

possible mode of pathogenesis. Although mutations in *LMOD2* have not been associated with human disease to date, *Lmod2* loss results in a lethal cardiomyopathy in mice. Since *KLHL40/41* mutations may result in altered *LMOD2* protein levels we recommend cardiac surveillance for *KLHL40/41* myopathy patients.

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Highly variable ultrastructural findings in KBTBD13-nemaline myopathy (NEM6)

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KBTBD13 myopathy (NEM6) is a rare autosomal dominant congenital myopathy characterized by early onset axial and proximal muscle weakness, poor exercise tolerance, and characteristic slowness of movements. Muscle biopsy analyses revealed the presence of numerous rods and unstructured cores, associated with type 1 fiber hypertrophy and type 2 atrophy. *KBTBD13* is a BTB/Kelch family protein whose role in skeletal muscle is largely unknown. In an attempt to better characterize histopathologic findings in *KBTBD13* muscles and suggest pathophysiologic mechanisms we performed detailed electron microscopy analysis in 13 muscle samples from affected subjects. The most frequent lesion, found in 10 samples, was the presence of subsarcolemmal and cytoplasmic clusters of rods. The rods originated from the Z-line, presented different shape, size and orientation. In 2 samples rods clusters were surrounded by a rim or disorganized sarcomeres. Cores of variable size areas were found in 6 samples, always in distinct fiber areas with respect to rods. Very interestingly, 3 samples also harbored variably sized granular protein aggregates resembling to desmin or granulo-filamentous protein structures. The samples with aggregates did not have cores areas. In conclusion, our *KBTBD13* ultrastructural analysis showed that rods are the most frequent histopathologic lesion, followed by the association of rods and cores. The unexpected finding of protein material surcharge derived from myofibril dissolution in 3 cases suggests a possible role of *KBTBD3* in intermediate filaments maintenance. Immunohistochemical and protein studies to assess intermediate filaments integrity in *KBTBD13* samples are ongoing to confirm this hypothesis.

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A mouse model with compound heterozygous nebulin mutations recapitulates the typical form of nemaline myopathy

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The typical form of autosomal recessive nemaline myopathy (NM) is most commonly caused by mutations in the nebulin gene (*NEB*). *NEB* mutations account for at least 50% of all NM cases worldwide, thus represent a significant disease burden. Most *NEB*-NM patients have a compound heterozygous genotype. Very few murine models have been developed for *NEB*-NM, and even fewer have mutations in *Neb* – instead many of the models are knock-outs of *Neb*. There is only one mouse model harbouring the equivalent of a patient mutation (exon 55 deletion), however this has a very severe phenotype. All homozygous mice die by postnatal day 8, thus the scope for analysing disease progression and therapies in this model is limited. None of the previously developed murine models have had compound heterozygous *Neb* mutations, similar to most human patients. Our aim was to develop a murine model that would more accurately match the underlying genetics of typical *NEB*-NM, and would be useful in elucidating the pathogenic mechanisms underlying the disease. Therefore, we have characterised a mouse strain with compound heterozygous *Neb* mutations, one a missense (*KINeb*^{Y2303H}) within a conserved actin binding domain and the other a nonsense mutation (*KINeb*^{Y935X}). Preliminary studies show this compound heterozygous model survives beyond 12 months of age, has perturbed body weight, decreased voluntary running ability, and striking skeletal muscle pathology, including nemaline bodies and cores. Other phenotypic assessments are ongoing. This new model, along with the corresponding parental lines with only the missense or the nonsense mutation, will be useful in deciphering the pathogenic mechanisms of *NEB*-NM. Moreover, this mouse model will be valuable for evaluating therapeutic approaches for *NEB*-NM, including gene-based therapies. Our results so far indicate that this mouse strain recapitulates the typical form of *NEB*-NM and will be a much-needed addition to the *NEB*-NM mouse model collection.

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Further insights in nemaline myopathy (NM) with hyaline masses

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Nemaline myopathies (NM) comprehend a heterogeneous group of rare congenital muscle conditions characterised by hypotonia, muscle weakness, skeletal deformities with nemaline bodies (rods) in muscle biopsy. Here we describe four NM patients with distinctive hyaline masses in the muscle biopsy in addition to the typical NM histological features. Age at onset varied from early childhood, presenting with myopathy, skeletal deformities and retard on developmental motor milestones, to late-onset-myopathy beginning in the third decade as progressive generalized amyotrophy and muscle weakness. All patients presented with a moderate restrictive respiratory impairment, but no cardiac involvement was observed. Muscle biopsy analyses revealed in all samples round-shaped centrally located protein aggregates corresponding to hyaline masses, clusters nemaline bodies and type 1 fibre predominance or uniformity. Electron microscopy confirmed that hyaline masses consisted of centrally located protein aggregates originating from the z-line extending longitudinally through several sarcomeres. Typical rods were found separated from the protein aggregates, often localized in the subsarcolemmal areas. Genetic screening revealed two pathogenic mutation in nebulin (*NEB*) gene (P14, Malfatti et al. 2014) and one single exon 151 *NEB* gene deletion in another. Molecular screening is ongoing for the other two patients. Similar histopathological findings were previously reported by Selcen et al. in 2002 in

two patients with cardiopathy and no genetic characterization. In conclusion we further characterized nemaline rods myopathy with hyaline masses and we enlarge the histopathological spectrum of NEB-related nemaline myopathies.

