metabolism can be simultaneously determined using rapidly interleaved arterial spin labelled and BOLD imaging, deoxy-myoglobin proton spectroscopy and phosphorus spectroscopy during exercise protocols. They provide valuable information on the respective contributions of oxygen supply, extraction and utilization to particular conditions or diseases.

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M.I.4
Discovering biomarkers for monitoring neuromuscular diseases: A turning point

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Neuromuscular hereditary disorders (NMDs) represent heterogeneous phenotypes intensively studied both in terms of accurate genetic definition and pathophysiology. Since therapeutic approaches are now becoming reality in some NMDs, clinical trials have been designed to track down correction of a decline in muscle function, requiring large samples and lengthy times. In addition, clinical outcome measures may not always be sensitive enough to detect small changes in disease progression/regression and after short treatment periods. It is therefore imperative to be able to document benefits of the treatment at the individual level to justify demanding, risky, and expensive treatments. Biomarkers can effectively increase our capacity of studying and monitoring NMDs. They can be defined as diagnostic if able to implement the genotype–phenotype correlation, as well as to label the disease course; pathophysiological, when they disclose or integrate common pathways, therapeutic (pharmacogenetic, pharmacodynamic, pharmacokinetic and safety) when they can be translated into clinical trials. Other disciplines, such as oncology, routinely use biomarkers as surrogate or enrichment endpoints of treatments.

Examples of genomic (DNA and RNA) and proteomic biomarkers as gene-expression-based or genome signatures will be described in two pathologies (dystrophiniopathies and collagen VI myopathies) considered as paradigmatic examples for biomarkers discovery in NMDs. Particular emphasis will be put on less/non-invasive biomarkers, a crucial point in the selected disorders but more in general in many NMDs, where invasive sometimes multiple procedures are often required in order to demonstrate the efficacy of the treatment. Moving toward biomarkers in more accessible human cells or fluids will minimize the invasiveness of the follow up allowing us periodical measurements. This is a turning point in the research of NMDs since new therapies are emerging.

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M.I.5
The dystrophic dogs as an excellent animal model of Duchenne muscular dystrophy (DMD)

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Introduction: Canine X-linked muscular dystrophy (CXMD) is caused by a point mutation at the splice acceptor site of exon 7 of the dystrophin gene, resulting in complete absence of dystrophin. To explore potential therapeutic approaches to DMD, we have established a beagle-based CXMD colony in Japan (CXMDJ). The phenotype of CXMDJ was similar, but a little milder than that of golden retriever CXMD. *Evaluation of antisense morpholino-mediated exon skipping using dog models:* Systemic delivery of morpholino antisense oligonucleotides targeting exon 6 and 8 of the canine dystrophin gene, efficiently recovered functional dystrophin expression at the sarcolemma of dystrophic dogs, and improved performance of affected dogs without serious side effects (Yokota et al., Ann Neurol, in press). We have also tested antisense morpholinos targeting exon 51 of the mouse dystrophin gene in mdx 52 mice, in which gene targeting had disrupted exon 52 of the mouse dystrophin gene. Systemic delivery of a combination of two morpholinos showed an excellent restoration of sarcolemmal dystrophin and amelioration of the phenotypes in the mice. *Significance of dystrophic dogs:* Overall, the results using dystrophic mice are more stable than those in dystrophic dogs, partly because dystrophic dogs show a variation in the phenotypes even in a colony. Moreover, one must carefully evaluate the therapeutic effects within a limited number of dystrophic dogs, which is another disadvantage of dystrophic dogs. In addition, the gene mutation in CXMD is not common in DMD patients, therefore we cannot directly apply the morpholino sequences optimized in dogs to many human DMD patients. Dystrophic dogs, however, have a severe progressive course and clinical signs similar to DMD, whereas mdx mice exhibit mild clinical features. Thus, a combination of mouse and dog dystrophic models would be ideal to evaluate the therapeutic effects.

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NEW INSIGHTS INTO NEUROMUSCULAR DISEASES; ORAL PRESENTATIONS

G.O.1
Diagnosis of muscular dystrophies at the nanometer scale

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The diagnosis of muscular dystrophies or the assessment of the functional benefit of gene or cell therapies can be difficult, especially for poorly accessible muscles, and it often lacks a single-fiber resolution. In the present study, we evaluated whether muscle diseases can be diagnosed from small biopsies using atomic force microscopy (AFM). AFM was shown to provide a sensitive and quantitative description of the resistance of normal and dystrophic myofibers within live muscle tissues explanted from Duchenne mdx mice. The rescue of dystrophin expression by gene therapy approaches led to the functional recovery of treated dystrophic muscle fibers, as probed using AFM and by in situ whole-muscle strength measurements. Comparison of muscles treated with viral or non-viral vectors indicated that the efficacy of the gene transfer approaches could be distinguished with a single myofiber resolution. This indicated full correction of the resistance to deformation in nearly all of the muscle fibers treated with an adeno-associated viral vector that mediates exon-skipping on the dystrophin mRNA.

