being performed twice on separate days to test reproducibility. The MR data acquisition is performed on a Philips 3T Achieva, Siemens Verio, or Siemens TIM Trio system. Reproducibility of the MR measures have been evaluated for the soleus using MRI-T2 (Day 1: 43 ± 9 ms, Day 2: 44 ± 9 ms; CV 2.2 ± 1.9%), T2 of 1H2O from spectroscopic relaxometry (Day 1: 30.3 ± 2.6 ms, Day 2: 30.4 ± 2.9 ms; CV 2.1 ± 1.5%), and the ratio of lipid/lipid + water using 1H-spectroscopy (Day 1: 0.32 ± 0.23 ms, Day 2: 0.32 ± 0.22 ms; CV 4.8 ± 2.8%). In summary, the MR measures implemented in this multisite study are highly reproducible in children with DMD and controls. These noninvasive measures show promise for evaluating disease progression and treatment in DMD subjects, and are continuing to be evaluated in this multi-center study.

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P1.42

Inter- and intra muscle fat fraction variability in DMD patients

B. Wokke a, J. van den Bergen a, C. Bos b, A. Webb b, I. Ginjaar c, A. Aartsma-Rus d, J. Verschuuren a, H. Kan b

a Leiden University Medical Center, Neurology, Leiden, Netherlands; b Leiden University Medical Center, Radiology, Leiden, Netherlands; c Leiden University Medical Center, Clinical Genetics, Leiden, Netherlands; d Leiden University Medical Center, Human Genetics, Leiden, Netherlands

Therapy development for Duchenne muscular dystrophy (DMD) highlights the relevance of noninvasive quantitative methods for therapy follow-up. MRI can be used to evaluate muscle pathology in DMD patients, like edema and fatty infiltration. We aimed to quantitatively assess fat fraction variation between and within lower extremity muscles of DMD patients using the 3-point Dixon MRI method. Ten DMD patients (age 10.5, b 2.5 years) and eight healthy age-matched controls were scanned. Eleven upper and seven lower leg muscles were analyzed in minimally 10 slices. Obtained fat fractions were correlated with age and approximated with linear regression to obtain an overview of the rate of fatty infiltration per muscle. For analysis of fat fraction variation within the muscle the two most proximal and distal slices were compared using a paired t-test. Fat fractions ranged from 3.7% to 92.5% with relative sparing of the semitendinosus, gracilis and sartorius and posterior tibialis muscle. The peroneus and posterior tibialis muscle showed the greatest variation of fat fraction increase per year, 14% per year (R2 = 0.87) in the peroneus and 5.6% per year (R2 = 0.74) in the posterior tibialis muscle. In the medial and lateral gastrocnemius and anterior and posterior tibialis muscle fat fraction variation between the most distal and proximal slices was present, respectively up to 30% in gastrocnemii and up to 18% in the tibialis muscles. We found a large variation in the fat fraction of the total muscles and between distal and proximal regions of the muscles. In this crosssectional study the yearly increase of fatty infiltration was quite different between muscles. Quantification of fatty infiltration could be useful for quantitative therapy follow-up, whereby analysis of only the most and least affected muscle might provide sufficient information to evaluate the disease process.

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P1.44

Development of heart failure in mice with low dystrophin levels

E.M. van der Phl a, M. van Putten a, V.D. Nadarajah a, A.M. van Opstal a, M.A. Hulsken a, P.A.C. ’t Hoorn a, L. van der Weerd b, A.M. Aartsma-Rus b

a Leiden University Medical Center, Human Genetics, Leiden, Netherlands; b Leiden University Medical Center, Radiology, Anatomy and Embryology, Leiden, Netherlands

Duchenne and Becker muscular dystrophy are X-linked myopathies caused by mutations in the DMD gene. Both Becker and Duchenne patients develop severe heart failure which eventually can lead to death. Several therapeutic approaches aiming at restoration of dystrophin are currently under investigation, but the heart appears to be a difficult target. Furthermore, it is unknown which dystrophin levels are sufficient to prevent or delay the onset of heart failure. To study this, we used MRI to assess the heart function of mdx-Xist mice, expressing low dystrophin levels (1–20%) as a consequence of non-random X-inactivation, mdx mice and two wild type mouse strains (C57BL/10ScSnJ and Xist–/–). Ejection fraction, cardiac output, stroke volume and end-diastolic volume of the left and right ventricle were assessed in mice aged 2, 6 and 10 months. The physiological parameters were complemented with analysis of serum biomarkers for heart failure. Troponin I levels were increased with age in dystrophic mice but no significant difference was observed between mdx-Xist–/– and mdx mice. NT-proBNP levels were essentially undetectable in the different groups of mice. CK levels decreased with age in dystrophic mice and were normalized towards wild type in mdx-Xist–/–. A dystrophin level dependent reduction of the degree of fibrotic tissue in the heart was observed in the 10 months old mdx-Xist–/– mice compared to mdx mice. Expression levels of genes involved in heart function, immunological and fibrotic processes revealed a dystrophin level dependent normalization towards wild type levels in the mdx-Xist–/– mice. The combined results suggest that dystrophin levels of <20% of wild type levels

