# Neuromuscular Research

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## Translational Research in Neuromuscular Diseases

Our Neuromuscular Research Laboratory, Clinic of Neurology and Department of Biomedicine, focuses on the elucidation of pathophysiological mechanisms involved in neuromuscular diseases and on the development of therapeutic strategies. Myotonic dystrophy type I (DM1) is a disabling neuromuscular disease with no causal treatment available. It is the most prevalent muscular dystrophy in adults, affecting about 1 in 10'000 individuals. This disease is caused by expanded CTG trinucleotide repeats in the 3' UTR of the dystrophia myotonica-protein kinase gene *(DMPK).* On the RNA levels, expanded (CUG)n repeats form hairpin structures that sequester splicing-factors, such as muscleblind-like 1 (MBNL1). Lack of available MBNL1 leads to mis-regulated alternative splicing of many target pre-mRNAs, causing multisystemic involvement in DM1.

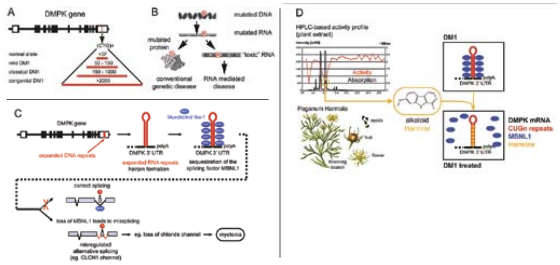


**Fig. 1:** The molecular basis of DM1 is an expansion of an unstable repeat sequence in the noncoding part of the DMPK gene (A). Severity of disease is correlated with the size of the repeat expansion (A). In DM1, the mutation is located in a noncoding region and does not alter the protein sequence, but leads to toxic RNA (B). The sequestration of the alternative splicing factor MBNL1 by toxic RNA leads to altered splicing of target pre-mRNAs like *CLCN1,* encoding muscle-specific chloride channel (ClC-1). This mis-splicing leads to ClC-1 deficiency and to myotonia (C). HPLC-based activity profiling identifies the alkaloid harmine as the active constituent in an extract from the roots of Peganum harmala capable of inhibiting the complex formation between expanded CUG repeat RNA and the splicing factor MBNL1 (D). (plant image from *www.drugs-forum.com;* cartoons adapted from *Herrendorff et al., JBC 2016; 291(33)17165–77* and *Kinter and Sinnreich, Swiss Med Wkly. 2014;144:w13916)*

In an effort to identify small molecules that liberate sequestered MBNL1 from (CUG)n RNA, we focused in a collaborative effort with the research group of Prof. Matthias Hamburger, Pharmazentrum Basel, specifically on small molecules of natural origin. We developed a DM1 pathomechanism-based biochemical assay and screened a collection of isolated natural compounds, as well as a library of over 2100 extracts from plants and fungal strains. HPLC-based activity profiling in combination with spectroscopic methods were used to identify the active principles in the extracts. Bioactivity of the identified compounds was tested in a human cell model and in a mouse model of DM1. We identified several alkaloids, including the beta-carboline harmine and the isoquinoline berberine, which ameliorated certain aspects of the DM1 pathology in these models. These compounds may provide pharmacophores for further medicinal chemistry optimization.

To investigate whether deregulation of central metabolic pathways such as AMPK/mTOR may also be implicated in the pathogenesis of DM1, we are currently examining human skeletal muscle biopsies, as well as human cell lines and DM1 mice challenged by different conditions. In parallel, we are analyzing whether DM1 muscle shows alterations in the autophagy flux and/or proteasome activity by biochemical and histological means. Lastly, we are testing the consequences of the modulation of these metabolic pathways on myotonia by *ex-vivo* electrophysiological methods. We recently found that AICAR, an agonist of AMPK, markedly reduces myotonia in DM1 mice; the underlying mechanisms are currently being investigated.

