

Nemaline Myopathy Type 2 (NEM2): Two Novel Mutations in the Nebulin (*NEB*) Gene

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Abstract

Nemaline myopathy is a type of the heterogeneous group of congenital myopathies. Generalized hypotonia, weakness and delayed motor development are the main clinical features of the typical congenital form. Histopathology shows characteristic nemaline rods in the muscle biopsy. Mutations in at least seven genes, including nebulin gene (*NEB*) proved to be responsible for this muscle disease. We present a boy with nemaline myopathy type 2 (NEM2) caused by compound heterozygosity for two novel mutations, a deletion and a duplication in the *NEB* gene. The deletion was inherited from the father and the duplication from the mother. Testing all family members supports genetic counseling.

Keywords

congenital myopathy, hypotonia, nebulin

Introduction

Congenital myopathies are a group of genetic muscle disorders with clinical, pathological and molecular heterogeneity.¹ Nemaline myopathy is a pathologically well-defined subgroup of congenital myopathies characterized by the presence of rod-like structures in the muscle fibers.² The disease presents a wide range of clinical variability and severity, although generalized hypotonia, muscle weakness, feeding difficulties and delay of motor milestones are almost always present.² All molecularly characterized forms of nemaline myopathy are autosomal, but inheritance can be recessive or dominant, and singleton cases may arise from de novo dominant mutations.^{2,3} To date, seven genes have been linked to this condition: α -tropomyosin (*TPM3*), nebulin (*NEB*), skeletal muscle α -actin (*ACTA1*), β -tropomyosin (*TPM2*), muscle troponin T1 (*TNNT1*), cofilin-2 (*CFL2*) and the recently identified *KBTBD13* gene, whose function is still unknown.²⁻⁴

Disease resulting from mutations in the *NEB* gene is classified as nemaline myopathy type 2 (NEM2; OMIM 256030), the most common form of nemaline myopathy.² NEM2 is a rare autosomal recessive condition characterized by early onset muscle weakness, which is most pronounced in the axial muscles and proximal limb-girdles. Weakness in facial and bulbar muscles commonly results in dysarthria.⁵ The course of the disease is nonprogressive or slowly progressive and life-expectancy depends on the severity of respiratory-muscle weakness. Mechanical ventilation is necessary for some patients and orthopedic complications are frequent.⁶ Mental development and intelligence are typically within the normal range.⁶ In this report we present the clinical and histological findings in a patient suffering from NEM2 and describe compound heterozygosity for two novel mutations in the *NEB* gene.

Case Report

Clinical Presentation

The patient, a boy was born at term from the fourth uneventful pregnancy. Birth weight was 3450 g and Apgar scores were 9 and 10 at 5 and 10 minutes, respectively. Floppiness and weak cry were noticed in the neonatal period. Generalized hypotonia, weakness and delayed acquisition of motor milestones were observed in early childhood. He began to walk with support at the age of 2 years. Deep tendon reflexes were reduced. There was no fasciculation in the tongue. He suffered also from recurrent respiratory tract infections. The serum creatine kinase activity was normal. The optic fundus and brain MRI were also normal. Tandem mass spectrometry and urine gas chromatography screening showed no evidence of inborn error of metabolism. Nerve conduction velocity was normal, and electromyography did not show any abnormalities. There was no homozygous deletion in exons 7 and 8 of the *SMN1* (Survival of Motor Neuron 1) gene. The possibility of a congenital myopathy emerged and muscle biopsy was performed at the age of 4 years. Severe respiratory tract infection and pneumonia led to respiratory failure later requiring tracheostomy and mechanical ventilation. His condition improved, however he needs ventilatory support during sleep. Cardiological examination did not show any abnormalities. The patient's cognitive abilities were appropriate for his age.

The patient's parents and his three siblings (a brother and two sisters) are asymptomatic. Informed consent was requested from the parents prior to the muscle biopsy and molecular genetic studies.

Muscle biopsy

The histological work up of the muscle biopsy tissue followed standard procedures. The majority of the muscle fibers were hypoplastic or atrophic with large variation in fiber size. Fiber necrosis,

regeneration, phagocytosis, or inflammatory cell infiltration were not noted. Gomori's trichrome technique detected prominent red-stained inclusion bodies in the fibers with variation in number and distribution. Gomori trichrome stained sections showed rod-shaped particles in the fibers (Figure 1a). Electronmicroscopy revealed that the rods appeared as electron-dense structures localized mainly along the thickened Z-lines (Figure 1b). These rod-shaped particles were identified as nemaline rods/bodies, and these findings were consistent with the diagnosis of nemaline myopathy.

