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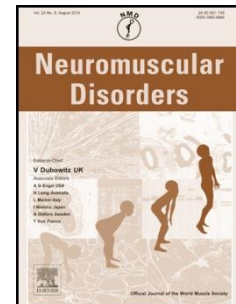
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**Clinically variable nemaline myopathy in a three-generation family caused by mutation of the skeletal muscle alpha-actin gene**

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## Highlights

- This paper describes a Finnish nemaline myopathy family with a dominant mutation in the skeletal muscle  $\alpha$ -actin gene *ACTA1*
- The family illustrates the clinical and histological variability of NM between patients sharing the same mutation.
- Only few *ACTA1* mutations segregating in more than two generations have been described.

## Abstract

We present here a Finnish nemaline myopathy family with a dominant mutation in the skeletal muscle  $\alpha$ -actin gene, p.(Glu85Lys), segregating in three generations. The index patient, a 5-year-old boy, had the typical form of nemaline myopathy, while his mother never had sought medical attention for her very mild muscle weakness, and his maternal grandmother had been misdiagnosed as having myotonic dystrophy. This illustrates the clinical variability in nemaline myopathy.

## Keywords

Nemaline myopathy; dominant inheritance; skeletal muscle alpha-actin; three-generation family; clinical variability

## 1. Introduction

To date, more than 200 different pathogenic variants have been identified in the  $\alpha$ -actin 1 gene (*ACTA1*). The vast majority, over 170 of these disease-causing variants, cause nemaline myopathy (NM), but variants in *ACTA1* may cause a wide spectrum of myopathies, clinically varying from lethal fetal akinesia to disorders with mild muscle weakness [1,2]. In addition to NM, *ACTA1* myopathies include actin myopathy, cap myopathy, congenital fibre type disproportion, core-rod

myopathy, intranuclear rod myopathy, zebra body myopathy, and progressive scapulo-peroneal myopathy. Recently, dominant mutations in *ACTA1* have been identified as the cause of a dominant distal myopathy with nemaline rods [3-11]. Correspondingly, the muscle histopathology of patients with *ACTA1* mutations is diverse. The spectrum encompasses nemaline bodies, actin filament aggregates, core structures, caps, fibre-type disproportion, zebra bodies, and dystrophic features. In some patients with muscle weakness, only minimal histological changes have been detected. Diagnostics based on muscle histopathology can thus be very difficult; the clinical and histological features overlap between entities, and one single muscle biopsy may show a combination of different histopathologies [9,12].

The most common myopathy related to *ACTA1* is NM. We have estimated that variants in *ACTA1* cause 23% of NM cases. *ACTA1* variants are the most common cause of sporadic NM, and a common cause of very severe (lethal) forms with fetal akinesia also. Most of the pathogenic variants in *ACTA1* are dominant (90%) and a majority of the dominant variants have arisen *de novo*. Of the sporadic cases with *ACTA1* variants, approximately 85% have been shown to be caused by *de novo* variants. Autosomal recessive variants are rarer (10%). Dominant variants inherited across two or more generations have been identified in less than 5% of *ACTA1* families, while mosaicism has been observed in a couple of families [12,13].

## **2. Clinical features in the three affected family members**

### *2.1. Clinical findings and molecular diagnostic investigations*

In the Finnish family described here, a 5-year-old boy underwent investigations because of generalised muscle weakness. His motor milestones were delayed but reached. The clinical examination showed lumbar hyperlordosis, myopathic facies and high-arched palate (Fig. 1A-D). A

diagnosis of NM was made on the basis of muscle biopsy findings including the presence of nemaline bodies (Fig. 2A). His NM was classified as typical.

The genetic cause was subsequently sought for by sequencing of the nebulin gene for the known Finnish variants, and of the entire *ACTA1*, resulting in the identification of a missense variant, p.(Glu85Lys), in *ACTA1*. This variant had previously been identified in two unrelated NM patients as *de novo* mutations, and thus the variant was known to be pathogenic [14]. DNA samples of the family were subsequently sequenced to check the segregation of the variant.

The same variant was identified in the mother of the index patient, and she was then clinically examined. She was found to have mild weakness of the neck flexors, grip strength and quadriceps muscles, consistent with the mild form of NM (Fig. 1E). A detailed history revealed that she had always had slight motor difficulties although she had reached her motor milestones without any observed delay. She had, however, never sought medical attention for her motor problems. Her biopsy, performed at the age of 36 years, showed numerous nemaline bodies (Fig. 2B).

