

subtypes. We have also identified two other classes of noncompetitive aptamers that are differentially selective to conformations of GluA2, a key AMPA receptor subunit that mediates excitotoxicity: one class uniquely inhibits the open-channel whereas the other inhibits the closed-channel conformation. To turn these aptamers into potentially useful drugs, we have now successfully generated a class of chemically modified aptamers that are biostable or resistant with ribonucleases so that these aptamers can be tested *in vivo*. Our results demonstrate the possibility of developing aptamers that have nanomolar affinity and are highly selective to both an AMPA receptor subunit and a unique receptor conformation. These aptamers are excellent water-soluble, nanomolar affinity templates for design of better inhibitors as drug candidates for a potential new ALS therapy.

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## DYSTROPHINOPATHIES: PHARMACOLOGICAL APPROACHES; POSTER PRESENTATIONS

### P3.39

#### Ca<sup>2+</sup>-permeable channel TRPV2 as a promising therapeutic target for muscular dystrophy and cardiomyopathy

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Disruption of dystrophin–glycoprotein complex caused by genetic defects of dystrophin or sarcoglycans results in muscular dystrophy and/or cardiomyopathy in human and animal models. Abnormal Ca<sup>2+</sup> handling has been thought to be a key molecular event in the pathology of these diseases. However, the detailed mechanism for Ca<sup>2+</sup> abnormality remains elusive. We have previously shown that the transient receptor potential cation channel TRPV2 is a principal candidate for Ca<sup>2+</sup>-entry pathways in dystrophic muscles. In order to determine whether TRPV2 is a crucial molecule for Ca<sup>2+</sup>-induced muscle damage, we developed two procedures to inhibit the endogenous TRPV2 activity, i.e., transgenic incorporation of dominant-negative TRPV2 and application of drugs inhibiting TRPV2. We found that these approaches significantly reduced the increase in basal intracellular Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>i</sub>) as well as the increase in [Ca<sup>2+</sup>]<sub>i</sub> induced by high Ca<sup>2+</sup> and TRPV2 agonist, 2-aminoethoxy diphenyl borate, which were observed in dystrophic muscles. These findings indicate that these treatments resulted in the robust inhibition of TRPV2. Furthermore, we observed the 40–70% amelioration of impaired muscle function such as an increased number of central nuclei and fiber size variability, fibrosis, apoptosis, elevated serum creatine kinase levels, and of reduced muscle performance in dystrophic animals. Similar beneficial effects were also seen in cardiomyopathic animals. These results suggest that TRPV2 is a principal Ca<sup>2+</sup>-entry route leading to a sustained [Ca<sup>2+</sup>]<sub>i</sub> increase and muscle degeneration, and that it is a common promising therapeutic target for muscular dystrophy and cardiomyopathy.

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### P3.40

#### Potential role of sirtuin-1 as druggable target in muscular dystrophy: effect of a chronic resveratrol treatment on *in vivo* and *ex vivo* pathological signs of dystrophic mdx mouse

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Sirtuin1 (Sirt1) is a NAD<sup>+</sup> dependent deacetylase modulating metabolic functions, reaction to stressors and longevity. Sirt1 also activates peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$  (PGC-1 $\alpha$ ), a key modulator of muscle metabolism; PGC-1 $\alpha$  overexpression in dystrophic mdx mice leads to milder signs of pathology and an improved function both in normal condition and after intense physical exercise (Handschin et al., Gen. Develop., 2007; Cantò et al., Nature, 2009). Then, drugs able to activate Sirt-1/PGC-1 $\alpha$  pathway may have positive effects in muscular dystrophy. We performed a proof-of-concept study by evaluating in treadmill-exercised mdx mice the effects of a chronic treatment with resveratrol (100 mg/kg; 6 days/week i.p. for 4–6 weeks), a known Sirt1 activator, in comparison with those of a similar treatment with  $\alpha$ -methyl-prednisolone (PDN 1 mg/kg i.p.). *In vivo*, resveratrol and PDN similarly counteracted the exercise-induced decrease of maximal and normalized fore limb strength, while a partial amelioration of resistance to exercise was observed. *Ex vivo*, the resveratrol treatment slightly ameliorated mechanical threshold, an electrophysiological index of calcium homeostasis, but did not exert any significant effect on isometric twitch and tetanic tension of EDL muscle. However, in contrast with PDN, a significant reduction of plasma creatine kinase and lactate dehydrogenase was observed in resveratrol-treated animals. Also, resveratrol caused a 70% reduction of fibres positive to dihydroethidium (DHE), a marker of superoxide anion production, in tibialis anterior muscle. An improvement of histology profile was observed in gastrocnemius muscle, along with a slight decrease of NF- $\kappa$ B positive fibres. The results suggest that resveratrol may exert protective effect in dystrophic muscles, likely by reinforcing the metabolic pathways that contrast oxidative stress (supported by Telethon-Italy and Charley's Fund).