a University of Messina, Department of Neurosciences, Psychiatry and Anaesthesiology, Messina, Italy; b University of Messina, Department of Radiology, Messina, Italy

The dual-echo dual-flip angle spoiled gradient recalled is a new MRI technique which allows to accurately quantify and display the muscle fat fraction (MFF). We recently showed that it provides an accurate fat quantification when compared to muscle biopsy findings in different neuromuscular disorders. Moreover it is easy to be used in pediatric population having a short acquisition time and is available in all MRI scanners. We prospectively evaluated the MFF of all thigh muscles in 20 ambulant Duchenne muscular dystrophy (DMD) patients. We correlated the MRI data with the results of selected outcome measures: Medical Research Council (MRC) scale, timed Gowers’ sign, time-to-run 10 m. The technique was able to quantify low amount of fat, not detected by the conventional qualitative T1-weighted techniques in younger boys even in absence of motor impairment. Defining the disease distribution with MFF color-coded maps, we showed that gluteus maximus had the highest mean MFF (46.3 ± 24.5%) and the gracilis had the lowest (2.7 ± 4.7%). A significant positive correlation was found between the mean MFF of all muscles and patients’ age (P < 0.005), MRC score (P < 0.001), timed Gowers’ sign (P < 0.03) and time-to-run 10 m (P < 0.001). We have demonstrated that MFF calculation and mapping are objective, sensitive and non-invasive tools to evaluate the degree and distribution of muscle fatty infiltration in DMD. This approach could be helpful in forthcoming clinical trials, particularly if focused on disease early phases. Moreover, the use of MFF maps may be of relevance in monitoring gene-therapy effects in specific muscle areas after local administration.

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Application of NMR spectroscopy in the study of mdx mouse
A.B. Martins-Bach a, A.C. Bloise b, S.R. Rabban b, M. Vainzof a
a University of São Paulo, BioScience Institute, São Paulo, Brazil; b University of São Paulo, Physics Institute, São Paulo, Brazil

The mdx mouse is the classical animal model for Duchenne Muscular dystrophy, showing similar molecular and protein defects. It is widely used to investigate the pathogenesis of the disease aiming the development of therapeutic strategies. The mdx, however, does not show the significant muscle weakness observed in humans, and the diaphragm muscle is usually used as a marker of progression, due to its severe pattern of degeneration. High resolution 1H nuclear magnetic resonance spectroscopy (MRS) was used in this work to study the metabolic profile of quadriceps and diaphragm muscles, comparing mdx and control mice at 3 and 6 months of age. 1D and 2D MRS techniques were used, and analysis of the spectra were done by direct comparison between peak areas and by principal component analysis (PCA). Among the 20 major identified metabolites, 14 showed significant differences between the groups. These metabolites could be important key biomarkers associated to natural aging in control mice and to the progress of the dystrophy in mdx mice, which involves continuous degeneration and regeneration of the skeletal muscle tissue. Glutamate, glutamine, and β-hydroxybutyrate were increased in mdx samples when compared to control mice, in contrast to carnosine, that was consistently decreased. Creatine, taurine, glycerol, carnitine and succinate also presented alterations. Mdx mice could be distinguished from control individuals in both ages and muscles. Additionally, mice from the same lineage were significantly different when comparing 3 and 6 months old animals for quadriceps samples, due to the effects of aging and dystrophy progression in quadriceps’ metabolism. These results suggest that MRS is a good and reliable tool to assess the degree of pathologic aging and dystrophy progression in quadriceps’ metabolism. These results suggest that this method offers a robust method of biomarker detection for trials of DMD therapies.

