

X-Linked centronuclear myopathy (XLCNM) is a rare and severe congenital myopathy characterised by generalised muscle weakness and abnormal nuclei positioning. Most affected boys die in their first year of life and survivors fail to achieve independent ambulation. It is caused by mutations in the *Mtm1* gene encoding myotubularin, a ubiquitously expressed phosphoinositide phosphatase. No cure exists and very few pharmacological avenues are being explored. Here, we treated *Mtm1*-null mice with tamoxifen (TAM), a drug that modulates estrogen actions and that we have shown earlier to be efficacious in dystrophic (*mdx5Cv*) mice, a model of Duchenne muscular dystrophy (DMD). We report that TAM is also effective in *Mtm1*-null mice, a model of XLCNM. Wild type and *Mtm1*-null mice were given normal chow or a TAM-supplemented chow starting at weaning. Non-treated *Mtm1*-null mice died at around 40 days. By contrast, about half of the *Mtm1*-null mice treated with clinically relevant doses of TAM survived beyond 365 days of age. Clinical scoring showed that the motor function of the affected mice was markedly improved. *In vivo* force recordings performed at D40 and D80 revealed that the force of treated *Mtm1*-null was significantly improved after only 3 weeks of treatment. Histological and electron microscopy analyses show partial rescue of muscle structure and triads, consistent with improved calcium homeostasis in FDB fibres. Quantitative PCR and western blots demonstrate reduction of BIN1 and DNM2, which act downstream of MTM1. In conclusion, we found that tamoxifen extends the lifespan of *Mtm1*-null mice up to 10-fold and rescues their motor skills. Collectively, these findings suggest that estrogen signalling is a key pathway that modifies disease severity in unrelated myopathies as diverse as DMD and XLCNM. Tamoxifen is safe and readily available. We believe that it deserves clinical evaluation for XLCNM.

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TH.O.20

Exhaustive characterization of the newly developed Duchenne muscular dystrophy rat model: a unique animal model for DMD which mimics the human disease at both the muscular and the cardiac levels

C. Huchet¹, G. Toumaniantz², T. Larcher³, B. Frayssé⁴, A. Lafoux⁵, S. Remy⁶, D. Caudal⁵, M. Allais⁴, E. Amosse², I. Anegón⁶, C. Le Guiner⁴
¹ Université de Nantes, Nantes, France; ² UMR INSERM U1087, Nantes, France; ³ UMR INRA 703, Nantes, France; ⁴ UMR INSERM U1089, Nantes, France; ⁵ Therassay Capacités, Nantes, France; ⁶ UMR INSERM U1064, Nantes, France

Duchenne muscular dystrophy (DMD) is a severe muscle-wasting disorder caused by mutations in the gene encoding dystrophin. The evaluation of potential therapeutic products requires relevant animal models exhibiting a phenotype very close to those observed in human patients. If both large and small animal species deficient for dystrophin (especially mice and dogs) have been extensively used for preclinical studies of DMD, they present some limitations, including the absence or very delayed development of cardiomyopathy. We recently generated a line of *dmd* mutated-rats (*Dmd^{mdx}*) using TALENs and initially characterized it at two stages of development. To complete this characterization, we performed a large natural history study, during which different groups of *Dmd^{mdx}* rats and littermate wild-type controls were followed over several generations and exhaustively evaluated at different time points (1.5, 3, 4.5, 7, 10 and 12 months). One supplemental group of *Dmd^{mdx}* rats was also used to document the lifespan, as well as the causes of death. We showed that life span of *Dmd^{mdx}* rats is significantly reduced. Weight, blood biomarkers concentrations, muscle strength and fatigue measured by grip force test, muscle calcium homeostasis and histology (including quantification of fibrosis) in skeletal muscles, diaphragm and heart, are all significantly impaired as soon as the age of 1.5 months and show a clear stepwise evolution along with age. Moreover, echo and electrocardiography approaches highlighted a significant and rapid concentric remodeling associated to an alteration of diastolic function, which progressed unfavorably with age towards systolic heart failure with rhythm disorders. In conclusion, with systematic and stepwise aggressive phenotypes at both the muscular and the cardiac levels, similar to what occurs in DMD patients, this unique and newly developed

Dmd^{mdx} rat model is now one of the best animal model for the preclinical evaluations of new treatments for DMD.

