Rewiring Decline: Targeting Early Neurodegeneration in Sanfilippo Syndrome Through Gene Correction and Molecular Parallels with Alzheimer's Disease

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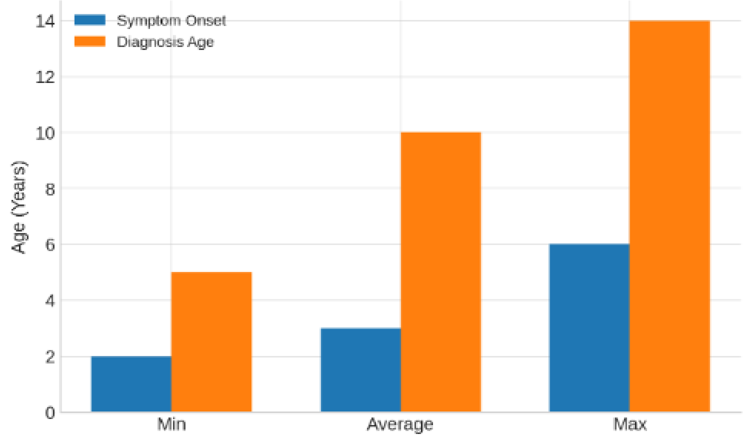
Specific Aims: This project is built around three aims: figuring out what's happening early in the brain, fixing the genetic glitch that causes it, and learning how to actually get that fix where it will do the most good.
Aim 1: Investigate Sanfilippo's early brain alterations and compare them to Alzheimer's. Gene expression and inflammatory profiles in brain samples from both disease conditions will be investigated. If the same signals were found, especially close to synapses or glial activity, it can mean that there's an overlap beneath. That correlation can help in learning more about both diseases and shine a spotlight on Sanfilippo more within the neurodegeneration community.
Aim 2: Repair the mutated gene in patient-derived neurons through CRISPR. By producing neurons from patient iPSCs and fixing the gene in situ, it can be seen if this restores enzyme function and reduces cell damage. Lysosomal accumulation and signs of improved neuron function will be watched very closely. If correction works at this level, it's a big step toward therapy.
Aim 3: Develop a delivery system that can cross the blood-brain barrier using monoclonal antibodies. Even if the gene is fixed, unless it's able to get to the brain, it won't be of any use. This objective puts labeled nanoparticle carriers to the test to get therapeutic material beyond the barrier. If it does, it's a game changer in the delivery of Sanfilippo treatments but also for other brain disorders. These aims aren't isolated; they build on each other. If successful, this research could offer more than just new data. It could bring us closer to real options for families who've been waiting too long.

Sanfilippo syndrome (MPS III) is a rare but extremely devastating neurodegenerative disorder in children. Even though it only affects about 1 in 70,000 newborns (Zelei et al., 2018), the impact on families is overwhelming. Sanfilippo belongs to a group of conditions called lysosomal storage disorders. The body lacks certain enzymes that are needed to break down a sugar-like molecule called heparan sulfate. Because of this, heparan sulfate starts to build up in the cells, especially brain cells, which leads to inflammation, damage, and eventually brain shrinkage. There are four types of Sanfilippo, each related to a different enzyme: SGSH, NAGLU, HGSNAT, and GNS (Neufeld & Fratantoni, 2013).

One of the most heartbreaking things about this disease is how subtly it starts. Most children are born looking healthy. It's usually between the ages of 2 and 6 when parents begin noticing changes, speech delay, hyperactivity, or behavior that seems similar to ADHD or autism.

Because of that overlap, many children get misdiagnosed or not diagnosed at all until much later. According to research, it takes over seven years on average to finally reach a proper diagnosis (Lawrence et al., 2013). During those years, the disease is already progressing. Kids start to lose cognitive skills, speech, and motor abilities and eventually need full-time care. Many don't live past their teenage years.

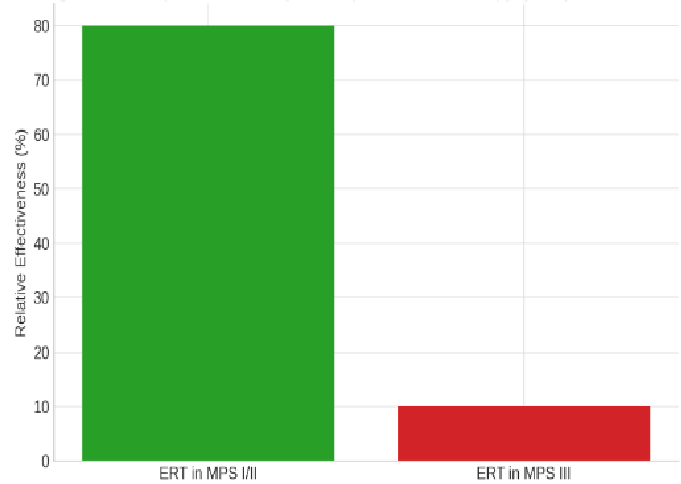
Figure 1: Delay Between Symptom Onset and Diagnosis in MPS III



