One of the key components in stabilizing and determining the lengths of the actin filaments of the skeletal muscle sarcomere and in the neurons of the brain is the gigantic protein nebulin. Its size varies from 600 to 900 kDa and correlates with the length of the actin filaments. A huge variation in length is produced by differential usage of the alternatively spliced exons 63–66, 143–144 and 166–177, among the 183 exons of the nebulin gene (*NEB*). Exons 63–66 are always expressed together as a block, while exons 143 and 144 give rise to two different transcripts varying between muscle types and in different developmental stages. Exons 166–177 are spliced independently. Over 150 recessive, mostly nemaline myopathy- (NM) causing mutations, but also mutations causing core-rod and distal myopathies have been identified to date; homozygous or both of the compound heterozygous mutations have been identified in 106 probands of the Helsinki cohort. Of these probands, 23 have a mutation in an alternatively spliced exon. Interestingly, at least twelve of them have clinical features unusual in NM, and two have core-rod myopathy. Vice versa, many probands in the Helsinki cohort with *NEB* mutations and unusual features such as core structures seen in their muscle biopsies have at least one of their two compound heterozygous mutations located in an alternatively spliced exon; three probands have mutations in exons 63–66, three in exons 143 or 144, and ten in exons 167–177. Three of these probands have both of their mutations in alternatively spliced exons. Further studies on the expression of alternatively spliced exons in different muscles combined with the clinical data of the patients with mutations in alternatively spliced exons might provide insights into the role and importance of these exons in different muscles and developmental stages, and explain some phenotypic features. The work is ongoing.

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P.9.9

A novel de novo mutation in ACTA1 causes a congenital myopathy with misleading type 1 fiber predominance and a peculiar MRI

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Nemaline myopathy is a genetically heterogeneous disease showing wide clinical variability. Disease severity and prognosis range from neonatal death to almost normal motor function. We report on a 19-year-old boy with reportedly absence of fetal movements. He was severely hypotonic at birth with dolichocephaly, weak facial movements, bilateral clubfoot and feeding difficulties, requiring nasogastric tube feeding; gastrostomy was applied at age 3 months and removed at 12. Motor milestones were delayed: walking at 24 months, with marked foot drop. He has always been unable to eat solid foods because of impaired chewing. Since the age of 8 he is on nocturnal BiPAP. A first muscle biopsy of the quadriceps at age 8 months, and a second biopsy of vastus lateralis performed at age 10 years, both showed variability in fiber diameter, marked type 1 fiber predominance (90%), with neither evidence of endomysial fibrosis nor nemaline bodies. A third biopsy of the right deltoid muscle at age 19 showed marked fibrosis and dystrophic features with proliferation of nemaline bodies. Current neurological examination showed a young intelligent man with nasal voice, generalized weakness, ptosis, and no limitation of ocular movements. The MRI showed marked involvement of glutei muscles, together with involvement of sartorius, tibialis anterior and peroneus longus. Sequencing of ACTA1 in blood DNA detected a novel heterozygous de novo variant. The new mutation affects a highly preserved Threonine. No mutations were detected in SEPN1 and TPM3. We report on a long follow-up in a patient with a congenital myopathy related to a de novo mutation in ACTA1. Of the 3 muscle biopsies performed, the first 2 were not contributory for diagnosis and the clinical presentation did not offer additional clinical clues. The last muscle biopsy performed in adulthood revealed nemaline rods prompting specific molecular investigations and ultimately allowing a genetic diagnosis.

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P.9.10

Deleting exon 55 from the nebulin gene induces severe muscle weakness in a mouse model for nemaline myopathy

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Nebulin – a giant sarcomeric protein – plays a pivotal role in skeletal muscle contractility by specifying thin filament length and function. Although mutations in the gene encoding nebulin (NEB) are a frequent cause of nemaline myopathy (NM), the most common non-dystrophic congenital myopathy, the mechanisms by which mutations in NEB cause muscle weakness remain largely unknown. To better understand these mechanisms, we have generated a mouse model in which Neb exon 55 is deleted ($Neb^{\Delta ex55}$) to replicate a founder mutation seen frequently in NM patients with Ashkenazi Jewish heritage.

$Neb^{\Delta ex55}$ mice are born close to Mendelian ratios, but show growth retardation after birth. Electron microscopy studies show nemaline bodies – a hallmark feature of NM – in muscle fibers from $Neb^{\Delta ex55}$ mice. Western blotting studies with nebulin-specific antibodies reveal reduced nebulin levels in muscle from $Neb^{\Delta Exon55}$ mice, and immunofluorescence confocal microscopy studies with tropomodulin antibodies and phalloidin reveal that thin filament length is significantly reduced. In line with reduced thin filament length, the maximal force generating capacity of permeabilized muscle fibers and single myofibrils is reduced in $Neb^{\Delta ex55}$ mice with a more pronounced reduction at longer sarcomere lengths. Finally, in $Neb^{\Delta ex55}$ mice the regulation of contraction is impaired, as evidenced by marked changes in cross bridge cycling kinetics and by a reduction of the calcium sensitivity of force generation. A novel drug that facilitates calcium binding to the thin filament significantly augmented the calcium sensitivity of submaximal force to levels that exceed those observed in untreated control muscle.

In conclusion, we have characterized the first nebulin-based NM model, which recapitulates important features of the phenotype observed in patients harboring this particular mutation, and which has severe muscle weakness caused by thin filament dysfunction.

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