P1.48
Serum matrix metalloproteinase-9 (MMP-9) as a biomarker for monitoring Duchenne muscular dystrophy (DMD) disease progression

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To identify biomarkers for monitoring progression and treatment outcome of Duchenne muscular dystrophy (DMD), we used ELISA assays to evaluate serum levels of Matrix Metalloproteinase-9 (MMP-9), Tissue inhibitor of metalloproteinase-1 (TIMP-1) and Osteopontin (OPN) in 63 DMD patients on corticosteroid therapy and compared these to age-matched controls. These potential biomarkers were selected for their role in the different aspects of muscle pathogenesis, including fibrosis and inflammation. Levels of MMP-9 and TIMP-1 were significantly higher in sera of DMD patients (p < 0.01) compared to healthy controls, whereas the OPN levels showed no significant difference. MMP-9 levels were also significantly higher in the older, non ambulant patients than in ambulant patients and are not affected by corticosteroid use. Longitudinal data from a smaller cohort of 9 DMD patients followed up for over 4 years shows that MMP-9, but not TIMP-1, increases significantly with age. Hence, MMP-9 is a biomarker for DMD disease progression and may be used for monitoring of disease severity and therapeutic efficacy in future clinical trials.

doi:10.1016/j.nmd.2011.06.808

P1.49
Mass spectrometry based clinical proteomics for biomarker discovery in Duchenne muscular dystrophy

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Current clinical trials in Duchenne muscular dystrophy (DMD) would greatly benefit from objective measures for therapeutic efficacy. In order to avoid repeated muscle biopsies, we explored the serum as an alternative source for molecular biomarkers to assess disease severity and response to therapy, hypothesizing that the disrupted muscle integrity will be reflected in the serum proteome. In this study a combination of different bottom-up mass spectrometry (MS)-based proteomics workflows was applied to maximize the number of identifications of proteins from the serum. The sample treatments were applied with a peptide ligand library (Proteominer®), Bio-Rad to reduce the large dynamic range in protein concentration and thus relatively enrich the low and medium abundant proteins. The Proteominer-treated serum was fractionated on a gradient 1D-SDS gel. After in-gel digestion the resulting peptide mixtures were analysed on an LC- ion trap mass spectrometer. Proteominer treatment of serum samples resulted in an eightfold increase in the number of protein identifications when compared to untreated serum. Moreover, combining data from different enzymatic digestions with Lys-N and trypsin along with LC–ESI-MS/MS using collision-induced dissociation (CID) and electron transfer dissociation (ETD) fragmentation methods improved peptide identifications with respect to sequence coverage. We compared serum samples from healthy controls and DMD patients. The identified proteins which may serve as candidate biomarkers for DMD will be discussed at the meeting.

doi:10.1016/j.nmd.2011.06.809

P1.50
Predictive markers of clinical outcome in the GRMD dog model of Duchenne muscular dystrophy

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It is well known that the disease course in Duchenne muscular dystrophy (DMD) patients is heterogeneous, varying from patient to patient. Such heterogeneity is also seen in the golden retriever muscular dystrophy (GRMD) dogs. In this context, identifying predictive markers of the clinical evolution would allow a better management of the model during therapeutic trials. This study aimed to identify biomarkers, able to predict the evolution of disease in the GRMD dogs towards one of the two clearly distinct clinical forms: the severe form (SF, loss of ambulation before the age of 6 months), and the moderate form (MF, no loss of ambulation). The proportion of circulating T-lymphocytes expressing high membrane levels of CD49d (the alpha-chain of the integrin VLA-4, previously defined by our group as a progression biomarker of human DMD), was assessed in seventeen 2 month-old GRMD dogs (5 SF, 12 MF). Clinical and functional tests (motor score (10 SF, 14 MF), and gait analysis using acceleratorometry at 2 months (9 SF, 10 MF), force measurement at 4 months (5 SF, 11 MF)) were also assessed to predict the two clinical forms of disease progression. The proportion of circulating CD4 + VLA4hi T-lymphocytes was significantly increased (p = 0.002) in SF dogs. Additionally, two month-old SF dogs were not able to maintain as frequent locomotor cycles as MF dogs (p = 0.007). A stride frequency lower than 2.3 s -1 was shown to be able to predict SF, with a Sp of 90% and a Se of 78%. This particular contractile behaviour probably reflects a more impaired ionic homeostasis in SF dogs. Accordingly, 100 ms post-tetanic relaxation level lower than 43.2%, was able to predict SF with a Sp of 91% and a Se of 100%. These predictive markers offer satisfying levels of specificity and sensitivity, which could be increased by combining the different markers, to reliably predict SF or MF. This combined strategy may be useful for better evaluating therapeutic trials.

doi:10.1016/j.nmd.2011.06.810

P1.51
Serum protein profiling in mouse models with Dystrophin deficiency using bead-fractionation, MALDI-MS and linear regression