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BIOMARKERS: POSTER PRESENTATIONS

P1.46

Quantification of immunofluorescent signal intensity in dystrophinopathy muscle specimens
L. Taylor a, Y. Kamino h, C. Rodesch c, K.M. Flanigan a
a Nationwide Children’s Hospital, Center for Gene Therapy, Columbus, OH, United States; b Nationwide Children’s Hospital, Columbus, OH, United States; c University of Utah, Salt Lake City, UT, United States

Duchenne muscular dystrophy (DMD) is usually associated with absent or nearly absent dystrophin expression at the sarcolemmal membrane. Quantification of very low levels of dystrophin signal in immunofluorescent studies of muscle biopsy sections presents a technical challenge. This is particularly true in the setting of clinical trials of therapies for DMD, including proof-of-principle drug trials, where the detection and quantification of what may be significant changes in levels of expression is important, even if absolute dystrophin levels remain low. We have developed a method of image analysis that allows reliable and semi-automated immunofluorescent quantification of low level dystrophin expression in sections co-stained for spectrin. Using a custom Metamorph script to create a contiguous region spectrin mask, we quantify dystrophin signal intensity only at pixels within the spectrin mask that presumably represent the sarcolemmal membrane. Using this method, we analyzed muscle biopsy tissue from patients with DMD, Becker muscular dystrophy (BMD), intermediate muscular dystrophy (IMD), and normal control tissue. Analysis of serial sections on multiple days confirms the reproducibility, and – allowing some exceptions predicted by genotype, particularly deletions including the N-terminal actin binding domain – comparisons of BMD and DMD samples suggest this quantification method can be used to distinguish between the two phenotypes. Normalized dystrophin: spectrin intensity ratios (expressed as a percentage of normal control tissue) correlate well with the dystrophin expression levels as determined by Western blot analysis. These results suggest that this method offers a robust method of biomarker detection for trials of DMD therapies.

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P1.47

CD49d is a disease biomarker and a potential therapeutic target in Duchenne muscular dystrophy
F. Pinto Mariz a, L. Rodrigues Carvalho b, A.P.Q. Araujo c, W. de Mello d, M.G. Ribeiro e, M.C.S. Cunha f, I. Riederer g, E. Negroni h, V. Mouly i, T. Voit i, I. Desguerre i, G. Butler-Browne k, W. Savino k, S.D. Silva-Barbosa l
a Thérapie des Maladies du Muscle Strié/Institut de Myologie, UM76, UPMC Université Paris 6; INSERM U974, Paris, France; b Laboratory of Thymus Research, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil; c Institute of Pediatrics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; d Cell Therapy and Orthopaedics Bioengineering Research Center, Traumatology and Orthopaedics National Institute, Rio de Janeiro, Brazil; e Necker Hospital, INSERM U-E10, Paris, France; f Department of Clinical Research, National Cancer Institute, Rio de Janeiro, Brazil

Duchenne muscular dystrophy (DMD) affects 1:3, 500 male births, and is caused by mutations in the dystrophin gene. Even though the genetic mutation results in decreased resistance of muscle fibers, the immune response may contribute to disease progression, through the generation of deleterious inflammatory infiltrates. However, the molecular mechanisms involved in lymphocyte migration into the muscle remain unclear. We studied a cohort of 71 DMD patients at different stages of the disease, and assayed by multicolor cytofluorometry and immunohistochemistry the expression levels of CD49d (the alpha 4 integrin chain of the fibronectin receptor VLA-4) in circulating and intramuscular T lymphocyte subsets. Additionally, we performed functional tests, which comprised transendothelial and fibronectin-driven migratory responses of blood-derived lymphocyte subsets in transwell chambers, as well as adhesion to human myotube monolayers. Increased percentages of circulating CD4+ and CD8+ T lymphocytes expressing high levels of CD49d correlated with both the severity and a more rapid evolution of the disease. CD49d + CD4+ and CD49d + CD8+ cells were also found in muscular inflammatory infiltrates, particularly in fibronectin enriched niches. Moreover, T cells from patients either with rapid progression or in an advanced phase of the disease exhibited higher transendothelial and fibronectin-driven migratory responses (when compared with mildly affected patients or control individuals), and increased adhesion to myotubes. These migratory responses were largely blocked with an anti-CD49d monoclonal antibody. CD49d can be applied as a novel biomarker to predict disease progression in DMD patients. Moreover, inhibition of VLA-4-mediated interactions can be envisioned as a novel therapeutic strategy for ameliorating the disease course of DMD patients.

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