**“Identification of genetic, biochemical and cellular factors influencing the course of mucopolysaccharidoses with particular reference to Sanfilippo disease”**

**Karolina Joanna Wiśniewska, M. Sc**

Mucopolysaccharidoses (MPS) are a heterogeneous group of hereditary metabolic disorders classified as lysosomal storage diseases. The leading causes of MPS are mutations in genes that encode lysosomal enzymes responsible for breaking down glycosaminoglycans (GAGs). As a result of the deficiency or complete lack of activity of these enzymes, GAGs accumulate in the cells of various tissues and organs, which in turn leads to their gradual damage and dysfunction. A wide range of clinical symptoms, such as facial dysmorphia, short stature, bone deformities, chronic joint pain, hepatosplenomegaly, corneal clouding, hearing loss, respiratory difficulties and cardiovascular complications, characterise MPS.

Until recently, 11 types and subtypes of MPS were distinguished, classified based on the type of stored GAG and the enzyme affected by the defect. In recent years, newly discovered, ultra-rare entities with an MPS phenotype have been described: MPS X and the so-called MPS-plus syndrome. MPS X is caused by a mutation in the *ARSK* gene*,* which encodes arylsulfatase K, leading to the accumulation of dermatan sulphate. MPS-plus syndrome (MPS-PS), on the other hand, is a disorder with a phenotype similar to MPS, in which elevated GAG levels are observed, but without a defect in any of the known lysosomal GAG-degrading enzymes. The pathomechanism of the disease remains unclear, despite the identification of a mutation in the *VPS33A* gene*,* whose product is involved in endocytosis and autophagy pathways. Due to the absence of a classic defect in a GAG-degrading enzyme and certain differences in the clinical picture (including renal dysfunction and haematopoietic abnormalities), MPS-plus syndrome is not currently included in the formal classification of MPS. Still, it is classified as *an "MPS-like disorder"*. Therefore, the current classification includes 12 "classic" types and subtypes of MPS, which are presented in **Table 1** along with their characteristics**.**

Additionally, MPS can be divided into neuropathic types (MPS I, II, III and VII), in which neurological symptoms predominate, and non-neuropathic forms (IV, VI, IX and X), in which the functions of the nervous system remain intact. Neuropathic symptoms occurring in severe forms of MPS I and II, all subtypes of MPS III and in MPS VII include cognitive impairment, aggression, hyperactivity, circadian rhythm disorders, insomnia, epilepsy, seizures, speech difficulties, hearing loss and personality changes.

Table 1. Characteristics of the types/subtypes of MPS described to date.

| MPS type/subtype | Common name | Mutated gene | Stored GAG | Neurodegenerative component |
|---|---|---|---|---|
| MPS I | Hurler, Scheie or Hurler-Scheie syndrome | *IDUA* | Dermatan sulphate, heparan sulphate | Yes (severe cases) |
| MPS II | Hunter syndrome | *IDS* | Dermatan sulphate, heparan sulphate | Yes (severe cases) |
| MPS IIIA | Sanfilippo syndrome | *SGSH* | Heparan sulphate | Yes |
| MPS IIIB | | *NAGLU* | | |
| MPS IIIC | | *HGSNAT* | | |
| MPS IIID | | *GNS* | | |
| MPS IVA | Morquio syndrome | *GALNS* | Keratan sulphate | May occur as a result of second- and third-order disorders ( ) |
| MPS IVB | | *GLB1* | Keratan sulphate, chondroitin sulphate | |
| MPS VI | Maroteaux-Lamy syndrome | *ARSB* | Dermatan sulphate | No |
| MPS VII | Sly syndrome | *GUSB* | Dermatan sulphate, heparan sulphate, chondroitin sulphate | Yes |
| MPS IX | Natowicz syndrome | *HYAL1* | Hyaluronic acid | No |
| MPS X | - | *ARSK* | Dermatan sulphate | Inconclusive data |