Genetics

In view of the morphological findings a search was initiated in collaboration with the commercial diagnostic company, Centogene GmbH (Rostock, Germany) to find the molecular genetic etiology of nemaline myopathy in this child. Tests for *ACTA1* and *TPM3* were negative. Eventually testing the *NEB* gene revealed two previously unreported heterozygous mutations: a deletion (c.24527_24528delCT p.P8176fsX8179; Figure 2a) in exon 174 and a duplication (c.24250_24253dupGTCA p.T8085fsX8100; Figure 2b) in exon 171. These mutations result in a frameshift and a premature termination codon, respectively, presumably leading to truncated nebulin protein. Further testing of the parents revealed that the father carries the deletion and the mother has the heterozygous duplication (Figure 3). One of the two sisters of the patient carries wild type alleles, while another sister and the brother are heterozygous for the duplication (Figure 3). Thus, the clinically unaffected family members carry either wild type alleles or only one of the mutant alleles; either the duplication or the deletion. The patient however is a compound heterozygous carrier of both mutations.

Discussion

Nemaline myopathies are a clinically and molecularly heterogeneous group of congenital myopathies.¹⁻³ The combination of characteristic clinical and histopathologic features are diagnostic for the disorder in most cases.¹⁻³ The presence of red inclusions detected with Gomori trichrome staining and of rod-shaped particles in toluidine blue stained tissue from the patient strongly suggest nemaline myopathy.¹⁻³ Ultrastructural studies reveal nemaline bodies as electron-dense, rod-shaped structures appearing as thickened Z-disks.¹⁻³ Muscle imaging by MR can be helpful to visualize the pattern of selective muscle involvement and guide in localizing the site of the biopsy.^{7,8}

The work up of a case with nemaline myopathy is further complicated by its heterogeneous genetic background: seven known causative genes have been linked to this condition and both autosomal dominant and recessive inheritance has been observed.^{2,9} Six of these genes encode proteins associated with sarcomeric thin filaments.² Recessive mutations in the *NEB* gene, located on chromosome 2q22-23 are the most commonly recognized cause of the disease.^{2,4} This gene has 182 exons and missense, nonsense and frameshift mutations have been reported.¹⁰ Hotspots have not been found and many patients proved to be compound heterozygotes for two mutations within the gene.^{2,10} The nebulin protein has a wide range of functions, including thin filament length specification and regulation of muscle contraction.⁴

The clinical, histological and molecular genetic findings in our patient are consistent with the typical congenital form of nemaline myopathy type 2, caused by mutations in the *NEB* gene.² Compound heterozygosity for two novel mutations was found. A 2-base deletion (c.24527_24528delCT, p.P8176fsX8179) was inherited from the father and a 4-base duplication (c.24250_24253dupGTCA, p.T8085fsX8100) from the mother. These novel mutations led to a

translational frameshift and a premature termination codon in the respective translated sequences and thus, presumably, to truncated nebulin protein.

Nemaline myopathy is a debilitating condition and further research is warranted in order to explore the details of the molecular pathology of this disorder. These efforts are complicated by the heterogenic molecular background of the disease and the fact that certain genes encode very large proteins, like nebulin. Molecular diagnosis however becomes available for more and more patients supporting preimplantation or prenatal diagnosis for subsequent pregnancies.² This case report extends the genetic profile of nemaline myopathy with two previously unreported mutations in the *NEB* gene.

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Author Contributions

AG and EH contributed to data collection and the first draft of the manuscript.

TH performed the evaluation of the muscle biopsy.

GG and HS cared for the patient

KF and NN carried out the mutation analysis of the parents and family members.

MS and LS were mentors who contributed equally to this work.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

The writing/research of this article did not require ethical approval.

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Figure legends

Figure 1. Nemaline myopathy. (a) Gomori trichrome staining of plastic-embedded semithin sections shows numerous rod structures in the muscle fibers. (b) Electron microscopy reveals that the rods are electron-dense structures.

Figure 2. Identification of two novel mutations (one deletion and one duplication) in the *NEB* gene. Direct sequencing of DNA isolated from the affected patient (II/4) revealed (a) a two-base deletion (c.24527_24528delCT) in exon 174 of the *NEB* gene resulting in a premature termination codon (p.P8176fsX8179) in the translated sequence and (b) a four-base duplication (c.24250_24253dupGTCA) in exon 171 of the *NEB* gene, resulting in frameshift (p.T8085fsX8100) in the translated sequence.