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TH.O.21

Connexin-based hemichannels are key factors in the pathological mechanism underlying dysferlinopathy

G. Fernández¹, J. Bevilacqua¹, A. Cardenas², J. Sáez³, P. Caviedes¹, L. Cea¹
¹ University of Chile, Santiago, Chile; ² University of Valparaíso, Valparaíso, Chile; ³ Pontificia Universidad Católica De Chile, Santiago, Chile

Dysferlinopathy onsets during the second and third decades of life, usually as progressive lower-limb weakness that later involves trunk and upper-limbs. Dysferlin is localized mainly in the sarcolemma and participates in membrane repair. However, in dysferlin-deficient (DD) mice the recovery of the membrane resealing function by expression of a mini-dysferlin does not arrest progressive muscular damage. The latter suggests the presence of dysferlin-dependent pathogenic mechanisms still unknown. In this regard, we have demonstrated a persistent *de novo* expression of functional connexin-based hemichannels (Cx HCs) in pathological conditions that affect skeletal muscles. Such membrane channels are permeable to Ca²⁺ and contribute critically to muscular damage. Connexins 40.1, 43 and 45 were localized in the sarcolemma of myofibers of human muscle biopsies from 5 unrelated dysferlinopathy patients. DD mice myofibers also exhibited positive immunostaining for Cxs 39, 43 and 45. In addition, an elevated Cx HCs activity, concomitant with elevated resting intracellular free Ca²⁺ levels, was observed in myofibers obtained from DD mice, compared to control myofibers. Further, we detected a lower performance of DD mice in rota-rod motor testing compared to control mice. Moreover, all these changes were prevented in triple knockout mice deficient in Cx 43 Cx 45, and dysferlin, suggesting that Cxs are relevant in the pathogenic mechanism of dysferlinopathy. Therefore, Cx HCs could be a most suitable candidate for pharmacological therapy.

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TH.O.22

Prion-like protein aggregation of desmin myofibrillar myopathies

C. Weihl, J. Bieschke

Washington University in St. Louis, St. Louis, USA

Protein aggregate myopathies (PAMs) are a large class of myodegenerative diseases. Their pathologies are due to the misfolding and aggregation of intracellular proteins in muscle cells. In some cases, these inclusions stain with Congo Red suggesting they are true amyloid. Desminopathies are a prototypical PAM that is caused by dominantly inherited mutations of the *DES* gene, which codes for the protein desmin. Healthy desmin forms type III intermediate filaments in muscle fibers; whereas desmin disease mutations affect intermediate filament structure by interfering with intermediate filament assembly. This leads to desmin aggregation in desminopathies, although desmin is also a principal component of aggregates in other PAMs. We hypothesize that desmin can intrinsically form amyloidogenic aggregates that template the conversion of non-aggregated desmin *in vitro* and *in vivo*. This pathomechanism would be similar to neurodegenerative disorders and suggest a prion-like aggregate phenomenon in myopathies. Consistent with this hypothesis, we demonstrate, for the first time, 1) that recombinant desmin and/or desmin fragments form amyloid *in vitro*; 2) that disease-associated point mutations dramatically increase desmin amyloid formation; 3) that aggregated desmin amyloids can “seed” the aggregation of naïve unaggregated desmin fragments *in vitro*; 4) that desmin aggregate “seeds” can be taken up by cells and incorporate into an existing desmin network *in vivo*; and 5) that aggregated desmin expressed in cell culture can “seed” the aggregation of naïve unaggregated desmin fragments *in vitro*. These data support a novel pathogenic mechanism of disease in PAMs in which desmin amyloids can