P1.5

Expression of dystrophin–glycoprotein complex (DGC) in muscles from DMD patients: Overview of 80 cases (9 days–12 years of age)

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The dystrophin-glycoprotein complex (DGC) is a group of tightly associated transmembrane and cytoskeletal proteins that forms a molecular bridge between dystrophin and the extracellular matrix. DGC is formed by dystrophin, dystroglycan-1 complex (α- and β-DG), sarcoglycans (α, β, γ and δ), sarcospan, syntrophins, neuronal nitric oxide synthase (nNOS) and dystrobrevins. DGC plays an important role in protecting muscle fibers from potentially damaging tissue stresses developed during muscle contraction. A recent report suggested that eccentric contractions or high tension exercises induced muscle injury, lacking dystrophin and secondary loss of DGC from the sarcolemma, which indicated that loss of dystrophin was one of the key points of muscle damage from eccentric exercise. DMD is caused by the absence of dystrophin, which means DMD patient after birth is the long natural history state of actual path of force transmission in skeletal muscles from eccentric exercise. To investigate whether expression of DGC is associated with progressive muscle weakness with increasing age in natural history of DMD over time, we analyzed expression of DGC proteins at the sarcolemma by using immunohistochemistry. 80 confirmed DMD cases were divided into 3 groups by clinical stage, in which 32 DMD patients were stage 1 (9 days–3 years old), 38 were stage 2 (4–6 years old) and 10 were stage 3 (7–12 years old). The study showed that there was no relationship between the lack of proteins and progressive muscle weakness with increasing age, although expression of α-DG, β-DG, sarcoglycans (α, β, γ and δ), nNOS and syntrophin at the sarcolemma at different stages of DMD patients had different degrees of deficiency or completely deficiency. We considered that deficiency of these proteins may occur before birth and the study also showed that further damage from high-resistance and eccentric exercise should be avoided to skeletal muscles of DMD patients.

doi:10.1016/j.nmd.2011.06.765

P1.6

The incidence of revertant and trace dystrophin expression in muscle biopsies of Duchenne Muscular Dystrophy patients with different exon deletions

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Duchenne muscular dystrophy (DMD) is caused by mutations in the DMD gene, in 70% of cases deletions of one or more exons, leading to disruption of the open reading frame (ORF). This results in the lack of dystrophin expression at the myofiber membranes. Yet in some patients trace dystrophin expression and/or revertant fibers have been observed. Revertant fibers exhibit strong positive dystrophin staining at the sarcolemma, thought to arise from frame-restoring alternative splicing. Less is known on the origin of the dystrophin traces, described as patches of below-normal dystrophin-positive areas visible at the sarcolemma of muscle fibers. We have collected immunofluorescence data on analysed cross-sections of pre-treatment tibialis anterior muscle samples from a series of DMD patients participating in our clinical studies on PRO051 and PRO044. Remarkably, the prevalence of revertant fibers for 12 DMD patients with different deletions flanking exon 44 was as high as ~13% of the total number of fibers. Trace dystrophin levels were present in approximately up to 50% of fibers. In contrast, in an equivalent number of DMD patients with deletions adjacent to exon 51, the prevalence of revertant fibers was rare and trace dystrophin expression was minimal. This variability in incidence and co-existence of revertant fibers and trace dystrophin in distinct DMD populations is highly relevant for clinical studies on therapies that aim to restore and monitor dystrophin expression, and confirms the value of comparative analysis of pre- and post- treatment muscle samples.