Figure 3. Genetic screening of the affected family. The patient (II/4) and his clinically unaffected father (I/1) carry the deletion in heterozygous form, while the other unaffected family members (I/2, II/1, II/2, II/3) carry the wild type allele. The patient (II/4) and his clinically unaffected mother (I/2), brother (II/2) and sister (II/3) carry the duplication in heterozygous form, while the other unaffected family members (I/1, II/1) carry the wild type allele. These results suggest that the deletion is of paternal origin and the duplication is of maternal origin. The patient (II/4) is, thus, a compound heterozygote for these two novel mutations.

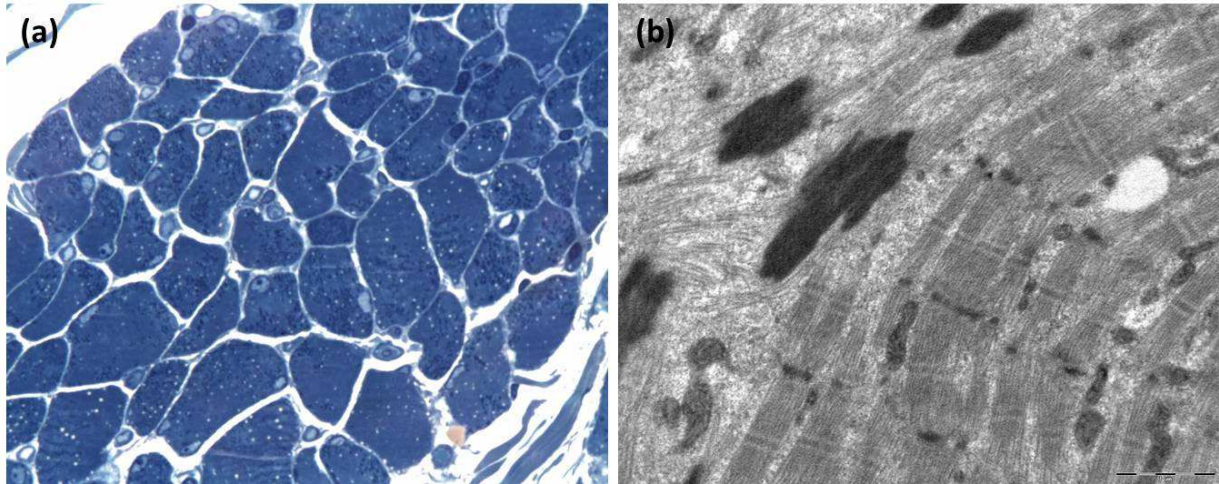


Figure 1.