As shown in Figure 1, the delay in diagnosis is a huge problem. The figure demonstrates that most children begin showing symptoms around age 6, but diagnosis often doesn't occur until age 14. Studies show that brain changes, like white matter loss and cortical thinning, actually happen before any symptoms are even visible (Nestrasil & Velodin, 2017). Right now, there's no effective way to catch the disease early. No reliable imaging markers, no approved blood tests. That means doctors usually catch it when it's too late to intervene meaningfully. Early treatment could make a difference, but we're missing that window.

Current treatments haven't been very effective. As shown in Fig. 2, enzyme replacement therapy (ERT), which has helped in other MPS disorders, doesn't work for Sanfilippo because the enzymes can't cross the blood-brain barrier (Noh & Lee, 2014). Figure 2 illustrates this limitation by depicting the blocked enzyme delivery at the endothelial interface of the brain. Some researchers are working on ways to inject the enzymes into the cerebrospinal fluid or design them to cross the barrier, but those are still experimental, and even when they work, the effects are often limited or temporary as well as invasive. Gene therapy seems like the most promising option. AAV (Adeno-Associated Viruses) vectors can be used to deliver healthy versions of the missing gene. Some early trials have shown this can improve certain symptoms; however, it's not perfect. The effects might not last

Figure 2: Comparison of Enzyme Replacement Therapy (ERT) Effectiveness



long in growing children, and there's also a risk of the immune system reacting to the virus used for delivery. CRISPR-Cas9 might be even better. Instead of adding a working gene, it fixes the mutation directly in the DNA (Poletto et al., 2020). CRISPR-Cas9 is a genome-editing tool derived from bacterial immune systems. It uses a guide RNA to direct the Cas9 enzyme to a specific DNA sequence, where it introduces a precise cut. The cell's natural repair machinery then modifies the target site, which can correct mutations responsible for genetic disorders. If done safely, it could offer a one-time, permanent solution, which feels like a real breakthrough.

Looking deeper into the biology, animal studies have revealed just how fast the damage happens. In mouse models of Sanfilippo type C, scientists found glial activation, mitochondrial failure, and widespread neuron loss by just five months of age (Martins et al., 2015). What's interesting is that these are the same kinds of brain changes seen in more common disorders like Alzheimer's and Parkinson's. So even though Sanfilippo is rare, studying it could teach us a lot about neurodegeneration in general.

What drives this proposal isn't just the science; it's what's happening to real people behind it. Sanfilippo syndrome is devastating. Families watch their children slowly lose cognitive and motor skills with no real treatment available. There's the emotional shock of the diagnosis, the slow progression, the financial pressure, and the lack of answers. A lot of families feel completely alone in this. That's what makes this work feel urgent.

Yes, it's about understanding the pathology, but it's also about pushing things forward. Earlier diagnosis could give families more time. Better delivery systems could finally target the brain. If CRISPR can correct the root mutation or reduce its damage, that's not just a lab success; it's potentially life-changing. Nowhere near finished, but the tools are better than they've ever been, and finally there's a way to approach this that feels both scientifically sound and deeply human.

Research Strategy

The proposal will address two connected aims to study mechanistic and therapeutic overlap between Sanfilippo syndrome (MPS III) and broader neurodegenerative disorders like Alzheimer's disease.

Aim 1: Investigate patterns of neuroinflammation and synaptic dysfunction in MPS III and compare with Alzheimer's disease.

One of the most consistent pathological features in MPS III is chronic neuroinflammation, induced by the accumulation of heparan sulfate in lysosomes (Martins et al., 2015). This deposition activates microglia, which share many of their phenotypic and transcriptomic markers with Alzheimer's microglia (Keren-Shaul et al., 2017; Hansen et al., 2018). To this end, we will first obtain RNA from postmortem brain tissue of MPS III and compare it to RNA from Alzheimer's and control brains. Differential gene expression analysis will enable the identification of whether general upregulation takes place among synaptic pruning-associated genes (like C1q, Trem2, and complement cascade genes) and neuroinflammatory pathways (like IL1β, TNF-α, and NFκB) (Hong et al., 2016; Sala Frigerio et al., 2019).