Diagnosis of MPS, after noticing symptoms that may suggest a metabolic disorder, begins with a biochemical analysis to detect excess GAG excreted in the urine. Confirmation of the diagnosis requires assessment of the activity of specific lysosomal enzymes in the patient's leukocytes or fibroblasts. Modern molecular techniques enable the identification of mutations in the genes whose dysfunction causes each type of MPS, allowing for precise determination of the type/subtype of the disease and initiation of appropriate treatment, if available. However, the diagnostic process is extremely difficult and time-consuming due to the rare occurrence of these diseases, the varied clinical picture (both between individual types and within a single type), and the progressive and multi-organ/systemic nature of the disease. Due to their rarity, the development of symptoms at different times and their similarity to other (more common) diseases, MPSs are often misdiagnosed. They are most often diagnosed as neurological disorders (i.e. autism spectrum disorders, psychomotor hyperactivity or intellectual disability), rheumatological and orthopaedic diseases (i.e. juvenile idiopathic arthritis, Perthes disease, rickets or muscular dystrophy). It can take years to make a correct diagnosis, and the diagnosis of MPS itself is still only half the battle. An important part of the diagnostic process is the proper identification of the type/subtype of the disease, which has a significant impact on planning and treatment availability. Diagnostic problems in patients with MPS, diseases with which they may be confused, and ways to prevent this phenomenon are described by my co-authors and I in a review article, Wiśniewska K, Wolski J, Gaffke L, Cyske

Z, Pierzynowska K, Węgrzyn G. (2022) Misdiagnosis in mucopolysaccharidoses. *Journal of Applied Genetics;* 63(3):475-495.

Registered MPS treatment methods primarily include *enzyme replacement therapy* (ERT*)* and *haematopoietic stem cell transplantation* (HSCT*)*. ERT involves administering the missing enzyme to reduce GAG storage, which alleviates some somatic symptoms. It is used in clinical practice in MPS I, MPS II, MPS IVA, MPS VI and MPS VII. However, it does not affect neurological symptoms (due to the inability of the enzyme molecule to cross the blood-brain barrier) or musculoskeletal disorders (due to the inability of the enzyme to reach bones and cartilage). HSCT, which involves transplanting healthy haematopoietic stem cells to rebuild the patient's haematopoietic and immune systems, is most commonly used in the youngest patients with MPS I and II. Research is being conducted on many other therapeutic approaches, which are currently in the clinical or preclinical trial phase. These include gene therapy, *substrate reduction* therapy (S*RT)* and molecular chaperone therapy. It should be noted that the available therapeutic methods are symptomatic treatments (with the exception of gene therapy), and their capabilities are severely limited and the effects are not entirely satisfactory.

It has long been believed that stored GAGs are the main, or even the only, cause of MPS. However, the fact that none of the therapies aimed at reducing GAG levels (ERT, SRT, gene therapy) led to a complete correction of symptoms began to raise doubts about the mechanisms of the disorders observed in the course of MPS. The wide spectrum of symptoms occurring among patients of different types also attracted attention. An example of such clinical variability is MPS III, characterised by neurodegeneration, severe neuropsychiatric symptoms and a relatively mild somatic component, and MPS IV, characterised by normal development of the central nervous system (CNS) but at the same time severe bone and joint disorders. Even if such a wide variability of symptoms may result from the storage of different types of GAGs (which cannot be ruled out), in MPS I and MPS II, where the same types of GAGs (dermatan sulphate and heparan sulphate) accumulate, their biochemical characteristics and symptoms may differ significantly. For example, corneal clouding occurs in patients with MPS I, but not in those with MPS II. On the other hand, skin changes (characteristic papules) are present in MPS II. Still, absent in MPS I. Hyperactivity and aggressive behaviour are also distinctive features of patients with the neuropathic type of MPS II, but not MPS I, where, even if there is significant cognitive impairment, patients are gentle and calm.

