biopsies exhibited, at least in part, distrophic features and the immunohistochemical staining for DYS showed abnormal expression of the protein. Both patients were submitted to cytogenetic study that revealed translocations with breakpoint at Xp21.2 presumably disrupting DMD gene and giving rise to DMD. Few cases of girls with DMD/BMD are reported in the literature. Most of these cases are related to preferential skewed X inactivaton or females with Turner syndrome and dystrophin mutation on remaining X chromosome. Molecular testing to DMD gene is able to detect deletion or duplication. Quantitative molecular test do not detect balanced X-autosome translocation patients. This limitation sometimes leads to a misdiagnosis of limb-girdle dystrophy in a girl with a dystrophinopathy. If the muscular biopsy exhibits an abnormal immunohistochemical expression of dystrophin and molecular tests show normal result, a cytogenetic test should be performed in order to exclude structural rearrangements involving X chromosome.

doi:10.1016/j.nmd.2011.06.781

PATHOPHYSIOLOGY: POSTER PRESENTATIONS

P1.22

Low dystrophin levels improve life expectancy, phenotype and functional performance in the *mdx/utrn* -/- mouse

M. van Putten, M.A. Hulsker, S.H. van Heiningen, V.D. Nadarajah, P.A.C. 't Hoen, G.J.B. van Ommen, A.M. Aartsma-Rus
Leiden University Medical Center, Human Genetics, Leiden, Netherlands

In Duchenne Muscular Dystrophy (DMD) patients, muscle fibers are susceptible to exercise induced injury due to the absence of functional dystrophin. Several therapeutic approaches aiming at restoring functional dystrophin are currently tested in clinical trials. It is unknown which minimum levels of dystrophin will be of functional benefit. *Mdx* mice are less severely affected than DMD patients due to utrophin upregulation, whereas mice without utrophin (*mdx-utrn* -/-) are more severely affected, die prematurely and thus closely resembles the DMD patients' phenotype. We generated *mdx-Xist*$^{\Delta hs}$*utrn* -/- mice in which skewed X-inactivation results in the expression of variable dystrophin levels in a utrophin negative background. We assessed and correlated dystrophin levels with life expectancy, muscle and heart function, fiber integrity and histopathology. Life span of *mdx-Xist*$^{\Delta hs}$*utrn* -/- females (n = 43) was significantly increased (60% was still alive at the age of 30 weeks) compared to *mdx-utrn* -/- mice, which do not live beyond 12 weeks. Serum biomarkers (CK, MMP-9, TIMP-1 and VEGF) were normalized towards wild type levels in young mice. Grip strength and two limb hanging wire performance improved by low dystrophin expression, and correlated negatively with biomarker levels. Heart function was determined by MRI at an age of 10 months. Since muscle fibers are susceptible to exercised induced damage, 20 *mdx-Xist*$^{\Delta hs}$*utrn* -/- females underwent a 12 week functional test regime (grip strength, rotarod, two and four limb hanging wire) to determine muscle function and fiber integrity during chronic exercise. Exercised *mdx-Xist*$^{\Delta hs}$*utrn* -/- mice were severely affected, developed kyphosis and died at a younger age than non-exercised mice. Age of death, functional performance, biomarker levels and respiration function correlated with dystrophin levels. We conclude our research that at least 10% of dystrophin is needed to rescue the dystrophic phenotype.

doi:10.1016/j.nmd.2011.06.782

P1.23

The effects of low dystrophin levels on muscle function and pathology

M. van Putten, M.A. Hulsker, S.H. van Heiningen, E. van Huizen, M. van Iterson, P. Admiraal, T. Messemaker, J.T. den Dunnen, V.D. Nadarajah, P.A.C. 't Hoen, A.M. Aartsma-Rus
Leiden University Medical Center, Human Genetics, Leiden, Netherlands

With several promising approaches for the treatment of Duchenne Muscular Dystrophy aiming at dystrophin restoration in clinical trials, there is an increasing need to determine which dystrophin levels are sufficient to restore muscle fiber integrity, protect against muscle damage and improve muscle function. To address this we generated a new mouse model (*mdx-Xist*$^{\Delta hs}$) with varying, low dystrophin levels due to skewed X-inactivation. Females of a mouse model carrying a mutation in the *Xist* promoter, which coordinates X-inactivation were crossed with dystrophin negative *mdx* males. During embryogenesis, the maternal X chromosome encoding a functional dystrophin gene will be preferentially (60–90%) inactivated, resulting in a higher level of X chromosomes with the dystrophin mutation. The *mdx-Xist*$^{\Delta hs}$ female offspring had dystrophin levels varying from 3–47% (mean 22.7, median 21.8, $n = 24$). Dystrophin positive fibers were spread randomly across the muscles. The dystrophin levels observed in the quadriceps were a benchmark for the other skeletal muscles, but not heart, in which lower levels were found. *Mdx-Xist*$^{\Delta hs}$, *mdx* and wild type females underwent a 12 week functional test regime, aiming to either assess muscle function (grip strength, rotarod, two and four limb hanging wire tests), or muscle function after chronic exercise (three times per week horizontal treadmill running, directly followed by a functional test). Overall, *mdx-Xist*$^{\Delta hs}$ mice outperformed *mdx*mice in the functional tests, had improved histopathology and normalized pro-inflammatory biomarker expression in a dystrophin level dependent manner. Functional test performance also improved after chronic exercise. Furthermore, dystrophin levels correlated with body weight and serum biomarker levels. Based on these findings, this easy to generate *mdx-Xist*$^{\Delta hs}$ mouse model offers great possibilities to study the effect of low levels of dystrophin on different outcome measures.

