muscle biopsy, sarcoidosis was confirmed, and tattoo pigmentation was prominent in that area. Afterward, the patient showed signs of improvement with the use of steroids. Sarcoidosis is a disease in which various symptoms may appear throughout the body, and when multiple damages, such as nerves and muscles, are found in patients with tattoos, suspicion is required, and related tests are needed for quick diagnosis.

http://dx.doi.org/10.1016/j.nmd.2023.07.137

P340

The MikroIBioM study - Comparison of gut microbiome of sporadic Inclusion Body Myositis (sIBM) patients and unaffected spouses

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Sporadic inclusion body myositis (sIBM) is a disorder with features of both inflammation and degeneration but yet without effective treatment. Influences of the gut microbiome on degenerative as well as inflammatory disorders and immune treatments are known. Curious whether the gut microbiome might influence the development or recalcitrance of sIBM, we appealed to sIBM patients and their unaffected spouses for stool samples and data on stool, gastrointestinal symptoms and nutrition. We included 22 patients (n=2 clinically, n=20 clinico-pathologically defined; m:f 18:4; 51 – 83 years) and 21 controls (m:f 8:13; 53 – 82 years) for 16S rRNA V3V4 metagenomic analysis of their stool samples. In addition, modified Gastrointestinal Symptom Rating Scale, IBM Functional Rating Scale and Bristol Stool Scale were recorded. Bioinformatic analysis was performed using Qiime2 and MicrobiomeAnalyst software packages. LEfSe and Random Forest analysis were used to identify biomarkers, specific to the patient and control group. PICRUSt was used to perform pathway analysis. Checking across all controls vs patient samples showed no significant differences for various alpha and beta diversity metrics. But a significant ($p < 0.05$) reduction in alpha diversity (observed features) was found for older (72+ years) patients compared to control. Increased abundances of the genera Lachnospira, Bacteroides, CAG-325, Defluviitaleacea (UCG-011) and Family XIII AD3011 group were detected for the patient group. Our high-level microbiome analysis did reveal significant differences of the gut microbiome in patients only for older subjects, indicating that a more refined analysis may be needed. Cause-effect relationships are notoriously difficult to determine for microbiome changes in diseases, and our sIBM findings are no exception to this.

http://dx.doi.org/10.1016/j.nmd.2023.07.138

DMD – TREATMENTS

P12

Comparison of U7snRNA-induced dystrophin expression following systemic delivery with AAV9 and AAVrh74 capsids

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We have developed a U7snRNA vector that targets DMD exon 2 to result in exclusion of this exon from the mature mRNA. Preclinical studies resulted in significant expression of full-length dystrophin in our duplication of exon 2 (Dup2) mouse model, and the scAAV9.U7.ACCA vector is now in a clinical trial (NCT04240314). Here we assess efficiency of AAV9 and AAVrh74 capsids by delivery of this transgene at clinically relevant doses, as judged by RT-PCR and by dystrophin expression. Both vectors were intravenously delivered in a blinded fashion at a dose of 8E13 vg/kg to Dup2 mice at 10-12 weeks of age (n=6 mice per group), mice were euthanized 4 weeks later for analysis of mRNA splicing by RT-PCR, dystrophin expression by both quantitative immunofluorescence (IF) and western blot (WB) analyses, and vector genome biodistribution by qPCR. Biodistribution by transgene-specific qPCR showed no significant difference between capsids. In tibialis anterior (TA), Quadriceps (Quad) and Triceps Brachii (Tri) muscles, mRNA splicing efficiency by RT-PCR showed that AAV9-encapsidated vector outperformed AAVrh74-encapsidated vector, with AAV9 resulting in 74-85% total therapeutic transcript and AAVrh74 resulting in 43-48%. Protein expression in skeletal muscle was consistently better in Dup2 mice treated with AAV9.U7.ACCA. In TA, percent dystrophin-positive fibers (PDPF) by IF analysis were 57.8% for AAV9 and 35.3% for AAVrh74, while in diaphragm AAV9 showed 68.2% PDPF vs. AAVrh74 with 49.4%. In cardiac tissue, AAVrh74 appeared superior, with PDPF of 91.8% versus 79.4% for AAV9. These results suggest that robust target engagement for alteration of splicing can occur with either serotype, presenting an additional clinical avenue for therapeutic delivery. However, the AAV9 capsid ultimately results in greater skeletal muscle dystrophin expression.