Attention should also be paid to MPS type III (Sanfilippo syndrome), which was given particular emphasis in the doctoral dissertation presented. MPS III, as one of two types of MPS (alongside MPS IV), has been divided into subtypes (MPS III A, B, C and D) due to its genetic

basis, a deficiency of a different lysosomal enzyme, but leading to the storage of the same GAG (heparan sulphate in MPS III). As in the case of MPS I and II described above, patients with different subtypes of MPS III can exhibit significant clinical variations. Autism spectrum disorders occur in almost 30% of patients with MPS IIIA and only 8% of patients with MPS IIIC. Epilepsy occurs in 17% of patients with MPS IIIA and 8% of patients with MPS IIIC. Similarly, facial dysmorphic features and hepatomegaly occur in 94% and 56% of patients with MPS IIIB and 85% and 39% of patients with MPS IIIC, respectively. These subtypes also differ markedly in the age of onset of specific symptoms. For example, the average age of diagnosis for MPS IIIB is 2.5 to 5 years, and for MPS IIIC it is 4.5 to 19 years. Life expectancy also varies considerably, ranging from 15 to 18 years for MPS IIIA, 17 to 19 years for MPS IIIB, and 19 to 34 years for MPS IIIC (no data available for MPS IIID).

All observations to date indicate that the accumulation of glycosaminoglycans (GAGs) is a key process in the pathogenesis of MPS; however, it cannot be the sole factor determining the clinical picture. Similarly, differences in the type of GAGs accumulated are a biochemical fact, but do not in themselves explain why individual types and subtypes of MPS manifest themselves with such a wide range and dynamics. Similarly, the differences in the type of GAGs accumulated are a biochemical fact, but do not in themselves explain why different types and subtypes of MPS manifest such a wide range and dynamics of symptoms, particularly concerning the nervous system.

A growing body of research indicates that the course of MPS is modulated by additional cellular and molecular processes that may act independently of GAG accumulation or develop secondary to it. These include, among others, redox imbalance, chronic activation of the inflammatory response, oxidative stress, and disturbances in gene expression regulation. These mechanisms can significantly modify the course of the disease, affecting the variety of symptoms and the rate of their progression.

Therefore, **my doctoral thesis aimed to identify genetic, biochemical, and cellular factors that may modulate the course of MPS, with particular emphasis on Sanfilippo syndrome.** This approach seeks to understand better why patients with theoretically the same enzyme defect and type of stored GAG may present with such a different clinical picture.

I conducted my research mainly on a model of skin fibroblasts taken from patients with all types/subtypes of MPS described up to 2020 (when I began my research). The characteristics of the cell lines used are presented in **Table 2**.

Table 2. Characteristics of the cell lines used

| MPS type | Defective enzyme | Type of mutation | Catalogue number ** |
|---|---|---|---|
| MPS I | α-L-iduronidase | p.Trp402Ter/p.Trp402Te | GM00798 |
| MPS II | 2-iduronate sulfatase | p.His70ProfsTer29 | GM13203 |
| MPS IIIA | N-sulfoglucosamine sulfhydrolase | p.Glu447Lys/p.Arg245His | GM00879 |
| MPS IIIB | α-N-acetylglucosaminidase | p.Arg626Ter/p.Arg626Ter | GM00156 |
| MPS IIIC | Acetyl-CoA:α-glycosaminide acetyltransferase | p.Gly262Arg/pArg509Asp | GM05157 |
| MPS IIID | N-acetylglucosamine 6-sulfatase | p.Arg355Ter/p.Arg355Ter | GM05093 |
| MPS IVA | N-acetylglucosamine-6-sulfate sulfatase | p.Arg386Cys/p.Phe285Ter | GM00593 |
| MPS IVB | β-galactosidase | p.Trp273Leu/p.Trp509Cys | GM03251 |
| MPS VI | N-acetylglucosamine-4-sulfatase (arylsulfatase B) | Not determined | GM03722 |
| MPS VII | N-acetylgalactosamine 4-sulfatase | p.Trp627Cys/p.Arg356Ter | GM00121 |
| MPS IX | Hyaluronidase | p.Glu268Lys/c.37bp-del;14bp-ins at nt 1361 | GM17494 |
| Control line | N/A | N/A | N/A |

*Catalogue numbers are consistent with the cell line description at the Coriell Institute.