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### P3.41

#### *In vivo* studies on the effects of EGCG, a major polyphenol in green tea, on a mouse model of Duchenne muscular dystrophy

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At WMS2009, we reported that epigallocatechin-3-gallate (EGCG) given to young mdx mice in their diet or by subcutaneous (s.c.) injection reduces their serum CK activities and the numbers of lipofuscin (LF) granules, a marker of oxidative stress, per unit volume of diaphragm muscle. Concomitantly, the specific phasic twitch and tetanic tensions of triceps surae muscles are increased. These results suggest that EGCG limits the degeneration of mdx muscles. To extend our previous findings, we have investigated the effects of EGCG on mdx mice using additional assessment criteria. The EGCG doses used were 180 mg/kg/day in the diet and 2.9 or 5.7 mg/kg/day for s.c. injection. The mice were treated for 5 weeks beginning when they were 3-weeks-old. The integrated locomotor activities of the mice were then measured, and selected muscles removed at autopsy. The mean % area of the connective tissue in sections of EDL muscles of mdx mice given dietary EGCG was 15  $\pm$  3% less than in mdx controls not given EGCG. EGCG injection at both doses did not alter the amount of connective tissue in either EDL or diaphragm muscles. Dietary EGCG administration also had no significant effect on the

amount of connective tissue in diaphragm muscles but the mean numbers of LF granules/mm<sup>3</sup> of diaphragm muscles of *mdx* mice treated orally with EGCG and subcutaneously with the higher dose of EGCG were reduced by 27 ± 6% and 22 ± 5% respectively. However, the lower injection dose had no effect. Both routes of EGCG administration did not alter the amount of LF in EDL muscles. Integrated locomotor activities of *mdx* mice only increased significantly (by 55 ± 18%) with the higher s.c. EGCG dose. These results suggest that decreased LF formation in involuntary diaphragm muscle is a more reliable marker for monitoring the effectiveness of EGCG treatment than that in voluntary EDL muscle. Our previous and present results indicate that EGCG is a potent drug for limiting the degeneration of dystrophin-deficient muscles.

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### P3.42

#### Contribution of reactive oxygen species generated through NADPH oxidase to abnormal calcium signals in dystrophic skeletal muscle cells: involvement of phosphatidylinositol-3-kinase and protein kinase C

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Although the molecular mechanisms leading to muscle degeneration in Duchenne muscular dystrophy are not fully understood, numerous studies point to a deregulation of Ca<sup>2+</sup> homeostasis and an increase in the generation of reactive oxygen species (ROS) in the pathogenesis of the disease. We show here that primary cultures of dystrophic myotubes generated more ROS than normal ones upon stimulation by KCl or phorbol 12-myristate 13-acetate. This increase was associated with a greater NADPH oxidase (NOX) activity in dystrophic myotubes. Under these conditions, NOX activation was mediated by both phosphatidylinositol-3-kinase/phospholipase C and protein kinase C pathways. We confirmed the presence of mRNA transcripts for NOX1, NOX2 and NOX4 isoforms in cultured myotubes. Dystrophic myotubes expressed greater mRNA levels for NOX2, as well as for p22<sup>phox</sup>, the membrane subunit associated with NOX1, NOX2 and NOX4, and for p40<sup>phox</sup>, p47<sup>phox</sup>, p67<sup>phox</sup>, the cytoplasmic subunits associated with NOX2, as determined by real-time RT-PCR. NOX4 mRNA was not changed. Moreover, ROS generated by NOX contributed to the increased Ca<sup>2+</sup> transients in dystrophic myotubes as the NOX inhibitors diphenylene iodonium and apocynin restored these transients to those of normal myotubes. This effect was associated with an increase of both plasma membrane Ca<sup>2+</sup> entry and Ca<sup>2+</sup> release from the sarcoplasmic reticulum. In conclusion, our findings support the idea that ROS elicited by NOX contribute to the abnormal Ca<sup>2+</sup> signals in dystrophic myotubes.

