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Case report

Two novel *nebulin* variants in an adult patient with congenital nemaline myopathy

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Abstract

Congenital myopathies are clinically and genetically heterogeneous disorders, which often remain genetically undiagnosed for many years. Here we present a 40-year old patient with an almost lifelong history of a congenital myopathy of unknown cause. Muscle biopsy in childhood revealed mild myopathic features and rods. Clinical examination on presentation at the age of 40 revealed a facial weakness, atrophy and weakness of the arm muscles and distal leg muscles with mild contractures of the foot flexors and the right elbow. Subsequently, the *nebulin* gene was identified as a putative candidate gene by linkage analyses, but sequence analysis only revealed one heterozygous splice site mutation in intron 73 (c.10872+1G>T). Therefore, "Next Generation Sequencing" was performed, which revealed a second pathogenic variant in exon 145 (c.21622A>C). Compound-heterozygous carrier status was confirmed via sequence analysis of the index patient's parents. Whole body muscle MRI showed a muscle involvement as previously described in *nebulin*-associated myopathies. Based on biopsy material, genetic analyses and muscle MRI, we identified two novel, compound-heterozygous variants in the *nebulin* gene after a 30 year clinical history, which cause a classical childhood type of nemaline myopathy.

Keywords: Congenital myopathy; Nemaline rods; *Nebulin (NEB)* associated myopathies; Next Generation Sequencing; Whole body muscle MRI

1. Introduction

Congenital myopathies are clinically and histopathologically heterogeneous disorders, which usually present at birth or in early childhood, although some patients develop symptoms later in life [1]. The three main categories of classical congenital myopathies comprise nemaline myopathies (NEM), core myopathies, and centronuclear myopathies [2]. NEM are characterized by the presence of rod-like accumulations of Z-disk-derived material, so-called "nemaline bodies" or "-rods", which can best be seen in the modified Gomori-trichrome stain [3]. NEM are associated with mutations in skeletal muscle α-actin (*ACTA1* [4]), nebulin (*NEB* [5]), tropomyosin 2 (*TPM2* [6]), tropomyosin 3 (*TPM3* [7]), troponin T1 (*TNNT1* [8]), cofilin 2 (*CFL2* [9]) and BTB/Kelch family of proteins (*KBTBD13* [2]), kelch repeat and btb/poz domains-containing protein 5 (*KLHL40* [10] and *KLHL41* [11]). Recently, *LMOD3* has been published as another gene for nemaline myopathy [12]. The most frequent disease-causing variants are autosomal dominant *de novo* mutations of *ACTA1* (>190 known, mostly missense mutations distributed across all of the 6 coding exons) and autosomal recessive variants of *NEB*. Up to now, 212 pathogenic variants of NEB have been described [5].

Clinically, NEM can be divided into severe congenital NEM, intermediate congenital NEM, typical congenital NEM, mild NEM with childhood onset and adult-onset NEM [13]. Because of the heterogeneity of NEM, diagnostic workup requires an interdisciplinary approach including neurological, histopathological, radiological and genetic assessment, and often takes a long time to diagnose the patients correctly.

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Here we present a patient with childhood onset congenital myopathy with two novel pathogenic variants in the *NEB* gene, which were uncovered by classical Sanger sequencing and "Next-Generation Sequencing" (NGS).

2. Case report

A 40-year old man presented with gait disturbances and a mild congenital myopathy since early childhood. His parents first noticed gait disturbances at the age of 12 months, and he never gained the ability to run. Symptoms were only slightly progressive, so that he was still able to walk independently at the age of 40 years. He did not suffer from any respiratory symptoms. There was no family history of myopathy or any other neuromuscular disorders. Both parents and his 44-year old brother did not report neuromuscular symptoms and were normal upon clinical examination.

Neurological examination revealed mild facial weakness with a facies myopathica and a mild palatum ovigale. There was no ophthalmoparesis or ptosis. Inspection of skeletal muscle and testing of muscle strength showed atrophy and weakness of the proximal and distal arm muscles as well as the distal leg muscles. In detail, muscle strength was symmetrically reduced in the shoulder muscles, deltoid, biceps and triceps muscles (MRC 4/5), in the hand- and finger extensors (MRC 3/5), slightly in the hip extensors and knee flexors (MRC 4 + /5 each) and in the foot extensors (MRC 3/5). He showed a moderate weakness of the neck flexors (MRC 3/5), mild contractures of the foot flexors and a moderate contracture of the right elbow. There was no rigid spine, axial atrophy or scapula alata. Deep tendon reflexes were absent. He showed a slight steppage gait.

Serum creatine kinase levels were slightly increased at the age of three years and normal at the age of nine years, nerve conduction studies showed no abnormalities. Electromyography revealed myopathic potentials with short duration and small amplitude at the age of three years.

A first skeletal muscle biopsy at the age of 3 years revealed a type 1 fibre predominance and few atrophic muscle fibres. The second skeletal muscle biopsy at the age of 9 years (right quadriceps muscle) revealed typical nemaline rods in the modified Gomori-trichrome-stain (Fig. 1A), muscle fibre atrophy of type 1 and type 2 fibres, and type 1 fibre predominance. Electron microscopy showed disruption of myofibrils and the presence of intramyofibrillar rods (Fig. 1B and C). Cores and intranuclear nemaline bodies were not reported.