Although fibroblasts may seem an unlikely model for studying neurodegeneration, they share several important characteristics with neurons. Both cell types originate from the ectoderm, suggesting that they share common molecular mechanisms, including signalling pathways and gene expression regulation processes. This feature is crucial for elucidating the pathogenesis of neurodegenerative diseases. Additionally, fibroblasts exhibit abnormalities in cellular pathways, including oxidative stress, autophagy, and metabolism, which are also essential factors in neurodegeneration. Fibroblasts may reflect systemic cellular changes that affect neuronal function, especially in genetic disorders where the consequences of mutations remain unclear. Furthermore, fibroblasts are located near blood vessels, in the meninges, and in the choroid plexus of the brain and spinal cord, where they play a key role in maintaining CNS function. It is also worth noting that fibroblasts are easily accessible through minimally

invasive procedures, allowing for regular monitoring of patients. Their stable cultures facilitate long-term studies, making them a valuable model for research into neurodegenerative mechanisms. I also conducted some of the research using tissues obtained from a mouse model of MPS IIIB.

I did not include MPS X, a type of MPS discovered only after I began my research, or MPS-plus- syndrome in my studies. Access to cells from patients suffering from these two types of MPS, even after the start of the project, was limited due to the very small number of patients (no more than 30 worldwide) and the fact that most of them come from Russia, which is affected by armed conflict.

As mentioned earlier, MPS is divided into 12 types/subtypes based on the type of GAG that is stored and the defective enzyme. In addition, MPS can be divided into neuronopathic types (MPS I, II, IIIA, IIIB, IIIC, IIID, VII) and non-neuronopathic types (MPS IV, VI, IX, X) based on the presence/absence of neurodegenerative features. This is an interesting division because, despite the seemingly identical cause of the disease, which is GAG storage, there is a clear difference in the occurrence of somatic symptoms and those related to neurodegeneration.

Currently, it is not possible to determine with certainty whether a patient with MPS will develop neurological symptoms. If they do, it is difficult to predict the rate of their progression or severity. The ability to predict this component would be of significant importance to patients and their families. Firstly, it would enable the early use of therapies aimed at protecting neurological functions, including intra-canal or intraventricular ERT and intra-canal gene therapy, which can target the central nervous system directly. Second, it would enable better planning of specialist care and rehabilitation, which could improve quality of life and slow the progression of symptoms. Early diagnosis of neurodegeneration risk would also help to adjust expectations and prepare carers for the specific challenges associated with progressive neurological changes. This is why it is so important to search for markers of neurodegeneration in MPS.

Using the RNA-seq library developed in previous years by the team I was working with, I performed a transcriptomic analysis to select genes whose expression levels, compared to control cells, are specific only to neuropathic types/subtypes of MPS (I, II, IIIA, IIIB, IIIC, IIID and VII), while showing no changes in expression levels in non-neuronopathic types. The results collectively showed over 300 transcripts that may be associated with nervous system disorders (specifically, 322 unique genes whose expression is significantly altered in at least

two neuropathic MPS types/subtypes, but not in non-neuropathic types). Transcripts with altered expression levels in at least five neuropathic MPS types/subtypes (without simultaneous expression level alterations in non-neuropathic types) include transcripts with increased expression, e.g. *PDIA3* (encoding protein disulphide isomerase A3, PDIA3) and those with decreased expression, e.g. *ARL6IP6 (*encoding a protein interacting with GTPase 6 similar to ARF 6, ARL6IP6). Disruption of ARL6IP6 function can lead to abnormal neuron differentiation and disturbances in the homeostasis of the mitochondria and endoplasmic reticulum. Additionally, this protein plays a crucial role in the processing of APP into β-amyloid. A reduction in its level may therefore explain the increase in the level of β-amyloid and its precursors, as well as their tendency to aggregate. Literature data on studies using the *PDIA3*$^{(-/-)}$ mouse model have shown that the absence of *PDIA3* significantly reduces the effectiveness of apoptosis, alleviates inflammation and oxidative stress, and also improves cognitive function and reduces the volume of contusion caused by trauma in a traumatic brain injury model. In addition, an age-dependent increase in PDIA3 levels in the brains of mice with Alzheimer's disease was demonstrated, in contrast to its decline with age in healthy mice. These data strongly suggest that the high level of *PDIA3* expression detected in the analysis of neuropathic cell types/subtypes of MPS may also contribute in part to neurodegeneration.

