IS EXON SKIPPING THE KEY TO CORRECT N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE DEFICIENCY? AN ANTISENSE OLIGONUCLEOTIDE THERAPEUTIC APPROACH

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INTRODUCTION: Mucolipidosis II (ML II) is a Lysosomal Storage Disorder caused by N-acetylglucosamine-1-phosphotransferase (GlcNAc-PT) deficiency, which impairs the trafficking of lysosomal hydrolases. Of all ML II mutations, c.3503_3504delTC in *GNPTAB* exon 19 is the most frequent, making it a good target for a personalized therapy. Here, we explored an innovative therapeutic strategy based on the use of antisense oligonucleotides (ASOs). Previously, in ML II patients' fibroblasts, we tested ASOs to induce exon 19 skipping in pre-mRNA, successfully generating an in-frame mRNA.

AIMS: Now, our aim is to determine whether this in-frame transcript leads to increased GlcNAc-PT levels improving ML II cellular phenotype.

Methodology: First, the GlcNAc-PT activity was measured in fibroblasts by a radioactive assay, but activity levels were similar in ML II and control fibroblasts (treated and non-treated) showing that the assay is not proper to measure endogenous levels. To overcome this, we designed 3 constructs: a WT (full *GNPTAB* cDNA), a del_ex19 (without the exon 19) and a mutant (with the mutation c.3503_3504delTC) that were transfected in HEK293T cells. Then GlcNAc-PT expression was analyzed by Western Blot (WB).

Additionally, we have measured the activity of several lysosomal hydrolases and evaluated the expression of α-galactosidase A (α-Gal) by WB after ASO treatment of control and patient cells.

To further help in the validation of this therapy we are also generating a novel GlcNAc-PT antibody in rabbits.

Results: Our results demonstrated that HEK293T cells were able to express all the constructs. The WB of both WT and del_ex19 constructs showed bands corresponding to the α/β precursor. However, only the WT construct expressed the β subunit, suggesting that there is no GlcNAc-PT activity in the absence of exon 19. As expected, the c.3503_3504delTC construct showed no expression, with no detectable α/β precursor band. We also observed a slight increase in the activity of various lysosomal hydrolases in ML II fibroblasts treated with the ASO, particularly 24h and 48h post-treatment. However, only the values relatively to the α-Gal were statistically significant, but the WB analysis using an antibody against this enzyme did not detect any band in ASO-treated ML II fibroblasts.

We also developed a novel antibody for GlcNAc-PT. Preliminary results revealed a band corresponding to the β-subunit in both control and ML II patient fibroblasts (unexpected), but in overexpression assays both WT and del_ex19 constructs presented α/β precursor bands. So, further assays are needed to assess their specificity.

Conclusion: Our ASO-based approach effectively promotes the skipping of exon 19. However, this strategy, as far as we have been able to prove, is not able to restore any GlcNAc-PT enzymatic activity. Further validation, including co-localization studies are planned to clarify these findings.