Bayesian Diagnosis of Amyotrophic Lateral Sclerosis (ALS) to improve Clinical Trial Eligibility
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Amyotrophic Lateral Sclerosis (ALS) is unique among the neuromuscular diseases (NMD) in that its diagnosis is clinical, and cannot be made with genetic tests, biopsies, or imaging. Current ALS diagnostic criteria reflect what “the experts” believe ALS is, not necessarily what ALS really is. Consequently, some 10 to 20% of ALS patients do not meet current diagnostic criteria. Bayesian diagnosis ignores expert opinion in favor of data; in this case we used a dataset of over 1000 patients, half with ALS, and half with conditions from which ALS must be distinguished. Using a logistic regression model, “Bayes Factors” (BF or odds ratios), were determined for the key clinical signs that distinguish ALS from other NMD; tongue atrophy, tongue fasciculations, deep tendon reflexes, Babinski sign, and muscle tone. The BF of a clinical sign is the odds ratio that a patient with that sign has ALS. The BF’s provide a relative weighting for each clinical sign, and can be combined when more than one sign is present, to provide a final “Odds of ALS” for that patient. When applied to a new dataset of patients with progressive weakness and motor disability suggestive of ALS, the Bayesian method shows that even people who, by current criteria, do not have ALS or who have “suspected or possible “ ALS, have extremely high “odds of ALS”. As new drugs become available for ALS trials, this Bayesian diagnosis will allow the inclusion of more subjects, especially those early in the course of the disease.

Analysis of exons 6 and 8 skipping in cells from an exon-7 deleted DMD patient: direct application of antisense sequences found in study with canine muscular dystrophy
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The multi exon skipping is expected to bring versatility in this approach to various mutations of Duchenne muscular dystrophy (DMD). We have previously tried a systemic administration of phosphorodiamidate morpholino oligomer (PMO) targeting exons 6 and 8 of the DMD gene in canine X-linked muscular dystrophy (CXMD), having exon 7 deletion of dystrophin mRNA. We successfully achieved recovery of dystrophin expression in skeletal muscle and muscle function (Ann Neurol. 2009;65(6):667-76.). To date, antisense chemical compounds used for exon skipping in DMD animal models have not been directly applied to that in DMD patient having the same type of exon deletions. We recently experienced a DMD patient having an exon 7 deletion, therefore, we evaluated the multi exon skipping approach in cells derived from the patient and compared the efficiency with cells from CXMD. We converted the fibroblasts of CXMD and the DMD patient to myogenic cells by MyoD transduction. PMO cocktails with antisense sequences targeting exons 6 and 8 were administered to either canine or human cells. Skipping of exons 6 and 8 occurred with the similar skipping efficacy between canine and human cells, but skipping of exon 9 was different between these cells, which did not alter the reading frame. The similarity of exon sequences of targeted region, and the antisense design methodology targeting identical sequence, may assure similar skipping efficiency between species.

National Registry of Duchenne Muscular Dystrophy Patients at a major Center in Turkey
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Between January to September 2009, we have completed the national registry of 173 Duchenne/Becker boys at our center. The overall statistics are as follows: Mean age 8.6 ±3.9 years, still ambulant 145 (83.8%) cases and lost to walk 28 (16.2%) cases. One hundred eighteen (68.2%) boys are on low dosage daily steroids plus vitamin D, 6(3.5%) boys never used this drug, whereas 17 (9.8%) boys used steroids before, however stopped in due course. We don’t have any knowledge about usage of steroid of 32 (18.5%) boys. Thirteen (7.5%) boys have cardiomyopathy and 6 (3.4%) of these are on cardiac drug treatment. There is 1 patient who is on night-time ventilation. One other patient received scoliosis surgery. Thirty-one (17.9%) have positive family history for this disorder. No deletion was found in 35 (20.2%) boys by multiplex PCR technique spanning 35 exons. 10 (5.8%) boys only had muscle biopsy and no genetic test. The remaining 128 (74%) were shown to have deletions. Eighty five (49.1%) patients had muscle biopsy. We continue to register our cases, now also the ones with spinal muscular atrophy.
Developing a skeletal muscle cell model for testing novel therapies in myotonia congenita

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This PhD project is part of an effort at the MRC Neuromuscular Centre to develop novel therapies for myotonia congenita by manipulating gene expression. Autosomal dominant and recessive myotonia congenita, characterized by impaired muscle relaxation, are caused by dysfunction and/or reduced expression of the skeletal muscle chloride channel, ClC1, owing to mutations in the CLCN1 gene on chromosome 7. Current therapies e.g. mexiletine compensate for the resulting muscle cell hyperexcitability by dampening sodium currents; none target the primary impairment of chloride conductance. A number of emerging therapies may be applicable in myotonia congenita. Certain small molecules (e.g. PTC124) can force translation past premature stop codons. For mutants that are trapped in the sarcoplasmic reticulum, molecules such as curcumin, which act on molecular chaperone systems, may improve trafficking to the sarcolemma and t-tubule. Mutations that cause dominant negative interactions should be susceptible to knock-down using shRNA constructs. The majority of chloride channel functional expression work has been in heterologous systems such as the Xenopus Oocyte, which do not recapitulate post-translational processing in skeletal muscle, and are inappropriate for testing potential therapies. Here we describe here our plans to develop a skeletal muscle expression system for studying ClC1 mutations and testing therapies. Specifically, we will use the PCDH1 lentivirus to express normal and mutant CLCN1 in cultured myotubes. Chloride current density will be measured with whole cell patch clamp. Trafficking of the channel will be studied with immunofluorescence.

