

phospholipid de novo biosynthetic pathway, demonstrating the pivotal role of phosphatidylcholine in muscle and brain.

doi:10.1016/j.nmd.2010.07.133

O.12

A new member of the BTB/Kelch family of proteins is mutated in nemaline myopathy type 6 (NEM6)

N. Sambuughin¹, S.Y. Kyle², M. Olive³, R.M. Duff², M. Bayarsaikhan¹, P. Sivadurai⁴, K.J. Nowak², F.L. Mastaglia², K. North⁵, B. Ilkovski⁵, B. van Engelen⁶, P. Lamont⁴, M.R. Davis⁴, N.G. Laing², L.G. Goldfarb⁷

¹Uniformed Services University, Bethesda, United States, ²University of Western Australia, Perth, Australia, ³Institut de Neuropatologia, Barcelona, Spain, ⁴Royal Perth Hospital, Perth, Australia, ⁵The Children's Hospital at Westmead, Sydney, Australia, ⁶Radboud University, Nijmegen Medical Center, Nijmegen, Netherlands, ⁷National Institute of Health, Bethesda, United States

We identified a putative BTB/Kelch protein that is mutated in nemaline myopathy type 6 (NEM6), an autosomal dominant congenital neuromuscular disorder characterized by the presence of nemaline rods and core-like lesions in the skeletal myofibers. Analysis of the Dutch and Australian–Dutch pedigrees previously reported by Gommans et al. [1] and the newly identified Spanish and Australian–Belgian families allowed to narrow the candidate region on chromosome on 15q22.31. Screening of 27 genes within the candidate region led to the identification of a previously uncharacterized NEM6 gene showing missense c.1170G>C (p.K390N) and c.1222C>T (p.R408C) mutations that perfectly co-segregate with the disease in the Spanish, and Dutch, Australian–Dutch and Australian–Belgian families, respectively. Screening of 14 other probands with core-rod myopathies identified a c.742C>A (p.R248S) mutation in another Australian family. The NEM6 mutations were not detected in >250 (p.K390N and p.R408C), or >50 (p.R248S), controls of different ethnic backgrounds. The approximately 1.5-kb mRNA of NEM6 has a single open reading frame encoding a protein with a calculated molecular mass of 49 kDa. The NEM6 protein contains a BTB/POZ domain and five Kelch-like repeats and is expressed strongly in skeletal and cardiac muscle. The BTB/POZ and kelch domain-containing proteins have been implicated in a broad variety of biological processes, including cytoskeleton modulation, regulation of gene transcription, ubiquitination, DNA binding, and cell migration. The functional role of the NEM6 protein in skeletal muscle and pathogenesis of nemaline myopathy are subjects of further studies.

doi:10.1016/j.nmd.2010.07.134

NEW THERAPEUTIC TARGETS FOR NEUROMUSCULAR DISORDERS 1; INVITED LECTURES, ORAL PRESENTATIONS

T.I.1

Pharmacological therapies in muscular dystrophies

K.M. Flanigan

Center for Gene Therapy, The Research Institute of Nationwide Children's Hospital, Columbus, United States

The prospect of gene replacement continues to hold great promise for the treatment of the monogenic muscular dystrophies. However, at least for the dystrophinopathies, the mainstay of treatment remains pharmacologic – corticosteroid therapy – although

three decades after they were first used, many issues regarding the use of steroids remain open. Other pharmacologic therapies hold promise, and are currently entering or completing trials. One approach is gene correction by exon skipping induced by antisense oligonucleotides; the promises and challenges of this potential therapy will be reviewed. A second approach stimulates therapeutic recoding of the mRNA at translation; examples include both the aminoglycoside gentamicin and newer orally available agents that stimulate readthrough of nonsense mutations. Mutation sequence context effects may play a role both in the disease phenotype associated with nonsense mutations, and with the response to these pharmacologic therapies. Other potential pharmacologic therapies entering clinical trials are not dependent upon a specific mutation class, but instead seek to modulate molecular pathways important to muscle development or maintenance, such as myostatin inhibition and utrophin upregulation. The rationale for and current status of trials in these and related therapies will be reviewed.

