showed that these dogs do not carry the GRMD mutation and the identification of their mutation is in progress. This shows that it is possible to have a large functional muscle despite the lack of dystrophin. Determination of the factors protecting these dogs from the severe effects of dystrophin deficiency could have importance for future therapeutic trials in PMD.

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O.3

Identification of a novel therapeutic pathway for Duchenne muscular dystrophy through utilization of the zebrafish model sapje

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Duchenne muscular dystrophy (DMD) is the most common neuromuscular disease of childhood, affecting approximately 1:3,500 males. DMD is associated with significant morbidity and early mortality, with loss of ambulation by age 13 and death in the 20s. Few adequate therapeutic interventions exist for DMD, and there is currently no cure for this devastating disease. In an effort to identify novel treatment approaches for DMD, we have undertaken a large-scale chemical screen in a zebrafish model of the disease. This model, called sapje, has a recessive mutation in the dystrophin gene that results in essentially no dystrophin protein expression. Sapje also has severe motor impairment, dystrophic muscle by histopathologic analysis, and early death. In addition, the sapje zebrafish, like all described zebrafish models of muscular dystrophy, exhibits an abnormal light scatter pattern when exposed to polarized light (i.e. abnormal birefringence). We have taken advantage of this easily screenable property of sapje to test the ability of a panel of 640 FDA approved drugs to ameliorate the dystrophic phenotype. To date, we have identified 3 drugs that prevent the development of abnormal birefringence. The first is aminophylline, a phosphodiesterase inhibitor previously demonstrated to improve aspects of the DMD phenotype in multiple models. The other two "hits" are modifiers of a monoamine neurotransmitter signaling pathway and have not previously been associated with DMD pathogenesis. Each compound delays or prevents the development of the dystrophic phenotype in the sapje zebrafish, and, in addition, rescues the motor defect and improves survival. We present the further characterization of these novel therapeutic targets, including dose–response analysis, studies related to interrogation of the relevant signaling pathway (both in the treated state as well as after exposure to the therapeutic agent), and extension of our findings to the mdx mouse model of DMD.

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O.4

Forelimb loco-regional injection of rAAV8-U7snRNA in GRMD dogs allows dose-dependant dystrophin expression and phenotypic correction

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In Duchenne Muscular Dystrophy (DMD) the selective removal by exon skipping of exons flanking an out-of frame mutation in the dystrophin messenger can result in in-frame mRNA transcripts that are translated into shorter but functionally active dystrophin. The goal of our project was to determine in the dog model of DMD, the GRMD, the effective dose of our therapeutic product defined as a recombinant Adeno-Associated Virus serotype 8 (rAAV8) expressing a modified U7 snRNA specific for the skipping of exons 5–10 of the GRMD dystrophin transcript. The mode of delivery was the locoregional high-pressure intravenous (IV) injection of a forelimb. Five groups of 2–3 GRMD dogs were exposed to different rAAV8-U7snRNA doses (from 2.5×10^{12} µg/kg to 2.5×10^{13} µ/kg diluted in two different volumes) and two dogs sham-injected with ringer-lactate. Each dog was followed 3 months after injection until sacrifice and full autopsy. The primary outcomes were the restoration of dystrophin expression and the improvement of the tissue pathology in the injected limb and in muscles behind the tourniquet. Results demonstrate high level (>80%) of dystrophin expression in dogs injected with 2.5×10^{13} µg/kg. In addition, significant dystrophin expression (5–20%) was observed in other muscles systemically. Lower levels of dystrophin expression (~30–40%) and (~10%) were observed in dogs injected with doses 5 or 10 times lower, respectively. An excellent correlation was found between the viral copy number/diploid genome and the dystrophin amount expressed. Strength improvement up to 50% was demonstrated in muscles with more than 45% dystrophin expression. In addition, we observed a dose-dependant correction of various RMN pathological indexes. This demonstrates the feasibility and the dose-dependant effect of locoregional rAAV8 U7snRNA approach in a large animal model of DMD and opens the way for a human trial targeting the upper limb of non ambulatory patients.

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CLINICAL ASPECTS: POSTER PRESENTATIONS

P1.1

Deletion of exon 26 of the dystrophin gene is associated with a mild Becker muscular dystrophy phenotype

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With the possible introduction of exon skipping therapy in Duchenne muscular dystrophy, it has become increasingly important to know the role of each exon of the dystrophin gene protein expression, and thus phenotype. In this report, we present two related men with an unusually mild BMD associated with an exon 26 deletion. The proband, a 23-year-old man, had slightly delayed motor milestones, walking one and a half years old. He had no complaints of muscle weakness, but had muscle pain. Clinical examination revealed no muscle wasting or loss of power, but his CK was 1500–7000 U/l. Muscle biopsy showed dystrophic changes. He had comorbidity with dystonia, slight mental retardation, low stature and neuropathy. The brother of the probands mother came to medical attention when he was 43 years old. He complained about muscle pain. On examination, a MRC grade 4 hip extention palsy and a discrete calf hypertrophy were noted. Creatine kinase was normal or raised maximally to 500 U/l. The muscle biopsy was myopathic with increased fiber size variation and many internal nuclei, but no dystrophy. No comorbidity was found. In both cases, Western blot showed a reduced dystrophin band. Genetic evaluation revealed a deletion of exon 26 of the dystrophin gene in both. This is the first description of persons carrying exon 26 deletions of the dystrophin gene. Assuming the probands comorbidity is unrelated, exon 26 deletion results in a very mild phenotype. This might be of interest in planning exon skipping therapy for Duchenne muscular

dystrophy. This report also shows that BMD may present with a normal CK.