Attenuation of adverse effects of prednisolone on δ-sarcoglycan-deficient cardiomyopathy by mineralocorticoid-receptor-antagonism

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An inflammatory component to Duchenne muscular dystrophy (DMD), Limb-girdle muscular dystrophy (LGMD) and other dystrophin-glycoprotein complex-associated muscular dystrophies has been well documented, and steroid therapy increases respiratory and skeletal muscle strength in DMD and sarcoglycan-deficient LGMD patients. In addition, retrospective studies in patients with DMD have suggested that steroids have beneficial effects on left ventricular function when assessed by echocardiography. We have however recently reported severe adverse myocardial effects of steroid treatment in the δ-sarcoglycan-deficient (Sgcd-null) mouse, a well characterised model of LGMD2F. We have therefore investigated the hypothesis that cross reactivity of prednisolone to cardiac mineralocorticoid receptors (MR) plays a role in the pathomechanism by co-administration of steroid-treated mice with the unselective MR-receptor antagonist Spironolactone. Animals were treated for 8 weeks from 8 weeks of age (prior to the development of overt signs of cardiomyopathy) with clinically relevant doses of prednisolone (1.5 mg/kg) and spironolactone (3.0 mg/kg). At the end of the treatment period animals were assessed by in vivo heart conductance catheter and their hearts processed for histological analyses. Our data show that spironolactone has beneficial effects on cardiac remodelling and contractility in Sgcd-null mice and that it prevents steroid-induced deterioration of cardiac haemodynamics and acute sarcolemmal damage, although not cardiac fibrosis. The elucidation of mechanisms underlying steroid-induced cardiomyopathy may have implications for the design of future treatment regimens for LGMD and DMD providing proof of principle for combinational therapies to prevent steroid-induced cardiac damage in patients.
Blocking calcium influx in mdx mice from developmental onset aggravates the dystrophic pathology

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This study aimed to identify the primary causes of calcium dysregulation in Duchenne Muscular Dystrophy, and to test a novel treatment strategy where calcium-channels are blocked from onset of embryonic development using streptomycin. Recent studies indicate that stretch-activated calcium-channels (SACs) are involved in DMD pathology. Specifically it was shown that inhibition of SACs in the young or adult mdx mouse ameliorates the pathological phenotype (Yeung et al. 2005, Iwata et al. 2009). The transient receptor potential (TRP) cation channels have been proposed as candidates for SACs. An intriguing study showed that over expression of the TRPC3 channel protein induces muscular dystrophy without sarcolemmal damage, suggesting that calcium influx is the secondary mediator of dystrophic myofiber degeneration (Millay et al. 2009). Therefore, in this study we treated pregnant mdx and control mice with streptomycin, a mild blocker of SACs, with continued treatment of the pups for 6 weeks up to 6 months of age. The idea was that inhibition of potentially over-active channels before and during muscle development could prolong onset and thereby alleviate the pathology even more. We observed an overall improvement of muscle pathology in 6 weeks old mdx mice, but with aggravation of the pathology at 10 weeks of age and continued worsening with increased age. Especially the heart displayed severe pathology at 6 months of age. Overall this proof-of-principle study suggests that the mdx/DMD pathology is very complex and long-term studies are very important.

Improved dystrophin gene analysis in Hungarian Duchenne/Becker muscular dystrophy families - Setting up the patient registry in relation with TREAT-NMD

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A comprehensive study of the Hungarian Duchenne/Becker muscular dystrophy (DMD/BMD) families is presented here. Our laboratory is the only one in Hungary which offers mutation screening for the entire dystrophin gene with Multiplex Ligation-dependent Probe Amplification (MLPA) technique for DMD/BMD patients and their female relatives. Deletions in the hot spots regions are identified by multiplex PCR, whereas, rare mutations are detected by Southern blot and MLPA techniques. Out of the analysed 203 male patients 58 % had deletions, whereas 8 % had duplications and in a small portion of the patients (1 %) point mutation has been confirmed. In 33% of the patients no mutation has been found so far, DNA sequencing will be performed later on. The distribution of the deletions in the dystrophin gene was following: 66 % in the major hot-spot region (ex44-ex52), 15 % in the minor hot-spot region (ex02-ex10), 13 % outside of the major and minor hot spot regions and 6 % of deletions comprised both hot spot regions (large deletions). The entire dystrophin gene and adjacent genes were deleted in two DMD patients. Out of the 95 female relatives, 41 proved to be carriers (with 2/3 being mothers and 1/3 being sisters of the patients), including 4 manifesting carrier females. Using MLPA method, a large portion of the Hungarian DMD/BMD patients and their female relatives were exactly genotyped. This enabled us to set up the national TREAT-NMD patient registry. Self reporting questionnaire form is used and 110 registration forms have been sent out per mail from 2007 on. Since then, 63 DMD patients with confirmed genetic mutation have been registered and all genetic and clinical data were entered into UMD. We contributed to the foundation of two Hungarian DMD patient organizations and have an effective collaboration with them in accordance with the purposes of the TREAT-NMD consortium. Further on, a constantly renewed http://treat-nmd.blog.hu provides information to help affected individuals and their families to understand diagnosis, genetic tests, care, new therapeutic developments and trials, and promotes TREAT-NMD registration.