The above-described results of research on *ARL6IP6* and *PDIA3* and other genes, suggesting their possible use as biomarkers of neurodegeneration in MPS, are described in the article: Wiśniewska K, Żabińska M, Szulc A, Gaffke L, Węgrzyn G, Pierzynowska K. (2024) The Role of Gene Expression Dysregulation in the Pathogenesis of Mucopolysaccharidosis: A Comparative Analysis of Shared and Specific Molecular Markers in Neuronopathic and Non-Neuronopathic Types of the Disease. *International Journal of Molecular Sciences;* 25(24):13447.

As I was interested in research on the functioning of the entire cell, I performed a similar analysis of neurodegeneration mechanisms, dividing it into the functioning of individual cell organelles. Using transcriptomic methods, I identified genes associated with the function and structure of individual cell organelles (based on the Ensembl database) whose expression levels are altered in neuropathic types of MPS compared to control cells, while remaining unchanged in non-neuropathic types of MPS. This analysis indicated that genes whose expression is disrupted in the neuropathic type of MPS are often associated with the structures or functions of the cell nucleus, endoplasmic reticulum or Golgi apparatus. Experiments using fluorescence and electron microscopy confirmed abnormalities mainly within the Golgi apparatus, which

appeared significantly more frequently in cells taken from neuropathic patients. The results of these analyses were discussed and compared with those found in other neurodegenerative diseases and neurological disorders in the article: Wiśniewska K, Gaffke L, Żabińska M, Węgrzyn G, Pierzynowska K. (2024) Cellular Organelle-Related Transcriptomic Profile Abnormalities in Neuronopathic Types of Mucopolysaccharidosis: A Comparison with Other Neurodegenerative Diseases. *Current Issues in Molecular Biology;* 46(3):2678-2700.

In the next stage of my research, I focused on two types of MPS – MPS III and MPS IV. Several factors dictated this choice. These are the only forms of MPS in which the division into subtypes is based on differences in the molecular defect, and not, as in the case of MPS I, on differences in the clinical course.

I found MPS III, in which CNS symptoms predominate, particularly intriguing. In this type, only heparan sulphate (HS ) is accumulated – a compound whose accumulation is associated with neurodegeneration in other diseases, such as Alzheimer's disease. Neurodegeneration is also what distinguishes types IVA and IVB, among others. Nervous system disorders may occur in the course of MPS IVB, although skeletal musculoskeletal disorders predominate in this type. For these reasons, MPS III and IV are an extremely valuable reference point in research on neurodegeneration in MPS.

First, using transcriptomic data obtained from a cellular model, I selected genes that showed significantly different expression between MPS III subtypes. The number of transcripts showing differential expression was highest between subtypes IIIA vs. IIIB, IIIA vs. IIID, IIIB vs. IIID, and IIIC vs. IIID, totalling over 400. I performed a similar analysis between MPS IV subtypes (IVA and IVB), in which the number of transcripts showing differential expression was less than 100. The cellular processes in which the products of these transcripts participate largely involve cellular metabolism and its regulation, as well as the cell's response to stimulation or cellular communication (according to the Ensembl database). Some of these transcripts showed very large, more than 16-fold differences in expression levels ($\log_2$fold change [FC]) > 4 or < -4), particularly between MPS IIIA and IIID, MPS IIIB and IIIC, and MPS IIIB and IIID. These transcripts include mRNA molecules derived from genes whose products are associated with ribosome functions (*RPLP2, RPL23, RPL10)*, maintenance of normal connective tissue structure (*COL4A1, COL4A2, COMP)*, involved in intracellular signalling (*NME2, WISP2, SRFP1)* and cell receptors and factors (*RARRES2, CRLF1, IGFBP5, TFPI2)*.