doi:10.1016/j.nmd.2010.07.135

T.I.2

Prospects of AAV-mediated gene therapy for neuromuscular disease

T. Voit

Institut de Myologie, Université Paris VI, UMR S 974, INSERM U 974, CNRS UMR 7215, Paris, France

AAV-mediated gene therapy has been successfully applied to different neuromuscular disorders at the level of the animal model. Different strategies have been followed such as U7- or U1-mediated exon skipping for Duchenne muscular dystrophy or for dysferlinopathy, AAV-mediated gene transfer for α -, γ - or δ -sarcoglycan deficiency or scAAV-mediated transfer of SMN1 into α -motoneurons for spinal muscular atrophy. The rapid translation into therapy of the Human is hampered by different constraints which include regulatory aspects but also technical issues such as large scale production under GMP conditions. We will describe further, hitherto unrecognized limitations due to the innate immune system which have a strong impact on the transfer of AAV therapy from the mouse model to the human. At the same time, AAV-mediated gene therapy offers a versatile tool which permits other types of gene defect correction such as long-lasting gene trans-splicing, exon inclusion, gene silencing, destruction of RNA aggregates or even other novel, not hitherto described applications. This technology is therefore on principle capable of addressing a large scale of different types of genetic diseases including those due to dominant or recessive alleles or diseases due to triplet repeat expansions. Proof of principle for several of these applications will be demonstrated. A further particular requirement for any type of gene therapy is target tissue specificity, and important new insights have been gained recently for several AAV serotypes regarding organ-specific transduction. Based on current state of the art AAV-based gene therapy concepts, strategies to develop this technology towards a clinical application will be discussed.

doi:10.1016/j.nmd.2010.07.136

O.13

Systemic delivery of AAV vectors to large animal models for DMD

J.S.C. Chamberlain

University of Washington, Neurology, Seattle, United States

Duchenne muscular dystrophy (DMD), is caused by mutations in the dystrophin gene. We are developing methods to deliver therapeutic genes to muscles throughout the body to either replace the missing dystrophin gene or to help compensate for the lack of dystrophin. We show that shuttle vectors derived from adeno-associated virus type 6 (rAAV6) are able to deliver genes to muscles throughout the body of adult mice when injected directly into the bloodstream. rAAV6 delivery results in highly efficient gene expression in skeletal and cardiac muscle that persists for the lifespan of the mouse. To accommodate the limited cloning capacity of rAAV vectors we have designed a variety of different micro-dystrophin vectors, and a recently modified micro-dystrophin with alterations in the hinge domains increases functionality. We have begun testing these AAV vectors in wild type and dystrophic dogs, and in wild type non-human primates. These studies revealed a cellular immune response directed against the AAV capsid proteins, but which could be blocked by short-term immune suppression, leading to long-term dystrophin expression. We have observed that delivery of AAV6 vectors into various veins and arteries of the dog results in efficient gene transfer to downstream muscles, but does not lead to whole body gene transfer. Instead, it appears that vector will need to be delivered into multiple vascular sites to target muscles body wide. These results suggest that a combination of intravascular AAV delivery coupled with transient immune suppression could lead to an effective therapy for DMD.

doi:10.1016/j.nmd.2010.07.137

O.14

Exon exchange approach to repair Duchenne dystrophin transcripts

S. Lorain, C. Peccate, M. Le Hir, G. Griffith, T. Voit, L. Garcia

Université Pierre et Marie Curie UMR S 974 – Inserm U974 – CNRS UMR 7215, Institut de Myologie, Paris, France