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“Core-rod” congenital myopathy with bilateral foot-drop. A challenging clinical and genetic diagnosis

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Core-rod myopathy (CRM) is a rare congenital myopathy, initially related to mutations in the *RYR1* gene and subsequently mutations in the *NEB* gene were described. We present a 3-year-old boy, followed-up for 12 years, who consulted because of motor developmental delay and muscle weakness since birth. Normal CPK, EMG suggested anterior horn disease. He showed: longiligneous aspect, high palate, pectum excavatum, mild generalized weakness, bilateral valgus feet, hyporeflexia, generalized hyperlaxity, waddling gait. No facial weakness, ophthalmoparesis and fasciculations were observed. The patient developed anterior tibial weakness, bilateral foot-drop and steppage gait. At 4 years, he presented with pneumonia and massive atelectasis. Mechanical ventilation was required, followed by nocturnal noninvasive ventilation (NIV). As progressive severe respiratory incapacity increased, daytime NIV was needed. A quadriceps muscle biopsy (3y 8mo) showed undifferentiated muscle type 1 with variation in fiber diameter. Rods were randomly distributed and/or accumulated in subsarcolemmal areas. Irregular oxidation defects were evident. In some fascicles, mainly in larger-size fibers, cores were observed, generally central and one each fiber. Electron microscopy showed rod structures, focal Z line streaming in few sarcomeres, small “minicore-like”. Congenital CRM was suspected. No DNA mutations were found in the *RYR1*-C-terminus domain. Sequencing of the entire *RYR1* transcript muscle biopsy showed a single heterozygous variant (de novo mutation). As the clinical features did not fit the *RYR1* phenotype, exome sequencing was performed showing two heterozygous variants in *NEB* gene responsible for the disease, agreeing with the clinical findings and previous descriptions. Clinical aspects -presence of foot-drop and progressive respiratory involvement- associated with the finding of CRM on muscle biopsy were the key to diagnosis. Foot-drop is a guiding and frequent sign in *NEB* myopathy.

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Unusual findings in a *TPM3* case

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Mutations in the gene *TPM3* (alpha-tropomyosin (slow)) is a rare cause of nemaline myopathy. Tropomyosin proteins regulate muscle contraction by controlling the binding of two muscle proteins, myosin and actin. Slow muscle α -tropomyosin is found only in type I fibers. Our case was given a diagnosis of ‘mild muscular dystrophy’ in 1968 when he was 18 years old. At that time, he was toe walking, unsteady on his feet and had pes cavus with demonstrable distal weakness. He represented at the age of 55 years with increasing

difficulties climbing stairs and slopes and noticed his balance had deteriorated and he had developed a high stepping gait. He also reported weakness in his grip but was able to do buttons and undo bottle tops without too much difficulty. He reported that his mother, who had subsequently died, had had clawed toes and he had no siblings. He had obvious proximal limb girdle weakness and more marked distal weakness and a creatine kinase level that was mildly raised at 300 IU/L, neurophysiology confirmed a sensory axonal neuropathy and his muscle biopsy is discussed. His initial muscle biopsy showed variation in fibre size, increased internal nuclei and excessive connective tissue at age 18 years. Semithin sections were reviewed; his biopsy showed wide variation in fibre size, many atrophic fibres and some with severe fibrillar disorganisation. Large clusters of nemaline rods are striking. Sparse abnormal mitochondria are seen. Zebra fibres are sparsely seen, mainly in regions of fibrillar disorganisation. No collections of actin filaments are seen. We present an unusual case of *TPM3* myopathy and the unusual muscle biopsy findings with Zebra bodies.

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Altered actin affinity – a possible disease-causing mechanism in *NEB*-related nemaline myopathy

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Nemaline myopathy (NM) is most commonly caused by recessive mutations in the nebulin gene (*NEB*). Nebulin is an enormous sarcomeric protein (600–900 kDa). Over 90% of the highly modular filamentous nebulin consists of super repeats, which are formed of seven simple repeats. Each super repeat has one tropomyosin-binding motif and seven actin-binding motifs. Missense mutations in *NEB* are common, and their effects often difficult to predict. Research into the nebulin protein has been hampered by its enormous size. Our previous studies have revealed that nebulin binds actin with notably higher affinity at both ends of the super-repeat region and, consequently, actin affinity is weaker in the central parts of nebulin. To study this further, we constructed a nebulin super-repeat panel, with which we were able to study the actin affinity of each of the 26 super repeats individually. The actin-binding experiments were performed as co-sedimentation assays. Currently, we are testing whether known disease-causing variants change nebulin–actin affinity. Our preliminary results show that the binding affinity of such variants can be either stronger or weaker compared with the actin affinity of the corresponding wild-type nebulin domains. This abnormal interaction between altered nebulin and actin is a possible pathogenetic mechanism in NM and related disorders. Our aim is to develop a tool for functional testing of missense mutations. Furthermore, our ongoing experiments are aiming to test whether the same variants have similar effects on nebulin–actin interaction when the variant is located in a super repeat with strong actin affinity compared with one with weak actin affinity. Understanding the pathogenetic mechanisms of NM, and learning more about the effects of *NEB* disease-causing variants is essential for diagnostics and the development of future therapies.

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A new *TNNT1* mutation in a non Amish patient with original muscle pathology findings

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We report the case of a 7 yo French-Canadian girl with rigid spine syndrome. She was born with severe arthrogryposis that evolved in infancy with rigid spine syndrome. Her muscle biopsy showed multiminicore. She also