Having shown that AFM can provide a quantitative assessment of the expression of muscle proteins and of the muscular function in animal models, we assessed myofiber resistance in the context of human muscular dystrophies and myopathies. Thus, various forms of human Becker syndrome can also be detected using AFM in blind studies of small frozen biopsies from human patients.

Interestingly, it also allowed the detection of anomalies in a fraction of the muscle fibers from patients showing a muscle weakness that could not be attributed to a known molecular or genetic defect. Overall, we conclude that AFM may provide a useful method to complement current diagnosis tools of known and unknown muscular diseases, in research and in a clinical context.

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G.O.2
A primary sequence motif underlying calpain 3 substrate cleavage

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Calpain 3 (CAPN3) is a member of the calpain family of cysteine proteases and is mutated in Limb Girdle Muscular Dystrophy (LGMD) type 2A. Three prevailing hypotheses exist for CAPN3 pathogenesis: (1) deregulated (in)activation of CAPN3 itself, (2) protease mislocalisation, and (3) deregulation of a limited number of substrates critically important for muscle fiber homeostasis. However, in the absence of knowledge how CAPN3 recognizes its substrates, large scale identification of its targets is impossible and consequently the exact pathomechanism of LGMD2A remains largely elusive. Using a combination of bio-informatics and biochemical analyses we identified a primary amino acid sequence motif underlying CAPN3 substrate recognition. This motif is common to all 11 reported CAPN3 substrates, and can transform a non-related protein into a substrate. The motif identifies >300 new CAPN3 targets with >90% accuracy. Among the new CAPN3 target proteins we identified the Protein Inhibitors of Activated Stats (PIAS) family of E3 sumoyl ligases as substrates for CAPN3 cleavage and observed that CAPN3 can negatively regulate PIAS3 sumoylase activity. Bioinformatic analysis of our predicted substrates suggest that CAPN3 functions as an orchestrator of rapid local changes in cyto-architecture.

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G.O.3
Contraction-dependent (FSHD1) and independent (FSHD2) epigenetic changes of D4Z4 unify FSHD

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Autosomal dominant facioscapulohumeral muscular dystrophy (FSHD) is the second most common myopathy in adults. FSHD is clinically characterized by progressive weakness and wasting of the facial, shoulder and upper arm muscles, often in an asymmetric fashion. Genetically, FSHD is associated with a contraction of the D4Z4 macrosatellite repeat in the chromosome 4q subtelomere in >95% of patients. In healthy individuals this polymorphic repeat can vary between 11-100 D4Z4 units, each unit being 3.3 kb in size. Patients with FSHD carry one allele with a D4Z4 repeat of 1–10 units. However, D4Z4 contraction is not sufficient to cause FSHD as it needs to occur on a specific genetic background: the subtelomeric 4qA161 haplotype. Contractions in other 4q haplotypes and in chromosome 10q carrying a highly homologous repeat are non-pathogenic.

We have performed a detailed DNA methylation study of the D4Z4 repeat and proximal sequences on chromosomes 4q and 10q. FSHD patients with a D4Z4 contraction (FSHD1) show *contraction-dependent* hypomethylation, which is restricted to D4Z4 and limited to the disease allele. Importantly, hypomethylation occurs irrespective of the genetic background of the repeat. Thus, control individuals with a D4Z4 contraction on a non-pathogenic 4q haplotype or on chromosome 10q also present with hypomethylation. In fifteen FSHD families without D4Z4 contractions (FSHD2), we observed D4Z4-restricted, *contraction-independent* hypomethylation on chromosomes 4q and 10q, including at least one copy of the pathogenic haplotype as seen in FSHD1. This implies that a genetic defect resulting in D4Z4 hypomethylation at chromosomes 4q and 10q underlies FSHD2. In conclusion, our data support the hypothesis that the combination of a chromatin change in D4Z4 on a 4qA161 haplotype unifies FSHD1 and FSHD2.

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G.O.4
α-Actinin-3 regulates muscle glycogen phosphorylase: A potential mechanism for the metabolic consequences of the common human null allele of *ACTN3*

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Approximately one billion people worldwide are homozygous for a stop codon polymorphism in the *ACTN3* gene (R577X) which results in complete deficiency of the fast muscle protein α-actinin-3. *ACTN3* genotype is associated with human athletic performance with the 577XX genotype being under-represented in sprint athletes and over-represented in endurance athletes. Using a knock-out mouse model of α-actinin-3 deficiency, we have previously demonstrated a shift in the properties of fast muscle fibres in α-actinin-3 deficient individuals towards slower fibre properties;of note there is increased activity of multiple enzymes in the aerobic metabolic pathway in the KO mouse muscle. This provides an explanation for the link between 577XX genotype and athletic performance and muscle function in humans.

We aimed to determine the molecular mechanism(s) underlying the metabolic changes seen in muscle in the absence of α-actinin-3. We show that a specific subset of structural muscle proteins is upregulated in the absence of α-actinin-3, including α-actinin-2, myotilin, desmin and γ-filamin. Although sarcomeric localisation of these proteins is not grossly affected we see cytoplasmic inclusions of myotilin in the knock-out mouse muscle suggesting subtle structural differences in muscles lacking α-actinin-3. Interestingly these inclusions also contain glycogen phosphorylase (GPh). We demonstrate