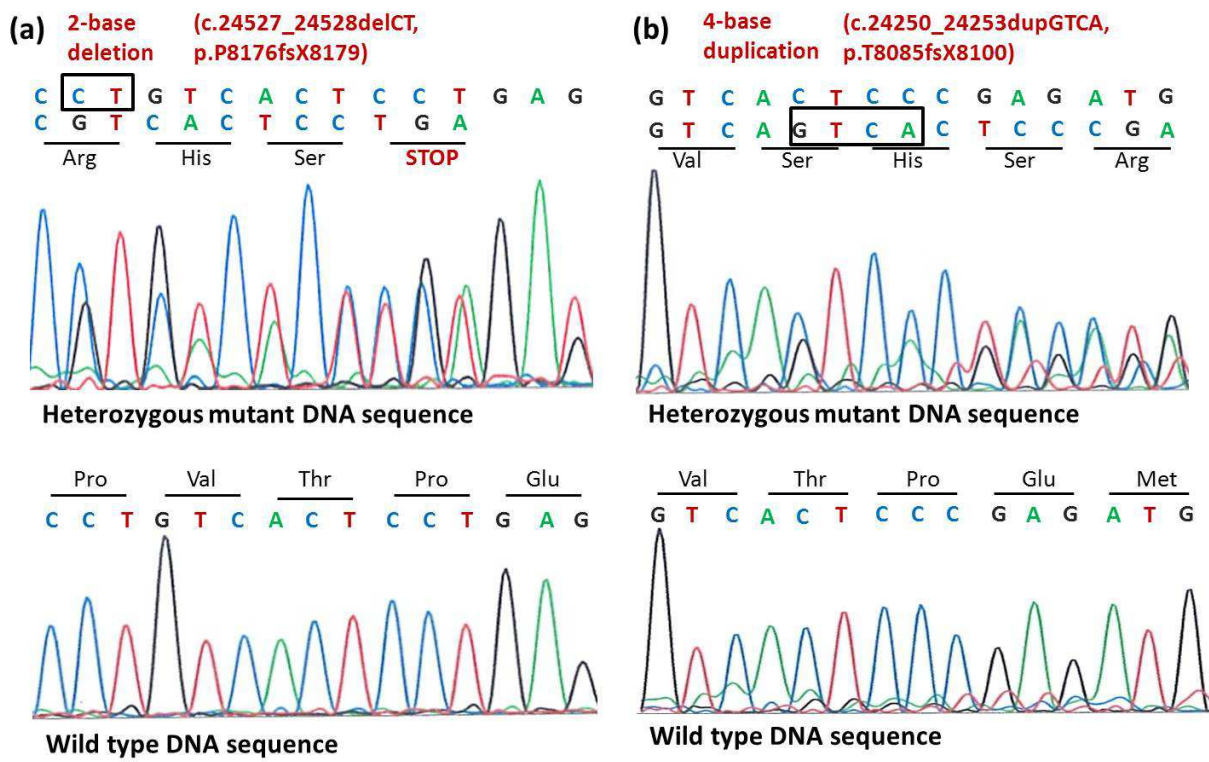


Figure 2.

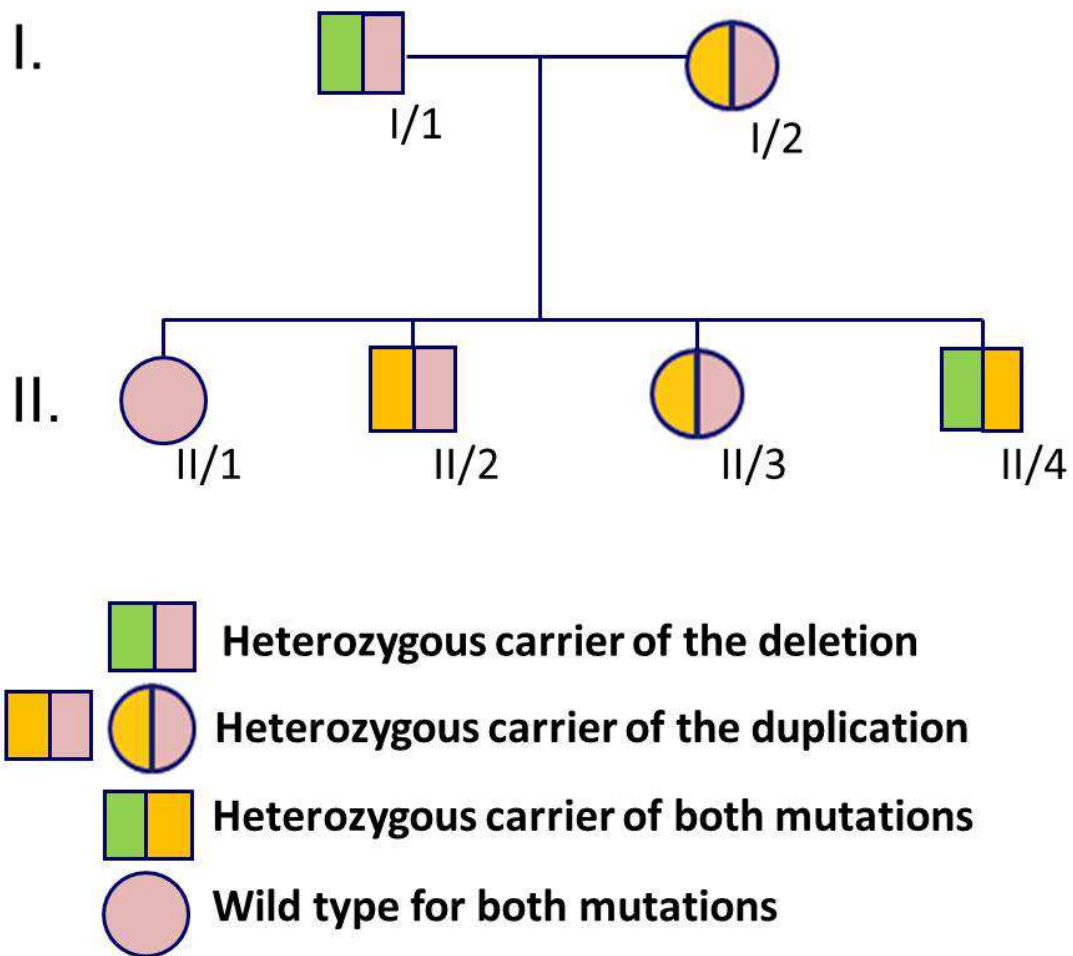


Figure 3.