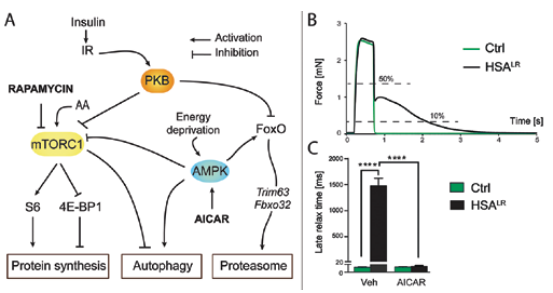


**Fig. 2:** A- Overview of the signaling pathways involved in proteostasis in muscle. B, C- *Ex vivo* measurement of the late relaxation time of EDL muscle reveals myotonia in HSA$^{LR}$ mice. Figures adapted from *Brockhoff et al. J Clin Invest. 2017 Feb 1;127(2):549–563.*

In a broader context we are interested in the disruption of the proteostasis network as a possible pathomechanism for diseases affecting skeletal muscle. In collaboration with the research group of Prof. Markus Rüegg, Biozentrum Basel, we are investigating the implications of mTOR deregulation on muscle homeostasis and are studying the function and regulation of atrogenes, in particular the regulation of the F-box protein Fbxo32, which represents a potential therapeutic target against muscle wasting diseases.

Dysferlin is a transmembrane protein involved in surface membrane repair of muscle cells. Mutations in dysferlin cause the muscular dystrophies Miyoshi Myopathy and Limb Girdle Muscular Dystrophy Type 2B. In the laboratory we aim at developing novel treatment strategies for these diseases. Mouse models are valuable tools to test novel therapeutic approaches. A prerequisite for successful animal studies using genetic mouse models is an accurate genotyping protocol. Unfortunately, the lack of robustness of the currently available genotyping protocols for the Dysftm1Kcam mouse, a widely used dysferlin knock-out mouse model, has prevented efficient colony management. Initial attempts to improve the genotyping protocol based on the published genomic structure failed. These difficulties led us to analyze the targeted locus of the dysferlin gene of the Dysftm1Kcam mouse in greater detail. We found that instead of a deletion, the dysferlin locus in the Dysftm1Kcam mice carried a targeted insertion. This genetic characterization enabled us to establish a reliable method for genotyping of the Dysftm1Kcam mouse, and thus has made efficient colony management possible. These results will help the scientific community to use the Dysftm1Kcam mouse model for future studies on dysferlinopathies.

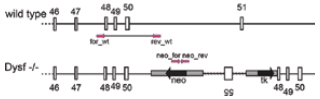


**Fig. 3:** Genetic characterization and improved genotyping of the dysferlin-deficient mouse strain Dysf tm1Kcam. Instead of a deletion, the dysferlin locus in the Dysf tm1Kcam mouse carries a targeted insertion. This genetic characterization enabled us to establish a reliable method for genotyping of the Dysf tm1Kcam mouse (red primers). (adapted from *Wiktorowicz et al., Skeletal Muscle2015, 5:32*)

## Connection to Clinical Practice

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**Interdisciplinary Neuromuscular Clinic**

At our interdisciplinary Neuromuscular Clinic at the Department of Neurology we care for patients affected by a broad range of neuromuscular diseases. In collaboration with our colleagues from pathology, genetics, plastic surgery, pulmonary medicine, rehabilitation, ergo-, physio- and speech therapy as well as social services, we provide clinical and electrophysiological evaluation, perform muscle and nerve biopsies with histopathological and biochemical workup, genetic workup and counseling, rehabilitation, ergo-/physio- and speech therapy as well as assistance in social matters. Novel clinical observations are being worked up scientifically and form the basis for translational research projects.

**Selected Publications**

Brockhoff M, Rion N, Chojnowska K, Wiktorowicz T, Eickhorst C, Erne B, Frank S, Angelini C, Furling D, Rüegg MA, Sinnreich M, Castets P. Targeting deregulated AMPK/mTORC1 pathways improves muscle function in myotonic dystrophy type I. J Clin Invest. 2017 Feb 1;127(2):549–563. doi: 10.1172/JCI89616. Epub 2017 Jan 9

Pröbstel AK, Schaller A, Lieb J, Hench J, Frank S, Fuhr P, Kappos L, Sinnreich M. Mitochondrial cytopathy with common MELAS mutation presenting as multiple system atrophy mimic. Neurol Genet. 2016 Nov 17;2(6):e121. eCollection 2016 Dec.

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Wiktorowicz T, Kinter J, Kobuke K, Campbell KP, Sinnreich M. Genetic characterization and improved genotyping of the dysferlin-deficient mouse strain Dysf (tm1Kcam). Skelet Muscle. 2015 Oct 13;5:32

Petersen JA, Kuntzer T, Fischer D, von der Hagen M, Huebner A, Kana V, Lobrinus JA, Kress W, Rushing EJ, Sinnreich M, Jung HH. Dysferlinopathy in Switzerland: clinical phenotypes and potential founder effects. BMC Neurol. 2015 Oct 6;15:182