doi:10.1016/j.nmd.2011.06.783

P1.24

Microarray analysis of two exceptional Golden Retriever Muscular Dystrophy (GRMD) dogs with no dystrophin and a mild course

N.M. Vieira, Y. Moreira, E. Zucconi, M. Valadares, M. Vainzof, S. Verjovsky-Almeida, M. Zatz
University of São Paulo, Sao Paulo, Brazil

The closest model to human Duchenne Muscular Dystrophy (DMD) is the Golden Retriever Muscular Dystrophy (GRMD) dog which has a splice site mutation in the dystrophin gene. These dogs present clinical signs within the first weeks of life involving the limbs as well as masticatory muscles and death occurs usually before 2 years of age. We recently reported the case of Ringo (almost 8 years-old), an exceptional GRMD dog showing an unusually mild course, able to run, jump and to breed naturally. One among his male offspring, Suflair (5 years-old), is also showing a relatively mild course. Histopathological and immunohistochemistry analysis from Ringo's and Sufflair's muscle biopsies show typical features of a dystrophic muscle, comparable to severely affected GRMD dogs. We are currently investigating what is protecting Ringo and Suflair from the mitigating effect of the dystrophin mutation. Our first approach was a microarray analysis comparing Ringo and Suflair muscle gene expression with severely affected and normal Golden Retriever dogs, all related to Ringo. This analysis revealed 66 genes that are differentially expressed between mildly and severely affected dogs and one among them shows the same expression level as normal dogs. This candidate gene was found to be under expressed in Ringo and Suflair as compared to severely affected dogs and overexpressed in severely affected dogs as compared with the normal dogs. An important observation is that the same expression pattern was observed in the microarray analyses of the mdx mice. Comparative studies represent a unique possibility to pinpoint protective gene(s) or mechanisms that are protecting these dogs from the severe

effects of dystrophin deficiency and could have importance for future therapeutic trials in Progressive Muscular Dystrophies.

doi:10.1016/j.nmd.2011.06.784

P1.25

Localization of chemokines and their receptors in muscle tissues from Duchenne muscular dystrophy patients

J.L. De Bleecker [a], K.K. Creus [a], J.J. Martin [b], B. De Paepe [a]
[a] *Ghent University Hospital, Department of Neurology, Ghent, Belgium;* [b] *Antwerp University Hospital, Department of Neuropathology and Born-Bunge Institute, Antwerp, Belgium*

In Duchenne muscular dystrophy (DMD), skeletal muscle infiltration by immune cells is correlated to disease severity and could be prognostic for disease progression. Yet, the precise mechanisms behind inflammatory muscle tissue damage remain poorly understood. Chemotactic cytokines or chemokines are key factors in the recruitment and activation of immune cells and may thus be involved in the build-up of inflammation in DMD. We studied a panel of chemokines in 9 patients using immunohistochemistry, immunofluorescence and in situ hybridization. CXCL1,2,3,8,11 were absent from normal but induced in DMD myofibers. Some, but not all, were regenerating or necrotic muscle fibers. CXCL11,12 and CCL2 were upregulated on DMD blood vessel endothelium. CD68 + macrophages express high levels of CXCL8, CCL2 and CCL5. In contrast, chemokine expression in infiltrating T-cells, B-cells and DCs was low or absent. Based on the spatial distribution and the selective expression pattern of distinctive chemokines, diverse functions in DMD muscle inflammation are suspected. On the one hand, chemokines are probably involved in inflammatory cell recruitment and macrophage cytotoxicity. On the other hand, certain chemokines may be beneficial in muscle damage control.

doi:10.1016/j.nmd.2011.06.785

P1.26

A comparison of metabolism and protein synthesis rates in young and adult dystrophic mdx and control C57Bl/10 mice

H.G. Radley-Crabb [a], M.D. Grounds [a], M.L. Fiorotto [b]
[a] *University of Western Australia, School of Anatomy and Human Biology, Perth, Australia;* [b] *Baylor College of Medicine, USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Houston, TX, United States*

