Recessive mutations in ANO5 were recently found to cause LGMD2L (MIM 611307) and MMD3 (MIM613319) myopathies. LGMD2L is characterized by late-onset progressive weakness of proximal limb muscles with prominent quadriceps atrophy and high serum Creatine Kinase, while the MMD3 distal myopathy is characterize by initial asymmetrical distal weakness in particular of calves muscles. A total of 13 published and unpublished ANO5 mutations were uncovered to date, consisting of 7 missense mutations, 2 nonsense mutations, 1 splice site mutation, 1 single base pair duplication, 1 small insertion/deletion, and 1 large deletion of two exons. We demonstrated that one splice site mutation introduces a premature termination codon and consequently triggered nonsense-mediated mRNA decay (NMD), which supported an underlining ANO5 loss-of-function. On electron microscopy, multifocal sarcolemmal lesions were observed in LGMD2L and MMD3 patients. ANO5's function is still unknown, but Ano1 and Ano2 function as calcium-activated chloride channel (CaCC). Ano5 is highly expressed in skeletal muscle and is over-expressed in muscles of mdx mice. Our data supports that Ano5 localizes to intracellular membranes in a striated pattern in close proximity to Z-disks, with a partial co-localization with SERCA1 (a sarcoplasmic reticulum marker) and OPA1 (a mitochondrial marker). Results from Western blots, subcellular fractionations, co-immunofluorescence studies and electron microscopy will be presented on human and wild type and KO mice to support the membrane bound sarcomeric localization. A hypothesis will be presented on the relationship between ANO5's intracellular localization and the documented multifocal sarcolemmal perforations and the membrane repair delay reported in some LGMD2L/MMD3 patients. To understand ANO5's function in skeletal muscle will shed light unto less charted pathways perturbed in muscular dystrophies.

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Mouse models of dominant *ACTA1* disease recapitulate human disease and provide insight into therapies

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Mutations in the skeletal muscle α-actin gene (*ACTA1*) cause a range of pathologically-defined congenital myopathies. Most patients have dominant mutations and experience severe skeletal muscle weakness, dying within one year of birth. Transgenic mice expressing dominant mutant *ACTA1*(D286G) were created. These $Tg(ACTA1)^{D286G}$ mice were less active than wildtypes. Their skeletal muscles were significantly weaker and showed various pathological lesions reminiscent of human patients, however they had a normal lifespan. Mass spectrometry revealed that $Tg(ACTA1)^{D286G}$ skeletal muscle contained ~25% mutant protein. $Tg(ACTA1)^{D286G}$ mice were crossed with hemizygous $Acta1^{+/-}$ knock-out mice to generate $Tg(ACTA1)^{D286G+/+}\cdot Acta1^{+/-}$ offspring: homozygous for the transgene and hemizygous for endogenous *Acta1*. Strikingly, the majority of these mice presented with severe immobility in the early postnatal period, requiring euthanasia. $Tg(ACTA1)^{D286G+/+}\cdot Acta1^{+/-}$ skeletal muscle contained extensive structural abnormalities, including nemaline bodies, actin accumulations and sarcomeric disarray. The mutant protein load in severely affected pups was 40–45% of total actin, whereas in the 20% of $Tg(ACTA1)^{D286G+/+}.Acta1^{+/-}$ mice that survived to adulthood the mutant load was 35%. Therefore we have created two dominant *ACTA1* disease mouse models, one mild, and one severe with a dramatically shortened lifespan. This is the first experimental, as opposed to observational, evidence that mutant ACTA1 protein load determines severity of disease. These data suggest that a small change in mutant load greatly affects disease severity and that altering the ratio of mutant to wildtype protein in muscle may be a therapy for patients with dominant *ACTA1* disease. Furthermore, ringbinden fibres were observed in transgenic mouse skeletal muscles, suggesting that perhaps patients with ringbinden of unknown genetic origin should be considered for *ACTA1* mutation screening.

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