CC-07

ANTISENSE-MEDIATED EXON SKIPPING FOR DUCHENNE MUSCULAR DYSTROPHY – CLINICAL TRIALS AND BEYOND

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Duchenne muscular dystrophy (DMD) is a severe, progressive muscle-wasting disorder, while Becker muscular dystrophy (BMD) is milder muscle disease. Both are caused by mutations in dystrophin, a protein, which stabilizes muscle fibers during contraction by linking muscle actin to the extracellular matrix. In DMD patients mutations disrupt the open reading frame, generating prematurely truncated, nonfunctional dystrophins. In BMD patients, mutations maintain the reading frame allowing production of internally deleted, partly functional dystrophins.

The exon skipping approach uses antisense oligonucleotides (AONs) to induce skipping of targeted exons during pre-mRNA splicing, with the aim of reading frame restoration, converting of the severe DMD into the milder BMD phenotype. This approach is mutation specific. However, as mutations cluster in a few hotspots, skipping of some exons applies to larger groups of patients (e.g. exon 51 skipping applies to 13%).

After obtaining proof-of-concept in cultured patient-derived cells, this approach was further optimized in animal models. In each case AON treatment resulted in targeted exon skipping and dystrophin restoration. In animal models this was accompanied by improved muscle function and quality. Proof-of-concept in patients was achieved in a clinical trial where 4 patients received local injections with an AON targeting exon 51 (coordinated by Prosensa Therapeutics). Dystrophin was restored locally for each patient.

Towards systemic application, studies in animal models revealed that dystrophic muscles facilitated uptake of 2OMePS AONs and that subcutaneous delivery was feasible. In a subsequent clinical trial, patients were subcutaneously injected with AONS targeting exon 51. Dystrophin was restored in a dose-dependent manner. All patients were enrolled in an open label extension study and have received subcutaneous AON injections at 6 mg/kg for almost 4 years. Two phase 2 and one a pivotal, double-blind, placebo-controlled multicenter trial for exon 51 skipping have recently been completed (coordinated by GlaxoSmithKline).

In parallel, preclinical studies to further optimize treatment regimens are in progress as well as clinical trials for additional exons for exon 44 skipping (PRO044, applicable to 6% of patients), exon 45 skipping and 53 skipping (PRO045 and PRO053, both applicable to 8% of patients).

The mutation specificity of the approach poses challenges to drug development regulations. A concerted effort of academic researchers, industry, regulators and patients is needed to adapt regulations to enable application of these personalized medicine approaches to rare diseases.

CC-08

DISCOVERING "X" IN THE MYOPATHIC EQUATION

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Congenital myopathies (CM) are a heterogeneous group of diseases, generally characterized by hypotonia and muscle weakness with onset at birth or during infancy and usually with a slowly progressive disease course. Scientific and technological developments in genomics over the last two decades have contributed to the identification of genetic causes for a significant number of myopathies. However, there are still several challenges to address both in diagnostics and in research. First, there is striking genetic and clinical heterogeneity associated to CM. In fact, although muscle biopsy is paramount for the diagnostic workup, pathognomonic findings such as cores, rods, central nuclei or fibre-type disproportion, are not gene-specific. In addition, a significant subset of these patients remains genetically unsolved, requiring further investigation that may lead to the identification of new genetic causes of CM.

Our recent research in congenital myopathies has focused on the mutational profile of the myotubularin gene (*MTM1*), which is defective in X-linked centronuclear myopathy (CNM). Male patients with MTM1 mutations are usually severally affected, presenting neonatal hypotonia and inability to maintain unassisted respiration. During the development and implementation of a mutation database for *MTM1* (http://www.lovd.nl/MTM1), we noticed that no large duplications had been reported. Large duplications in *MTM1* were screened by the MLPA technique in a small group of uncharacterized CNM Portuguese patients. A large duplication spanning exons 1 to 5 was identified in a boy with a mild CNM phenotype. Further characterization revealed that this duplication causes an in-frame deletion at the mRNA level (r.343_444del). Results obtained using a low-coverage next generation sequencing (NGS) approach showed that this genomic duplication extends into the neighbouring MAMLD1 gene and subsequent analysis unveiled the presence of a *MTM1*/*MAMLD1* fusion transcript [1].

This work demonstrates that it is clinically relevant to screen large *MTM1* duplications in CNM patients since this type of mutation may account for some cases that remain genetically unanswered, as was recently validated by the publication of additional cases. It also demonstrates how different analytical approaches are often required to solve the genetic complexity of congenital myopathies; the further application of NGS technology in these disorders shall be exemplified.

References

[1] Oliveira, J.; Oliveira, M.E.; Kress W.; Taipa, R.; Pires, M.M.; Hilbert, P.; Baxter, P.; Santos, M.; Buermans, H.; den Dunnen, J.T.; Santos, R. (2013). Expanding the *MTM1* mutational spectrum: novel variants including the first multi-exonic duplication and development of a locus-specific database. European Journal of Human Genetics. 21(5): 540-549.