Trans-splicing strategies for mRNA repair involve engineered transcripts designed to anneal target mRNAs in order to interfere with their natural splicing, giving rise to mRNA chimeras where endogenous mutated exons have been replaced by exogenous replacement sequences. A number of trans-splicing molecules have already been proposed for replacing either the 5' or the 3' part of transcripts to be repaired. Here, we show for the first time the feasibility of RNA surgery by using a double trans-splicing approach allowing the specific substitution of a given mutated exon. As a target, we used a minigene encoding a fragment of the mdx dystrophin gene enclosing the mutated exon (exon 23). This minigene was cotransfected with a variety of exon exchange constructions, differing in their annealing domains. We obtained accurate and efficient replacement of exon 23 in the mRNA target. Adding up a downstream intronic splice enhancer DISE in the exon exchange molecule enhanced drastically its efficiency up to nearly 50% of repair. These results demonstrate the possibility to fix up mutated exons, refurbish deleted exons and introduce protein motifs, while keeping natural untranslated sequences, which are essential for mRNA stability and translation regulation. Conversely to the well known exon skipping, exon exchange has the advantage to be compatible with any type of mutations and more generally to a wide range of genetic conditions. In particular, it allows addressing disorders caused by dominant mutations.

doi:10.1016/j.nmd.2010.07.138

NEW THERAPEUTIC TARGETS FOR NEUROMUSCULAR DISORDERS 2; ORAL PRESENTATIONS

O.15

24 week follow-up data from a phase I/IIa extension study of PRO051/GSK2402968 in subjects with Duchenne muscular dystrophy

N. Goemans¹, M. Tulinius², G. Buyse¹, R. Wilson³, S. de Kimpe⁴, J. van Deutekom⁴, G. Campion⁴

¹University of Leuven, Leuven, Belgium, ²University of Gothenburg, Gothenburg, Sweden, ³Spica Consultants Ltd., London, United Kingdom, ⁴Prosensa Therapeutics, Leiden, Netherlands

Objective: To evaluate the efficacy and safety after 24 weeks of treatment with PRO051/GSK2402968 in boys with Duchenne muscular dystrophy (DMD). **Background:** DMD patients suffer from progressive muscle degeneration due to mutations in the DMD gene and resulting absence of functional dystrophin in the muscle cell wall. PRO051/GSK2402968 is an antisense oligonucleotide compound which induces exon 51 skipping during pre-mRNA splicing and produces novel dystrophin expression in a subpopulation of DMD patients. **Design/methods:** 12 DMD patients (11 ambulatory, 1 non-ambulatory at study entry) completed a dose-escalation Phase I/IIa study (Netherlands Trial Register #124) and entered an open-label extension study. All subjects received weekly subcutaneous injections of 6 mg/kg of PRO051/GSK2402968 in the extension study, regardless of earlier dose. All subjects were at stable steroid doses during the study. Study visits were performed at baseline and 4 weekly thereafter. **Results:** All patients completed 24 weeks of treatment and reported treatment-emergent AEs. Mild proteinuria was detected in all boys at some point during continued treatment, confirmed as greater than the upper limit of normal in 2 boys in subsequent 24 h collection. There were no severe treatment-related AEs. Two serious AEs (not treatment related) were reported. The most common AE was local injection site reaction, none were considered severe. A raised GGT level was observed in one patient. The mean (SD) 6MWD in patients increased from baseline by 36.8 (59.8) m ($n = 10$) in this heterogeneous population. Two boys, aged 9 and 11 observed a 115 m increase over the 24 week period. **Conclusion:** PRO051/GSK2402968 6 mg/kg administered weekly by subcutaneous injection was generally well tolerated across 24 weeks of treatment in DMD patients with mutations/deletions correctable by exon 51 skipping. Renal and hepatic function warrant further monitoring. Encouraging gains in the 6MWD were observed in some boys.

doi:10.1016/j.nmd.2010.07.139

O.16

Current progress and preliminary results with the systemic administration trial of AVI-4658, a novel phosphorodiamidate morpholino oligomer (PMO) skipping dystrophin exon 51 in Duchenne muscular dystrophy (DMD)

S.B. Shrewsbury¹, S. Cirak², M. Guglieri³, K. Bushby³, F. Muntoni²

¹AVI BioPharma, Clinical, Bothell, WA, United States, ²Institute of Child Health, London, United Kingdom, ³Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom

Objective: AVI BioPharma in collaboration with the MDEX consortium, UK, have identified a PMO to skip dystrophin RNA exon 51 in patients with DMD, restore the reading frame and enable expression