http://dx.doi.org/10.1016/j.nmd.2023.07.139

P13

Comparison of U7snRNA-induced dystrophin expression following systemic delivery with AAV9, MyoAAV 2A, and MyoAAV 3A capsids in the Dup2 mouse

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Duplications of DMD exon 2 are the most common duplication mutations causing Duchenne muscular dystrophy. We have developed a vector (scAAV9.U7.ACCA) carrying four copies of U7 small nuclear RNA (U7snRNA) that targets exon 2, resulting in its exclusion from the mature mRNA. This vector is now in a clinical trial (NCT04240314). Here we evaluate the efficiency of exon 2 skipping and dystrophin restoration by U7snRNA delivered using two recently described myotrophic AAV capsids (MyoAAV 2A and MyoAAV 3A) and compare their efficacy to AAV9. Each vector was delivered intravenously at three doses (1E11, 1E12, and 1E13 vg/kg) in a mouse model of exon 2 duplication. Mice were treated at 11–15-weeks of age and euthanized 4 weeks post-injection for analysis. Tissues were analyzed for DMD splicing by RT-PCR, and dystrophin expression by quantitative immunofluorescence (IF) analysis and western blot (WB). Vector biodistribution was assessed by qPCR. In the gastrocnemius, at the highest dose, AAV9 resulted in 42% of exon 2 skipping and 16% of dystrophin protein expression by western blot, comparable to the middle dose of MyoAAV 2A (41% skipping and 11% protein) and MyoAAV 3A (37% skipping and 15% protein). In contrast, at the highest dose, MyoAAV 2A demonstrated 92% skipping and 14% protein, and MyoAAV 3A resulted in 90% skipping and 26% protein. IF analysis was consistent with RT-PCR and WB results. Comparable results were seen in the diaphragm. In the gastrocnemius, biodistribution of MyoAAV 2A and MyoAAV 3A was similar at all doses, and 10-fold higher than AAV9. At the highest dose, MyoAAV 2A and MyoAAV 3A were detected at 15 and 19 vector copies per diploid genome, respectively. In the heart, MyoAAV 2A and MyoAAV 3A likewise showed 10-fold higher biodistribution than AAV9. Our results support the improved muscle tropism of the MyoAAV capsids and suggest that use of these capsids may both improve efficiency of U7snRNA-induced splice alterations at a given dose and diminish the dose required for a therapeutic effect.

http://dx.doi.org/10.1016/j.nmd.2023.07.140

P14

U7snRNA-mediated exon skipping as a powerful therapeutic tool for the treatment of DMD

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Duchenne muscular dystrophy (DMD) is a progressive X-linked muscle disease caused by mutations that disrupt the open reading frame (ORF) of the DMD gene, resulting in the absence of dystrophin, a muscle-specific structural protein crucial for muscle cell integrity. The gene corrective therapy using U7 small nuclear RNA (U7 snRNA) resulting in a selective exclusion of exon 2 in DMD patients who carry the Dup2 mutation is an approach that is already in clinical trial. Here, we developed a viral-based exon-skipping platform utilizing rAAV to encapsidate a single copy of U7snRNA targeting the splice acceptor (SAS), donor (SDS) or enhancer (ESE) sites of the following DMD exons: 12, 17, 18, 19, 21 and 46. All sequences were designed using Human Splicing Finder database. Infection and assessment of exon skipping was performed in Immortalized tet-inducible-MyoD fibroblasts (FM) cells derived from healthy or DMD patient primary fibroblasts. Treatment with doxycycline allows FM cells to be transdifferentiated into myoblasts, and then myotubes, allowing characterization of DMD mRNA splicing and dystrophin expression. In this study, we used FM cells with exon duplications and deletions amenable to adjacent exon skipping. The FM cell lines were treated with various rAAV.U7snRNA doses and then they were differentiated for 7-14 days. The untreated FM cells from healthy patient were used as a control. To detect the exons skipping level at each dose, RT-PCR was performed using exon-specific sets of primers. The data revealed that most sequences targeting individual DMD exons showed a dose-dependent exon skipping in the tested cells. This skipping results in WT or in-