doi:10.1016/j.nmd.2011.06.766

P1.7

Early death following minor trauma in Duchenne muscular dystrophy

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Before corticosteroid (CS) treatment, males with Duchenne muscular dystrophy (DMD) died of respiratory and/or cardiac failure in the second to third decade of life. With improved pulmonary care, cardiac care and CS therapy, lifespan has improved significantly. We describe 4 males with DMD who died within 36 h of sustaining mild trauma. All 4 were nonambulatory, obese, medically stable and had osteoporosis prior to falling. Three of the 4 were taking daily CS. Three boys were 14 years old and one was 23. Three presented to hospital within 2 h and one at 23 h with rapid physical and neurologic deterioration after falling out of their wheelchair. None were wearing a seatbelt. None had fractures identified by X-ray. All 4 met the clinical criteria for acute respiratory distress syndrome (ARDS): acute onset, severe dyspnea, hypoxemia and diffuse bilateral lung infiltrates. All 4 boys had 2 of 3 major clinical criteria for Fat Embolism Syndrome (FES): respiratory insufficiency and cerebral involvement. Autopsy findings were consistent with ARDS in 2 and FES in 1. No staining for fat was performed in the 2 cases with ARDS. One family declined an autopsy. These histories suggest that all 4 boys had FES causing ARDS and death. ARDS may be associated with fat emboli, trauma and fractures. FES has been reported following minor trauma without fractures. All 4 had risk factors and clinical findings of ARDS and FES. Their decreased pulmonary reserve might have contributed. Both obesity and osteoporosis result in increased bone marrow fat. This is likely a risk factor for fat emboli after trauma. FES and/or ARDS may be difficult to reverse in these high risk boys. This report is an important reminder that seatbelts should always be worn, even at home.

doi:10.1016/j.nmd.2011.06.767

P1.8

The effect of joint hypermobility on children with neuromuscular disorders: Considerations for natural history studies and the interpretation of outcome measures

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Joint contractures are recognised features of neuromuscular disorders and are a major cause of functional difficulty. What has not been considered for both ambulant and non-ambulant children is the effect of inherent or incidental joint hypermobility on function. Studies of benign joint hypermobility suggest an incidence of 1:10 in the Caucasian population, 1:4 in the Asian population. Joint laxity in the "normal" population causes delay in motor milestones, difficulty with fine motor tasks, increased fatigue and increased joint pain and injury. We previously studied the effect of laxity on Duchene Muscular Dystrophy function (Main, unpublished). In 25 pairs of age matched boys, not on steroids, the boys with flat foot posture walked for up to two years longer than their aged matched pair. It appeared that reduced contracture development in some DMD boys facilitated longer ambulation. We are currently establishing the impact

of laxity on outcome measures such as grip strength measured by myometry in the normal population (children from 4 years to adults). Our results suggest that children and adults with upper limb joint laxity have reduced power in grip when compared to values for normal. In children with neuromuscular disorders, the problems of joint laxity, when combined with weakness, can prevent or delay functional activities including propping, getting to sitting, crawling and pulling to stand. It can delay walking, cause tip-toeing, knee pain and fatigue. At the same time in some conditions such as DMD joint laxity can confer advantages. We suggest that joint laxity, which appears to confer both advantages and disadvantages to children with neuromuscular disorders, must be considered when looking at natural history studies as it might allow the further stratification of different populations of patients.

doi:10.1016/j.nmd.2011.06.768

P1.9

Glucocorticoid therapy in a non-ambulant six year old boy with Duchenne muscular dystrophy

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Duchenne muscular dystrophy is a X-linked progressive muscular dystrophy with an incidence of about 1:3500 male newborns. Symptoms usually appear before the age of 6 but also as early as infancy. They include delay of motor, mental and speech skills. The boys usually show pseudohypertrophic calf muscles as well as worsening muscle weakness, frequent falls, positive phenomenon of Gowers, a dull gait and loss of ambulance around nine to ten years of life. There is no known cure so far, but a drug therapy with steroids to start at the age of five in usually ambulant patients is recommended to slow the decline of muscle strength and function. We report on a boy first seen in our outpatient clinic at the age of two years. In the context of a common cold blood samples were taken and showed a markedly increased result of creatine kinase level of about 36,000 U/l. Genetic analysis (sequencing) showed a deletion of two bases in exon 27 in the dystrophin gene leading to an out-of-frame-mutation confirming the diagnosis of Duchenne muscular dystrophy. Until this age the boy showed a motor delay in sitting stable (age 27 months), in crawling (age 30 months), in babble (age 12 months). Until the age of six he was not able to walk independently, only a few steps with assistance. Nevertheless we decided to treat the patient with daily Deflazacort (0.9 mg/kg daily). The next four to six weeks he started to walk independently and by that he improved his social skills. Despite missing values or standards in non ambulant patients with a very early manifestation of Duchenne muscular dystrophy a medical treatment with steroids should be considered. Although there is no evidence, we think the acquisition of ambulance might be a consequence of steroid therapy.