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### P3.43

#### Long term administration of green tea polyphenols and pentoxifylline to *mdx*<sup>5Cv</sup> dystrophic mice: differential effects on muscle function and kyphosis

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DMD is a fatal muscle disorder caused by the absence of dystrophin and characterized by progressive muscle wasting. Weakness of the back muscles often causes postural alterations, impairing patients' respiratory function. Oxidative stress likely contributes to the pathogenesis. We have previously shown that antioxidants such as green tea polyphenols (GTP) and EGCG (the major GTP component) improved muscle structure and function of the *mdx*<sup>5Cv</sup> mouse, a model for DMD. Pentoxifylline (PTX), a non-selective phosphodiesterase inhibitor and TNF  $\alpha$  release inhibitor was used as a positive control and also proved efficacious. EGCG is about to be clinically evaluated on DMD patients. In this context, we investigated the effects on dystrophic mice of long-term oral administration (for 15 months, from 3-weeks to about 16-months of age) of GTP, EGCG, and PTX. As assessed at the end of the treatment period with a wire test (involving fore- and hind-limb force), the motor function was improved with the following potency: PTX > GTP > EGCG. Using micro-computed tomography, we determined that kyphosis, the characteristic spine deformity occurring in old dystrophic mice as a result of back and thorax muscle weakness, was prevented by GTP, to a lesser extent by EGCG, but not by PTX. Tomography also allowed us to identify calcifications of the Achilles tendons, which, to the best of our knowledge, have never been reported so far. These calcifications were about twice larger in dystrophic mice than in normal mice and were diminished with the following potency: GTP > EGCG ~ PTX. Similarly, GTP and EGCG were more potent than PTX at reducing creatine kinase in the plasma (an index of muscle membrane fragility) and at preventing the loss of force induced by repetitive tetanic contractions. Our results suggest that GTP/EGCG and PTX exert different effects on different muscle groups. A combination of EGCG and PTX may provide more benefit than each compound alone.

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### P3.44

#### Green tea polyphenols enhance the motor performance of the *mdx*<sup>5Cv</sup> dystrophic mouse and normalize calcium influx in muscle fibres

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Duchenne muscular dystrophy (DMD) is a fatal muscle disorder caused by the absence of dystrophin and characterized by progressive muscle wasting. Oxidative stress and excessive calcium influx are thought to contribute to the pathogenesis. Indeed, we have previously shown that dietary interventions with powerful antioxidants such as green tea polyphenols (GTP) and EGCG (the major GTP component) improved muscle structure and function of the *mdx*<sup>5Cv</sup> mouse, a model for DMD. As clinical trials are being conducted with EGCG on DMD patients, we report additional therapeutic effects of GTP and EGCG on the dystrophic mouse. As in our previous study, 3-weeks old mice were given for 5–8 weeks a chow enriched with GTP, EGCG or pentoxifylline (PTX), a non-selective phosphodiesterase inhibitor and TNF  $\alpha$  release inhibitor used as a positive control. GTP, EGCG, and PTX ameliorated spontaneous locomotor activity and performance in a wheel running assay, and decreased plasma creatine kinase activity (a marker of muscle membrane fragility). The manganese quench technique was used to measure the influx of calcium into muscle fibres isolated from FDB muscles and loaded with the calcium probe Fura-2. Pre-treatment of the mice with GTP, EGCG, or PTX reduced by up to two-thirds the excessive calcium influx in dystrophic muscle fibres in resting conditions. In contrast, acute exposure to GTP, EGCG, or PTX of fibres