

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

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O.12

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

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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.

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

T.I.1

Pharmacological therapies in muscular dystrophies

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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.

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T.I.2

Prospects of AAV-mediated gene therapy for neuromuscular disease

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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.

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O.13

Systemic delivery of AAV vectors to large animal models for DMD

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