After identification of the dominantly inherited mutation in the family, a DNA sample of the boy's deceased maternal grandmother was also sequenced for the variant. She had presented at the age of 44 years with muscle weakness. She had always worn high heels and had contractures of the Achilles tendons. EMG showed myopathic features while CK levels were normal. At the time, she was misdiagnosed as having myotonic dystrophy. The grandmother also had arthrosis, gall stones, hypothyreosis, high blood pressure and kidney stones. She died of cancer of the urinary tract at the age of 68 years. On re-examination of the muscle biopsy, it was found to show nemaline bodies (Fig. 2C). Her diagnosis was confirmed by the identification of the same *ACTA1* variant in her. She can in retrospect be thought to have had the mild form of NM. Her father was said to have had weak legs, and he had to use his arms for getting up from a chair.

## 2.2. Histology

Muscle biopsy of the vastus lateralis had been done in the three affected family members. In all three biopsies, Gömöri trichrome staining revealed abundant cytoplasmic nemaline bodies (Fig. 2 A-C). Fibre size variability (Fig. 2D-F) was less apparent in the proband's biopsy (Fig. 2D) than in mother's (E) and grandmother's (F) biopsies. Only the grandmother's biopsy showed a clearly increased number of internal nuclei as well as split fibres (Fig. 2F). In addition, on NADH staining (Fig. 2G-I) a few core-like structures were seen in the biopsy of the grandmother (Fig. 2I), while core structures were not seen in the biopsies of the proband or mother (Fig. 2G, H). Myosin double staining of myosin heavy chain fast and slow (Fig. 2J, K) showed type 1 fibre uniformity and abnormal fibre size variability in the proband (Fig. 2J). The mother's biopsy showed mild fibre type 1 predominance, type 1 fibre atrophy and type 2 fibre hypertrophy. In addition, several immature hybrid fibres were present (Fig. 2K). The grandmother's biopsy is no longer available for double stainings. However, the pathologist's report mentions fibre type 1 predominance and atrophy. Electron microscopy was performed on muscle samples from the proband and mother. Both had typical intracytoplasmic nemaline rods. No intranuclear rods were found.

## 3. Variant prediction using the in silico tool

The *ACTA1* variant p.(Glu85Lys) was analysed using the HOPE tool (<http://www.cmbi.ru.nl/hope>), which predicts the 3D model of the protein of interest using WHAT IF web services. HOPE predicts how the variant affects the protein structure and function. Amino acid residue 85 is a glutamic acid in wild-type (wt) actin, located on the surface of the protein, this residue being 100 % conserved. The glutamic acid at position 85 forms a hydrogen bond with threonine at position 128, and a salt bridge with lysine at position 120. The function of the residue at position 85 is not known, neither is it known whether Glu85 binds to other molecules. The variant discussed here changes a

glutamine to a lysine, p.(Glu85Lys). Glutamic acid is smaller than lysine, and it is positively charged, while lysine is negatively charged. Lysine instead of glutamine at position 85 is unable to form the hydrogen bond and the salt bridge present in wt actin. The variant therefore changes the structure of the protein and likely disrupts the correct function of the protein.

#### 4. Discussion

To our knowledge, the family described here is the first NM family with a pathogenic dominant variant of *ACTA1* present in three generations. In this Finnish NM family, the variant p.(Glu85Lys) was found to be present in a 5-year-old boy, his mother and his maternal grandmother, who had probably inherited it from her father. All these family members were found to have NM, verified by muscle biopsy.

The same variant has been described in the literature as a *de novo* mutation in two unrelated patients of American origin [14]. Thus, the variant was known to be pathogenic, and therefore, further mutational analyses were not performed in the current family.

Both of the patients described in the previous publication [14] were boys with the typical form of NM. Hypotonia was noted at the age of three months in patient 349-1. At the age of three years he was ambulant, and had no respiratory or feeding issues at the time. Electron microscopy was not performed. The second patient, 349-2, showed hypotonia at birth. His motor milestones were delayed; he achieved sitting at the age of 9 months and walking at 16 months. He, too, was ambulant at the age of three years and did not have feeding or respiratory problems at the time. His muscle biopsy showed marked type 1 fibre predominance, moderate fibre-size variability, scattered and group atrophy, and numerous nemaline bodies [14].

