibitors) as potential therapies will also be a significant focus of research in the coming years. While these compounds won't fully cure the disease or correct the underlying defect in lysosomal targeting, they can be used to slow the progression of symptoms in many tissues. Preliminary studies using pharmacological agents in the various MLII animal models including zebrafish show promise.

In conclusion, impressive progress has been made over the last decade towards the understanding of the underlying pathogenesis in this disease and many molecular aspects of the enzyme involved. New insights into the cell- and tissue-specific effects of impaired lysosomal targeting will hopefully translate into novel approaches for therapy.