While the high variability in the expression of genes determining the proper function of connective tissue is not surprising (it has already been described in the context of MPS pathogenesis), the significant number of such genes involved in ribosome functions is a new discovery. Ribosomes are essential organelles responsible for protein synthesis, a key step in the gene expression process. The results obtained suggest significant variation in protein synthesis efficiency, which may contribute to the differing disease progression observed in patients with the four subtypes of MPS III. Furthermore, *RPL10* gene expression abnormalities have also been identified in other neurological disorders, such as autism spectrum disorders, X-linked microcephaly, X-linked synodromic intellectual disability, and epilepsy. Therefore, the reduced expression of these genes detected in MPS IIIA compared to MPS IIIC and IIID could, to some extent, explain the more frequent occurrence of autistic behaviours and more severe epilepsy in children with the former subtype of Sanfilippo syndrome than in other subtypes. Another example of a gene associated with epilepsy is *RARRES2,* which encodes chemerin, high levels of which are detected in the blood of children with idiopathic epilepsy as a prognostic factor. My transcriptomic analyses have shown that the expression level of this gene is elevated in MPS IIIA compared to IIID, and epilepsy is more common in the former subtype than in the latter. As previously mentioned, no significant changes in the expression levels of individual genes were observed between MPS IVA and IVB fibroblasts. In fact, the phenotypic differences between patients with different MPS IV subtypes are much smaller than between different MPS III subtypes. I therefore suggest that changes in gene expression patterns may determine the diversity of disease progression in patients suffering from different MPS III subtypes. Article by Wiśniewska K, Gaffke L, Krzelowska K, Węgrzyn G, Pierzynowska K. (2022) Differences in gene expression patterns, revealed by RNA-seq analysis, between various Sanfilippo and Morquio disease subtypes. *Gene;* 812:146090 describes the above-summarised research results and points to the possibility of a correlation between global gene expression patterns and the development of different symptoms in patients suffering from various subtypes of MPS III.

In the second stage of the study, I identified transcripts that undergo common changes in expression levels relative to control cells (taken from healthy individuals) in all subtypes of MPS III (IIIA, IIIB, IIIC, and IIID) and IV (IVA and IVB). No analysis of gene expression abnormalities common to all subtypes of MPS III and MPS IV had ever been conducted before. The results of this analysis identified 45 transcripts with similar changes in expression levels across all subtypes of MPS III, as well as up to 150 such transcripts in both subtypes of MPS IV, in relation to control cells. By identifying those transcripts with the highest fold change in

expression ($log_2FC > 3$ or $< -3$), I noticed three that showed high levels of expression changes in all subtypes of both MPS III and MPS IV compared to control cells. These are the overexpressed genes *PFN1* and *MFAP5*, as well as the underexpressed gene *MMP12*. These genes encode profilin (PFN1), microfibril-associated protein 5 (MFAP5) and matrix metallopeptidase 12 (MMP12), respectively. In cells of all MPS III and IV subtypes, I performed immunodetection of these three proteins using immunofluorescence and Western blotting techniques, which indicated an increase in PFN1 and MFAP5 levels in MPS III and IV cells and a decrease in MMP12 levels in MPS III cells. Since these transcripts changed expression levels in all types/subtypes of MPS III, it seemed interesting to investigate the relationship between their levels and the levels of stored GAGs. The level of GAG in MPS cells was reduced by one of the flavonoids, genistein, which inhibits the autophosphorylation of the epidermal growth factor receptor (EGFR), ultimately leading to the inhibition of gene expression encoding GAG synthetases. Under these conditions, the levels of PFN1, MFAP5, and MMP12 proteins were re-evaluated, and the results of this analysis indicated that as GAG levels decreased, PFN1 levels also decreased. Furthermore, silencing *PFN1* gene expression also resulted in a decrease in GAG levels, suggesting a possible new therapeutic pathway.

Profilins encoded by the *PFN1* gene are small proteins that interact with the cytoskeleton, influencing actin polymerisation. They are also involved in membrane transport, cell signalling, transcription and autophagy. The cytoskeleton plays a vital role in synaptogenesis and neurotransmission, and is also involved in the development of the nervous system and the plasticity of the mature brain. Cytoskeleton disorders are often observed in mental illnesses such as schizophrenia, bipolar disorder, autism or severe depression. This is very important because behavioural problems are one of the neuropathic symptoms of MPS. Furthermore, patients with MPS III are sometimes misdiagnosed as having autism spectrum disorders.