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P1.2

Phenotypic profile of dystrophinopathy patients with deletion of exons 3–7 of the dystrophin gene

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Deletion of exons 3–7 is an out of frame deletion predictive of Duchenne muscular dystrophy (DMD) but is commonly associated with a milder phenotype. An accurate clinical profile of such patients would be of value for prognosis and patient selection for clinical trials. To characterize the clinical phenotype of dystrophinopathy patients with deletion of exons 3–7. IRB approved retrospective case series' review. Patient characteristics: 14 ambulatory males with deletion of exons 3–7. Mean age at last visit – 11.1 yrs (range 5.7–15.8); mean CK (4–10 yr) – 13310 (4269–26,020); FH DMD in 6/14 – mean age of loss of ambulation (LOA) 12.9 yr (10–18) in 5; and deaths at 19–44 yrs. Muscle biopsy (3/14) – decreased dystrophin. MRI pelvic muscle (13/14) – mild to moderate fatty changes. Steroid therapy (daily deflazacort): start mean age 7.1 (3.0–11.1 yr); mean duration 3.9 yr (0.5–12.8); mean dose at last visit 0.5 mg/kg/day (0.2–0.8). Neuromotor function: history of delayed walking >16 months – 2/14 (brothers with hypotonia); 12/14 with onset of motor abnormalities between ages 1.5–5 and 2/14 between ages 6–8; presenting difficulties – toewalking (4/13); slow/abnormal run (8/13); fatigue (1/13); difficulty in rising from the floor - 0/14. Gower's maneuver (from sit to stand, $n = 13$) – mean 1.8 s (1.1–2.9); 30 feet run ($n = 14$) – 3.79 s (2.8–6.72); 13/14 with ability to jump with both feet and climb up steps with reciprocating pattern; 14/14 with antigravity neck and trunk flexion from supine; 12/14 able to hop on one leg. Cardiopulmonary function: normal 14/14. Neurocognitve function: normal 11/14. Patients with deletion of exons 3–7 who present with mild motor difficulties by age 5 have minimal motor limitations on follow up (on steroid therapy) and normal cardiopulmonary function. This clinical profile of a milder phenotype would have implications for prognosis and clinical trials.

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P1.3

Spectrum of point mutations in Czech DMD/BMD patients and their phenotypic outcome

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Duchenne and milder Becker muscular dystrophies (DMD/BMD) are X-linked recessive neuromuscular diseases, both caused by mutations in the DMD gene. DMD is typically associated with mutations causing premature stop codon creation, while BMD is usually related to in-frame deletions or duplications. In our study population, excluding 60% of deletions and 5% of duplications detected in DMD gene, we identified 63 different point mutations in 69 DMD/BMD patients and DMD/BMD female carriers. For mutation screening on the RNA level, we used reverse transcription-PCR, protein truncation test, and DNA sequencing. For screening on DNA level, we used PCR and sequencing. We describe patients with a mutation creating a premature termination codon but with a mild BMD phenotype, which present three different ways of rescuing the DMD phenotype. In one patient we detected the insertion of a repetitive sequence AluYa5 in intron 56, which led to skipping of exon 57. On the other hand, we present two patients with mutations deep inside the intron leading to severe DMD phenotype by creating pseudoexon and leading to frame-shift. Among presented mutations, there are 32, which are unique for Czech population. This work was supported by Grants MSMT LC06023 and 2B08060.

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P1.4

Molecular profile of 307 Portuguese patients with dystrophinopathy, including 39 new variants

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Mutations in the dystrophin gene (*DMD*) give rise to the allelic Duchenne or Becker muscular dystrophies. Besides providing a differential diagnosis for adequate clinical follow-up and management, the molecular characterization of these patients is becoming increasingly important in light of the recent and promising mutation-based therapeutic approaches. Due to the size and complexity of *DMD*, as well as the diversity of mutation types, molecular analysis requires a combination of techniques that enable the detection of gross deletions, duplications and the more subtle point mutations. In the course of our diagnostic service provided on a national basis, a total of 307 patients, representing 282 unrelated families, have been characterized at the molecular level. We identified 174 different mutations, where the distribution according to type was found to be in agreement with that reported in the literature for large cohorts. Also as expected, approximately 1/3 of the cases were shown to be *de novo* occurrences, as ascertained among the "sporadic" cases (25/82). These neo-mutations were comprised by 18% deletions, 6% duplications and 6% point mutations. We describe a total of thirty-nine undocumented variants, three of which were detected in obligate carrier female relatives of deceased patients. These new variants include 9 gross deletions, 8 gross duplications and 22 smaller mutations (deletions, duplications, *delins* rearrangements and nonsense or splice-site substitutions). Comprehensive analysis often involved expression studies at the mRNA level to help delineate breakpoint junctions, to identify altered splicing and ultimately to provide an explanation for apparent exceptions to the reading frame rule. This detailed molecular characterization is also important for the purpose of including our patients in the DMD National Registry, which will be articulated with the TREAT-NMD Global Database.

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