Single-cell RNA sequencing and spatial transcriptomics will be used to map glial heterogeneity and assess which brain regions exhibit overlapping damage in MPS III and Alzheimer's. Shared spatial distributions of neuronal loss or gliosis that are found would suggest conserved vulnerability (Mathys et al., 2019). The caveat here is that most MPS III samples are derived from pediatric cohorts, while Alzheimer's tissue is geriatric. That temporal gap will need to be treated with caution when making inferences.

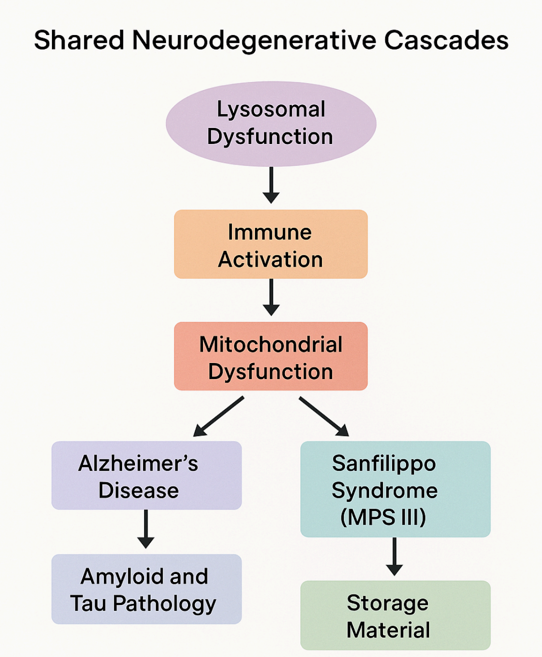


Figure 3
Shared Neurodegenerative Cascades

This objective is summarized in Figure 3, showing the shared neurodegenerative cascades of the two diseases. The figure shows that lysosomal dysfunction leads to immune activation, mitochondrial collapse, and consequent downstream neuronal death in both Alzheimer's and MPS III (Settembre et al., 2013; Zilka et al., 2012). Most importantly, the figure also shows where the pathways split, with attention to variation in amyloid deposition and tau pathology in Alzheimer's compared with storage material in MPS III. These sites of variation will guide our hypothesis testing.

Objective 2: Evaluate the therapeutic potential of CRISPR correction and monoclonal antibody delivery in MPS III models

Since MPS III is a monogenic disease, it is logical to try to correct the causative mutations directly using CRISPR/Cas9. We propose to use an adeno-associated virus (AAV9) vector system for CRISPR construct delivery against the SGSH gene (for MPS IIIA) into a mouse model (Ou et al., 2020). The sgRNAs will be constructed to target the most common pathogenic mutations. The constructs will be delivered intrathecally, and editing efficiency, behavioral recovery, and storage material accumulation will be analyzed.

In parallel, the possibility of using monoclonal antibodies (mAbs) that are engineered to cross the blood-brain barrier (BBB) for multiple purposes in MPS III will be explored. These mAbs are engineered to target transferrin receptors (TfR) for crossing the BBB via transcytosis, taking mechanisms previously demonstrated in Alzheimer's models (Niewoehner et al., 2014). The antibodies will be fused with anti-inflammatory payloads or even enzymes that target lysosomes. While this approach is not gene-corrective, it may slow neuroinflammation, which is a primary cause of neurodegeneration in MPS III (Koeberl et al., 2020).

It shall be verified by administering intranasally and intravenously TfR-targeting mAbs into MPS III mice and quantifying brain uptake via fluorescent labels. Cytokine levels and lysosomal diameter in neurons and glia will be analyzed at sacrifice. uccessful, this might assist in substantiating the broad hypothesis that technologies for neurodegenerative disease are reusable in the lysosomal storage diseases. In contrast, failure of mAb penetration or therapeutic ineffectiveness will define the limits of BBB-penetrating technologies in pediatric lysosomal disease.

Together, these two goals are aimed at testing in a direct fashion the convergence of pathophysiology and whether technologies developed for one neurodegenerative disease can be translated across others. The findings could dictate the evolution of future hybrid therapies that fuse gene editing with immunomodulation.

Conclusion

This proposal is aimed to bridge the gap between a pedietric disease disorder, Sanfilippo syndrome, and more well-understood neurodegenerative diseases like Alzheimer's. By exploiting the convergence of shared molecular mechanisms of initial neuroinflammation and synaptic degeneration, we can make novel and critical insights into understanding the disease pathogenesis. Employing CRISPR gene editing and innovative monoclonal antibody delivery technology, this research moves beyond mere discovery to actionable, targeted therapies.

Notably, this study is not single-condition focused; it's a paradigm change. If it works, it will not only improve therapeutic options for Sanfilippo but will also provide the foundation for treatments that can be applied across many neurodegenerative diseases. This is a timely and critical chance to bring cutting-edge science directly into positive clinical action for families who now have no other viable option.

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