Mutations in the *PFN1* gene are also detected in patients with other neurodegenerative diseases, including amyotrophic lateral sclerosis, fragile X syndrome, spinal muscular atrophy, Huntington's disease, Parkinson's disease, and adrenoleukodystrophy. This fact seems particularly interesting, as protein aggregates are a common feature in many of these diseases. Some reports in the literature suggest that an increase in PFN1 expression may contribute to their formation. It has been demonstrated that some mutated PFN1 proteins exhibit prion-like properties and function as factors that trigger the conversion of TDP-43 protein into toxic conformational states.

In summary, the genes listed above and their products may be involved in common pathways of pathogenesis of different types/subtypes of MPS, with PFN1 levels appearing to be related to GAG levels. The above-presented research results concerning mainly PFN1 level disorders in MPS III and MPS IV cells are described in the article: Wiśniewska K, Żabińska M, Gaffke L, Szulc A, Walter BM, Węgrzyn G, Pierzynowska K. (2024) Shared Gene Expression Dysregulation Across Subtypes of Sanfilippo and Morquio Diseases: The Role of PFN1 in Regulating Glycosaminoglycan Levels. *Frontiers in Bioscience (Landmark Ed.);* 29(12):415.

The results mentioned above concerning profilin level disorders in MPS cells and their involvement in the pathogenesis of other neurological diseases prompted me to perform a comprehensive analysis of the levels of proteins known for their tendency to form aggregates in various neurodegenerative diseases. In MPS III cells of all subtypes, I performed immunodetection using immunofluorescence techniques and western blotting of beta-amyloid and its precursor (APP protein) levels, tau protein and its hyperphosphorylated form (p-tau), as well as alpha-synuclein and TDP-43 protein (b TDP-43 protein has never before been studied in the context of MPS pathogenesis). The results of these analyses indicated elevated levels of APP, β-amyloid, tau, and TDP-43 proteins in all MPS III subtypes, and elevated levels of p-tau and α-synuclein in all subtypes except MPS IIIC. Furthermore, aggregates formed by β-amyloid and tau, visible under a fluorescence microscope, were present in all MPS III subtypes, and aggregates formed by p-tau, TDP-43 and α-synuclein were present in all MPS III subtypes except IIIC. Elevated levels of the proteins mentioned above were also observed in the brains of mice modelling MPS IIIB.

Since the GAG level marked in the cells was the lowest of all the lines studied in MPS IIIC (although still significantly higher than in the control cells), the question arose as to the involvement of GAG in the formation of protein aggregates. Therefore, as before, the GAG level in MPS cells was reduced using genistein, and the levels of APP, β-amyloid, tau, p-tau, TDP-43, and α-synuclein, as well as their aggregates, were re-examined. Surprisingly, the results of these experiments indicated a reduction in the levels of all the above-mentioned proteins and the aggregates they form in cells treated with genistein, except α-synuclein, whose elevated level remained independent of GAG levels. This suggests a clear link between the formation of aggregates of certain proteins and GAG levels, which raises a number of questions about the role of GAG in the formation of such aggregates. The results of these experiments are described in the article : Wiśniewska K, Rintz E, Żabińska M, Gaffke L, Podlacha M, Cyske Z,

Węgrzyn G, Pierzynowska K. (2024) Comprehensive evaluation of pathogenic protein accumulation in fibroblasts from all subtypes of Sanfilippo disease patients. *Biochemical and Biophysical Research Communications;* 733:150718.

The final element of my doctoral thesis was a literature review on behavioural disorders and sleep problems in MPS III, i.e. symptoms that are particularly characteristic of this condition and highly burdensome to both patients and their families. By analysing the available data, I sought to explain the underlying causes of these disorders, which involve overlapping pathological processes in OUN. Potential mechanisms include HS accumulation and the resulting neurodegeneration, chronic inflammation, oxidative stress, mitochondrial dysfunction, and disturbances in neurotransmitter metabolism. All these factors lead to the disruption of brain functions and structures responsible for controlling behaviour, emotions, and regulating sleep-wake cycles.