doi:10.1016/j.nmd.2011.06.769

P1.10

Comparative pharmacokinetics (PK) in primates and humans of AVI-4658, a phosphorodiamidate morpholino oligomer (PMO) for treating DMD patients

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AVI-4658 is a PMO that skips dystrophin exon 51, restores the reading frame and enables dystrophin expression in a substantial subset of Duchenne muscular dystrophy (DMD) patients. To characterize the plasma PK and understand the PK/Pharmacodynamic relationship, data has been collected in non-dystrophic non-human primates (NHP) and DMD patients. This paper studied the PK profile of AVI-4658 to assess whether the non-metabolized PMO PPK behavior is similar between animals and humans, and to better guide development assumptions for progressing additional PMOs into development. (1) Cynomolgus monkeys were dosed IV with 0, 5, 40 or 320 mg/kg (Maximum Feasible Dose) and 320 mg/kg subcutaneously weekly for 12 weeks, with blood and urine collected for 24 h post 1st and 11th dosing as part of a GLP IND-enabling toxicology study. (2) 19 DMD patients were dosed with 0.5, 1.0, 2.0, 4.0, 10.0 or 20.0 mg/kg weekly for 12 weeks, with blood and urine collected after 1st, 6th and 12th doses. The PK profile of AVI-4658, a neutral, non-metabolized 30-mer PMO, was consistent across species and time points with similar $T_{1/2}$ (NHP: 1.6–3.9 h; DMD: 1.6–3.6 h); dose-proportional C_{max} and AUC and steady clearance (NHP: 3.6–6.7 mL/min/kg; DMD: 3.9–10.2 mL/min/kg). The subcutaneous dosing in NHP at MFD provided at least 100% bioavailability. AVI-4658, the first PMO for DMD, demonstrates consistent PK across dose, species and disease states, with rapid urinary elimination (unmetabolized) from the plasma. If preclinical data suggests consistent behavior across different sequences and different length oligomers, this may support acceleration of the development of other PMOs for additional DMD genotypes.

doi:10.1016/j.nmd.2011.06.770

CARDIORESPIRATORY: POSTER PRESENTATIONS

P1.11

Comparison between courses of home mechanical ventilation patients with muscular dystrophy and mechanical ventilation inpatients

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In Japan, there are 27 hospitals specializing in muscular dystrophy treatment, which have managed wards for inpatients with muscular dystrophy and other neuromuscular disorder. We have been conducted survey of inpatients of these wards and home-mechanical ventilation patients (HMV patients) with muscular dystrophy and neuromuscular disorder annually since 1999, to construct the muscular dystrophy database. To evaluate efficacy of mechanical ventilation therapy for HMV patients and ventilation-dependent inpatients (MV inpatients) with those wards. By using these databases, we analyzed the courses of HMV patients and those of MV inpatients of wards, those of both groups started mechanical ventilation after 1999. Examination points are mechanical ventilation periods, outcome and caregiver (for HMV patients). HMV patients group included 434 patients; 262 patients with Duchenne muscular dystrophy (DMD), 60 myotonic dystrophy (MD), 14 spinal muscular atrophy (SMA), and so on. MV inpatients group included 583 inpatients; 339 DMD, 103 MD, 16 SMA, and so on. The range of mechanical ventilation introduction age for HMV patients was $6.3 \sim 72.8$ years old (mean 37.6), and that of MV inpatients was $10.0 \sim 76.0$ years old (31.7). The number of NPPV introduction cases of HMV patients was 417, and that of MV inpatients was 418. Survival analysis showed that 75% life time of HMV patients was 1689 days, while that of inpatients surpassed the observation period. The number of death cases of HMV patients was 57, and that of MV inpatients was 65. The former included more sudden death cases than latter, and had some accidental cases. Caregivers for 80% of HMV patients were patients' families. The course of HMV patients was fairly good. However, burden of caregivers was