GAA deficiency in Pompe disease is alleviated by exon inclusion in iPS cell-derived skeletal muscle cells

Pim Pijnappel1,2,3, Erik Van Der Wal1,2,3, Atze Bergsma1,2,3, Holm Zaehres4, Hans R Scholer4, Ans T Van Der Ploeg2,3, Marcos J Arauzo-Bravo4

1Clinical Genetics, Erasmus University Medical Center, the Netherlands, 2Erasmus University Medical Center, Center for Lysosomal and Metabolic Diseases, the Netherlands, 3Department of Pediatrics, Erasmus University Medical Center, the Netherlands, 4Department of Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, Germany

Pompe disease is a monogenic disorder caused by mutations in the acid-alpha glucosidase (GAA) gene and leads to progressive skeletal muscle wasting. Current enzyme replacement therapy has disadvantages including incomplete efficacy, heterogeneous response, and very high costs. The common Caucasian c.-13-32T>G (IVS1) GAA mutation occurs in 90% of adults and 50% of children with Pompe disease. It causes exon 2 skipping during pre-mRNA splicing resulting in mRNA degradation, but also allows some residual wild type splicing. We reasoned that the promotion of exon inclusion in IVS1 patients may restore wild type GAA expression and provide a basis for an alternative treatment option. To this end, a screen was performed using lentiviral-mediated U7 snRNA expression of antisense oligonucleotides. Hits were tested using PMO-based antisense oligonucleotides in patient-derived primary fibroblasts. In addition, patient-derived iPS cells were differentiated using a transgene free procedure to generate purified myotubes. The U7 snRNA screen resulted in a number of target sequences in the GAA pre-mRNA that showed enhancement of exon inclusion and GAA enzymatic activity when repressed by an antisense oligonucleotide. Two of these sequences enabled the design of PMO-based antisense oligonucleotides. These restored canonical GAA splicing: splice product-specific RT-qPCR showed increased expression of wild type GAA mRNA and a concomitant decreased expression of aberrantly spliced products. Importantly, the GAA enzymatic activity in Pompe patient-derived myotubes was enhanced to levels above the disease threshold. We anticipate that antisense oligonucleotide-mediated exon inclusion may be developed into an alternative treatment option for childhood/adult onset Pompe disease.