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Disease biomarker discovery is generally aided by using easily obtainable patient samples which hold components with high diagnostic and prognostic value. In the case of Duchenne Muscular Dystrophy (DMD) serum sampling is generally less invasive (compared to, e.g., muscle biopsy), easily standardized and can be included during routine clinical visits. The presence of biomarkers for DMD in serum is likely as the absence of dystrophin leads to muscle damage, which in turn may release proteinaceous material with high biomarker potential into the serum. In this study biomarker discovery was attempted by Mass Spectrometry (MS) based serum protein profiling with the aim to identify biomarkers that are discriminant for DMD disease severity. Three mouse models were used in this work; wild type mice (wt), dystrophin-deficient mice (mdx), and mice with intermediate levels (1–47%) of dystrophin, (mdx-Xist<sup>ΔMHC</sup>). To profile the serum proteome of healthy and dystrophic mice we combined magnetic bead-fractionation (C18 and WCX) and MALDI-MS proteomic analysis with wavelet spectral preprocessing and generalized linear regression models (GLM). After data treatment, 200 peaks were extracted from the mass spectra with which the GLM could provide a small set of discriminatory peaks for dystrophin deficient mice. The most discriminant peaks were found at m/z 1325, 1797, 1822, 3152, 3652, 4526 (up-regulated), and 2953, 3909 (down-regulated). In part, these results corroborate with previous findings identifying the activation peptide of coagulation factor XIIIa as a possible biomarker for DMD. Also, the model could confidently classify the mouse groups based on disease severity, as the mdx-Xist<sup>ΔMHC</sup> mice were grouped into an intermediate class between the wt and mdx group. The identified and disease discriminant peaks will be discussed at the pathophysiology of DMD and their role as potential biomarkers.

Anecdotal information from families and health care providers suggests a wide variation in services received by patients with DBMD. To document the type, frequency and setting of rehabilitation related therapy services received by patients with DBMD in population based samples from four states. The MDSTARNet is a multisite collaboration that conducts population based surveillance for individuals with DBMD and annual surveys with their primary caregivers. From April 2007 to May 2008, 372 eligible caregivers from four states (AZ, CO, IA and western NY) were identified. Two hundred (53.7%) completed a telephone interview. The interview included questions about the type of provider seen including- physical therapist (PT), occupational therapist (OT) and speech therapist (ST) and the frequency and location (clinic, home, school) of services received over the past 12 months. Frequencies among states were compared with χ2 tests. Statistical significance was set at p < 0.05. The caregivers reported receipt of PT (72%), OT (39%) and ST (17%) services. School was reported most frequently as the setting where PT (47%), OT (78%) and ST (89%) services were received. There were significant differences among states in regards to the location of receipt of physical therapy services. The results of this population based survey document and support the anecdotal information from families and providers regarding variation in services.

Abstracts / Neuromuscular Disorders 21 (2011) 639–751

Bio-NMD: Discovery and validation of biomarkers for neuromuscular diseases (NMDs) – An EU funded FP7 project

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Bio-NMD is an EU-funded project which aims to identify and validate biomarkers for neuromuscular diseases. It focuses on DMD, BMD and Collagen VI myopathies. It is a collaboration of 12 partners, coordinated by the University of Ferrara. The focus is on low-invasive molecular biomarkers that can be measured in body fluids like cells, plasma or serum since this would circumvent the need for (repeated) invasive muscle biopsies. The project also includes the implementation of novel high throughput technologies and an integrative analysis platform. A set of methods will be developed for high-throughput analysis of animal and patient samples including by whole exome sequencing, targeted re-sequencing of candidate genes, whole transcriptome, short RNA analysis, and high-throughput immunoassay testing. A platform has been developed to analyse and mine the flow of data in the context of NMD. Ariadne MedScan<sup>®</sup> technology was used to build a literature-derived biological knowledge base focusing on NMD. This knowledge-rich environment will help to capture NMD mechanisms and will facilitate translational research activities for the identification, prioritisation and validation of the different types of candidate biomarkers from disease diagnostic biomarkers to treatment efficacy biomarkers for personalised medicine. The most promising biomarkers will be further validated in animal models and human samples/cells. Then, for the genomic BMs related to response to drugs, qualification process at EMA will be requested. Qualified biomarkers will be ready for ongoing and further clinical trials. Now half-way through its three year funding, this poster will report on the progress made so far including sample and clinical data collection from cohorts, significant developments, plans for the future of the project and beyond. We detail potential implications for future research into NMDs and for treatment monitoring and describe the tools developed during the course of the project.

doi:10.1016/j.nmd.2011.06.812

Rehabilitation therapy services received by patients with Duchenne/Becker muscular dystrophy (DBMD): Data from the muscular dystrophy surveillance, tracking and research network (MDSTARNet)

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Anecdotal information from families and health care providers suggests a wide variation in services received by patients with DBMD. To document the type, frequency and setting of rehabilitation related therapy services received by patients with DBMD in population based samples from four states. The MDSTARNet is a multisite collaboration that conducts population based surveillance for individuals with DBMD and annual surveys with their primary caregivers. From April 2007 to May 2008, 372 eligible caregivers from four states (AZ, CO, IA and western NY) were identified. Two hundred (53.7%) completed a telephone interview. The interview included questions about the type of provider seen including- physical therapist (PT), occupational therapist (OT) and speech therapist (ST) and the frequency and location (clinic, home, school) of services received over the past 12 months. Frequencies among states were compared with χ2 tests. Statistical significance was set at p < 0.05. The caregivers reported receipt of PT (72%), OT (39%) and ST (17%) services. School was reported most frequently as the setting where PT (47%), OT (78%) and ST (89%) services were received. There were significant differences among states in regards to the location of receipt of physical therapy services. The results of this population based survey document and support the anecdotal information from families and providers regarding variation in services.

doi:10.1016/j.nmd.2011.06.813

Rehabilitation equipment use reported by families of patients with Duchenne/Becker muscular dystrophy (DBMD): Data from the muscular dystrophy surveillance, tracking and research network (MDSTARNet)

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