Dystrophic skeletal muscle is subject to many metabolic abnormalities that can greatly impact on the maintenance of muscle mass and function. This research thoroughly investigates the metabolic differences between young (4 week old) and adult (14 week old) dystrophic mdx and control C57Bl/10 mice specifically measuring; energy expenditure, activity level, body composition and protein synthesis rates. Striking differences were observed in the metabolic processes between both young and adult dystrophic and control mice. Young mdx mice have 'stunted' growth; they are significantly lighter with reduced fat free mass (lean muscle). Young mdx mice also have a significantly increased protein synthesis rate (whole muscle samples and isolated myofibrillar fraction) and increased metabolic rate (Heat kcal/24 h/kg ffm). The acute onset of myofibre necrosis and regeneration at 3–4 weeks of age appears to have a significant effect on many parameters related to muscle mass and function, and metabolic rate in young mdx mice. There is significant 'catch-up' growth in mdx mice between 4 and 14 weeks of age, with 14 week old adult mdx mice being heavier, with significantly increase muscle mass and bone length than C57Bl/10 mice. The adult mdx mice are very lean, with significantly increased fat free mass (lean muscle) and increased protein synthesis rates (whole muscle samples and isolated myofibrillar fraction). However, metabolic rate (Heat kcal/24 h/kg ffm), energy intake (kcal/24 h/kg ffm) and activity levels are both significantly decreased compared to control mice. These findings have many implications for understanding the basic pathology of DMD especially with respect to susceptibility to myofibre damage of both growing and mature muscle and impact on potential therapeutic interventions to protect dystrophic muscle from damage.

doi:10.1016/j.nmd.2011.06.786

P1.27

Mutation-directed studies on the function of the dystrophin ZZ domain

A. Vulin [a], B. Maiti [b], L. Taylor [a], Y. Kaminoh [a], T. Simmons [a], K.M. Flanigan [a]
[a] *The Research Institute at Nationwide Children's Hospital, Center for Gene Therapy, Columbus, United States;* [b] *Washington University, Department of Neurology, St. Louis, United States*

Duchenne Muscular Dystrophy (DMD) is an X-linked recessive disorder due to mutations in the DMD gene encoding the dystrophin protein, which plays an important role in muscle fiber integrity. Although the dystrophin protein has been studied in details, little is known about the function of the ZZ domain of the protein, a cysteine-rich zinc-finger domain near the C-term. This domain has been implicated in forming a stable interaction between dystrophin and β-dystroglycan, but other potential binding partners have not been well investigated. Missense mutations in DMD are quite rare; most are associated with BMD (the less severe form of DMD), and only 0.3% of DMD cases are due to missense mutations. Eleven point mutations have been identified in the ZZ domain but their consequence is not well studied. To date, studies have only addressed effects on β-dystroglycan binding, with discrepant results. In some ZZ missense patients, a sizeable amount of dystrophin localizes to the membrane, suggesting β-dystroglycan may be intact, and interactions with other proteins mediate disease. We are taking three complementary approaches to characterizing ZZ domain function. First we seek to delineate the effect of three ZZ mutations on several known and candidate binding partners, including β-dystroglycan, myospryn and calmodulin via site-directed mutagenesis, we have introduced ZZ mutations into constructs encompassing the cysteine rich and C-term domains. Co-IP of native or co-expressed candidate binding partners were performed. Our preliminary data suggest ZZ mutations have no effect on binding to known partners; confirmatory experiments are in progress. Second, we are using a mass spectrometric approach to identify novel binding partners of the dystrophin cysteine-rich domain. Finally, we are analyzing the effects of missense mutations on the II and III structure of the ZZ domain using both in silico modeling and circular dichroism studies.

doi:10.1016/j.nmd.2011.06.787

P1.28

Dystrophin mediates melanocytes attachment to dermal-epidermal junction in human skin

C. Pellegrini [a], F. Gualandi [a], E. Manzati [a], L. Merlini [a], M.E. Michelini [b], L. Benassi [c], A. Ferlini [a], N.M. Maraldi [d], P. Sabatelli [e]
[a] *Department of Experimental and Diagnostic Medicine, University of Ferrara, Ferrara, Italy;* [b] *U.O. Chir Ped – Azienda Ospedaliera, Università di Ferrara, Ferrara, Italy;* [c] *Clinica Dermatologica, Università di Modena e Reggio Emilia, Modena, Italy;* [d] *Biologia Cellulare Muscoloscheletrica, IOR, Bologna, Italy;* [e] *IGM-CNR, Unit of Bologna, c/o IOR, Bologna, Italy*

Dystrophin is a subsarcolemmal protein critical for the integrity of muscle fibers by linking the actin cytoskeleton to the extracellular matrix via the dystroglycan complex (DGC). DGC also occurs at dermal–