As a first genetic approach at the age of 38 years, we performed a haplotype analysis of the known candidate genes for NEM. Genetic linkage analyses using samples from the affected index patient, his unaffected brother and parents excluded linkage to four known loci for NEM, i.e. *ACTA1*, *TPM2*, *TPM3* and *TNNT1* with DNA marker sets of the known NEM-associated genes [14]. Markers at the NEB locus (MIM*161650) were compatible with linkage to this locus as the unaffected brother carried a different maternal allele (Fig. 1D).

Subsequent Sanger sequence analysis, which was performed in 75 of the 183 exons of the *NEB* gene, revealed a splice site mutation in intron 73 (c.10872+1G>T, NCBI: NM_00127

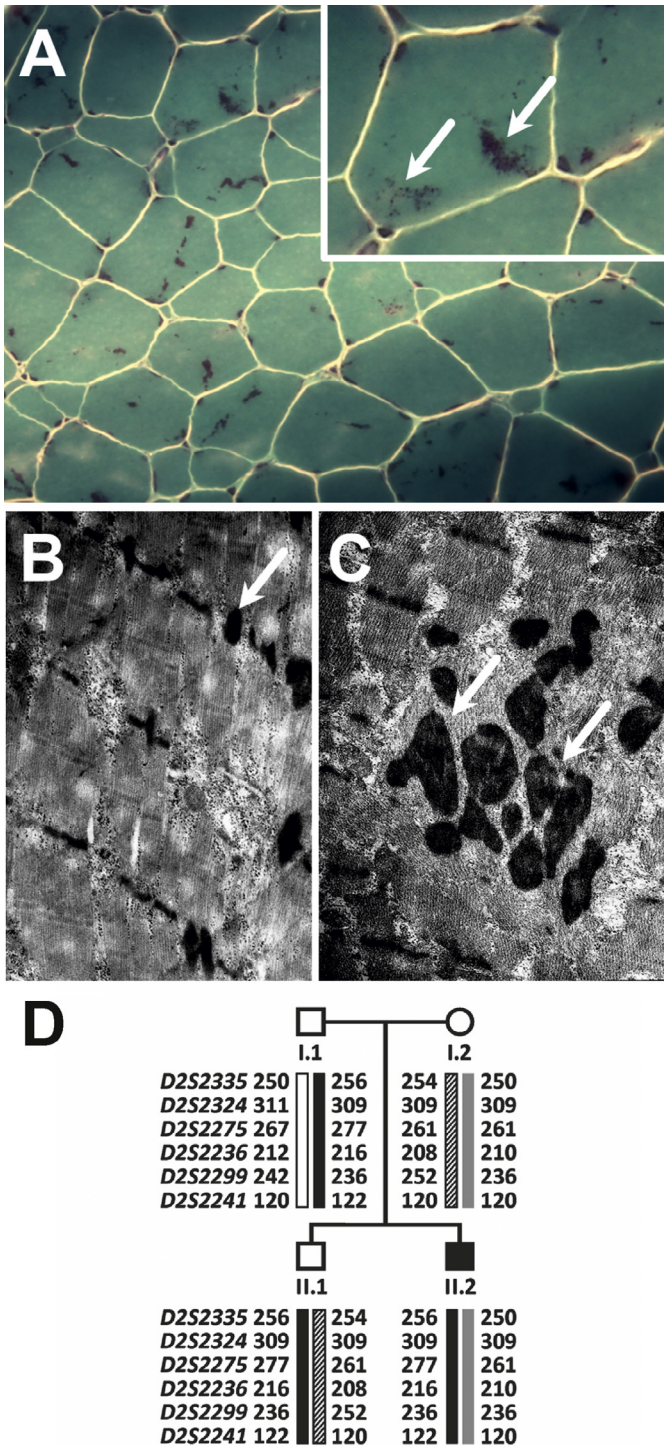

Fig. 1. Nemaline rods in skeletal muscle biopsy of the index patient at the age of 9 years taken from the right quadriceps muscle. A) Fresh-frozen sections stained with Gomori-trichrome-stain (magnitude: 10-fold and 20-fold (insert in the right upper corner)), showing multiple intracellular rods (white arrows). B) and C): electron microscopy. Ultrastructural analysis showed intermyofibrillar rods (white arrows) with disruption of myofibrils (magnitude: B): 1:4400, C): 1:7000). D) Linkage analysis of the index patient (II.2) and his family with DNA markers flanking the *nebulin* gene. Chromosome region 2q23.3 haplotyping of the family with NEM in the index patient, using six marker loci surrounding the *NEB* gene. The *NEB* gene is located in between the DNA markers *D2S2275* and *D2S2236*. The maternal marker *D2S2275* is not informative. The index patient has a different maternal allele than his unaffected brother. Linkage was therefore possible to the *NEB* gene locus.