The most characteristic symptoms are psychomotor hyperactivity, impulsivity, aggressive outbursts, stereotypical movements, anxiety and sleep problems, such as difficulty falling asleep, frequent awakenings, reduced total sleep time or reversal of the circadian rhythm. Importantly, these symptoms very often appear in the early stages of the disease, before apparent somatic symptoms develop. In the review, I also discussed the possibilities of alleviating these disorders – both pharmacological (including antidepressants, antipsychotics, melatonin) and non-pharmacological, such as environmental modifications, maintaining consistent sleep rituals, behavioural therapies, and psychological support. However, I pointed out that the effectiveness of these methods varies and that there is still a need for well-designed clinical trials in this area. In this paper, I aim to focus primarily on the daily problems associated with the disease that patients' families face, highlight the most significant ones, and discuss potential ways of dealing with them. This article is a paper by: Wiśniewska K, Wolski J, Anikiej-Wiczenbach P, Żabińska M, Węgrzyn G, Pierzynowska K. (2025) Behavioural disorders and sleep problems in Sanfilippo syndrome: overlaps with some other conditions and importance indications. *Eur Child Adolesc Psychiatry;* 34(6):1795-1816. This work is particularly important to me because it highlights an aspect that is often overlooked in research on the disease's mechanisms and potential causal therapies. Although the search for effective causal treatments remains a priority, it should not be forgotten that until such therapies are available, it is necessary to simultaneously develop and improve symptomatic treatments that currently improve the quality of life of patients and their families.

To summarise the research presented, it can be said that the discoveries described in my doctoral thesis provide new insights into the molecular mechanisms underlying the pathogenesis of MPS and the mechanisms of differentiation between the neuropathic and non-neuropathic course of the disease. Furthermore, my research has revealed numerous differences and similarities between the various subtypes of MPS III at every level. In addition, the research as a whole points to numerous similarities between neuropathic types of MPS and other neurodegenerative diseases, which may indicate new directions for research into treatment methods.

## List of publications included in the doctoral dissertation

1. Wiśniewska, Karolina et al. “Misdiagnosis in mucopolysaccharidoses.” *Journal of applied genetics* vol. 63,3 (2022): 475-495. doi:10.1007/s13353-022-00703-1
2. Wiśniewska, Karolina et al. “The Role of Gene Expression Dysregulation in the Pathogenesis of Mucopolysaccharidosis: A Comparative Analysis of Shared and Specific Molecular Markers in Neuronopathic and Non-Neuronopathic Types of the Disease.” *International journal of molecular sciences* vol. 25,24 13447. 15 Dec. 2024, doi:10.3390/ijms252413447
3. Wiśniewska, Karolina et al. “Cellular Organelle-Related Transcriptomic Profile Abnormalities in Neuronopathic Types of Mucopolysaccharidosis: A Comparison with Other Neurodegenerative Diseases.” *Current issues in molecular biology* vol. 46,3 2678-2700. 21 Mar. 2024, doi:10.3390/cimb46030169
4. Wiśniewska, Karolina et al. “Differences in gene expression patterns, revealed by RNA-seq analysis, between various Sanfilippo and Morquio disease subtypes.” *Gene* vol. 812 (2022): 146090. doi:10.1016/j.gene.2021.146090
5. Wiśniewska, Karolina et al. “Shared Gene Expression Dysregulation Across Subtypes of Sanfilippo and Morquio Diseases: The Role of PFN1 in Regulating Glycosaminoglycan Levels.” *Frontiers in bioscience (Landmark edition)* vol. 29,12 (2024): 415. doi:10.31083/j.fbl2912415
6. Wiśniewska, Karolina et al. “Comprehensive evaluation of pathogenic protein accumulation in fibroblasts from all subtypes of Sanfilippo disease patients.” *Biochemical and biophysical research communications* vol. 733 (2024): 150718. doi:10.1016/j.bbrc.2024.150718
7. Wiśniewska, Karolina et al. “Behavioural disorders and sleep problems in Sanfilippo syndrome: overlaps with some other conditions and importance indications.” *European child & adolescent psychiatry* vol. 34,6 (2025): 1795-1816. doi:10.1007/s00787-025-02661-5