The largest single actinopathy pedigree published to date depicts an extended family of six generations with a dominant, progressive scapulooperoneal neuromuscular disorder, shown to be

caused by the *ACTA1* variant p.(Glu197Asp). No nemaline rods or actin aggregates were observed in the muscle biopsies examined [10].

The family described here is one more example of how the clinical and histological features of NM may be variable, even between family members sharing the same mutation. This variability is likely due to both environmental and genetic modifying factors.

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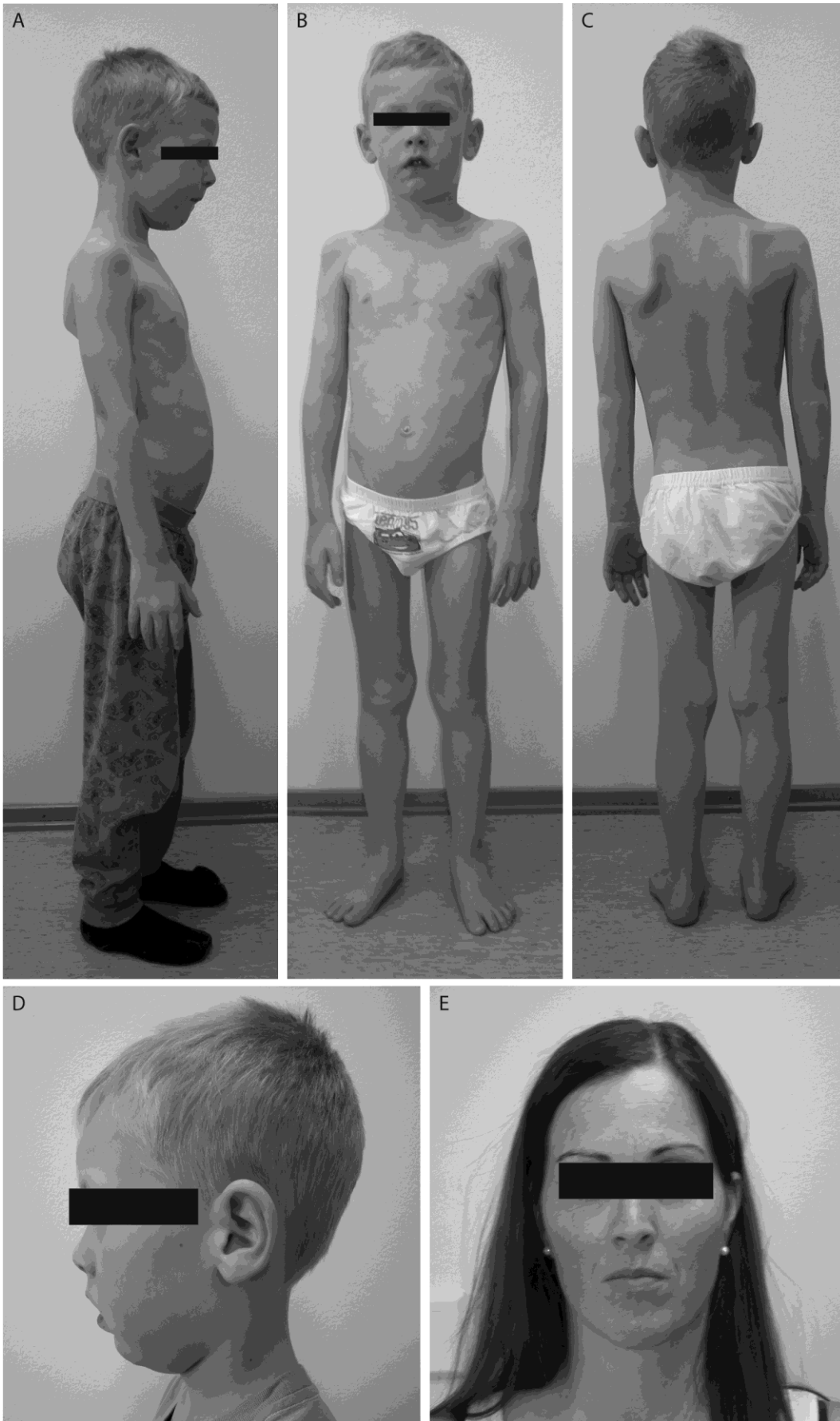
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**Figure Legends:**

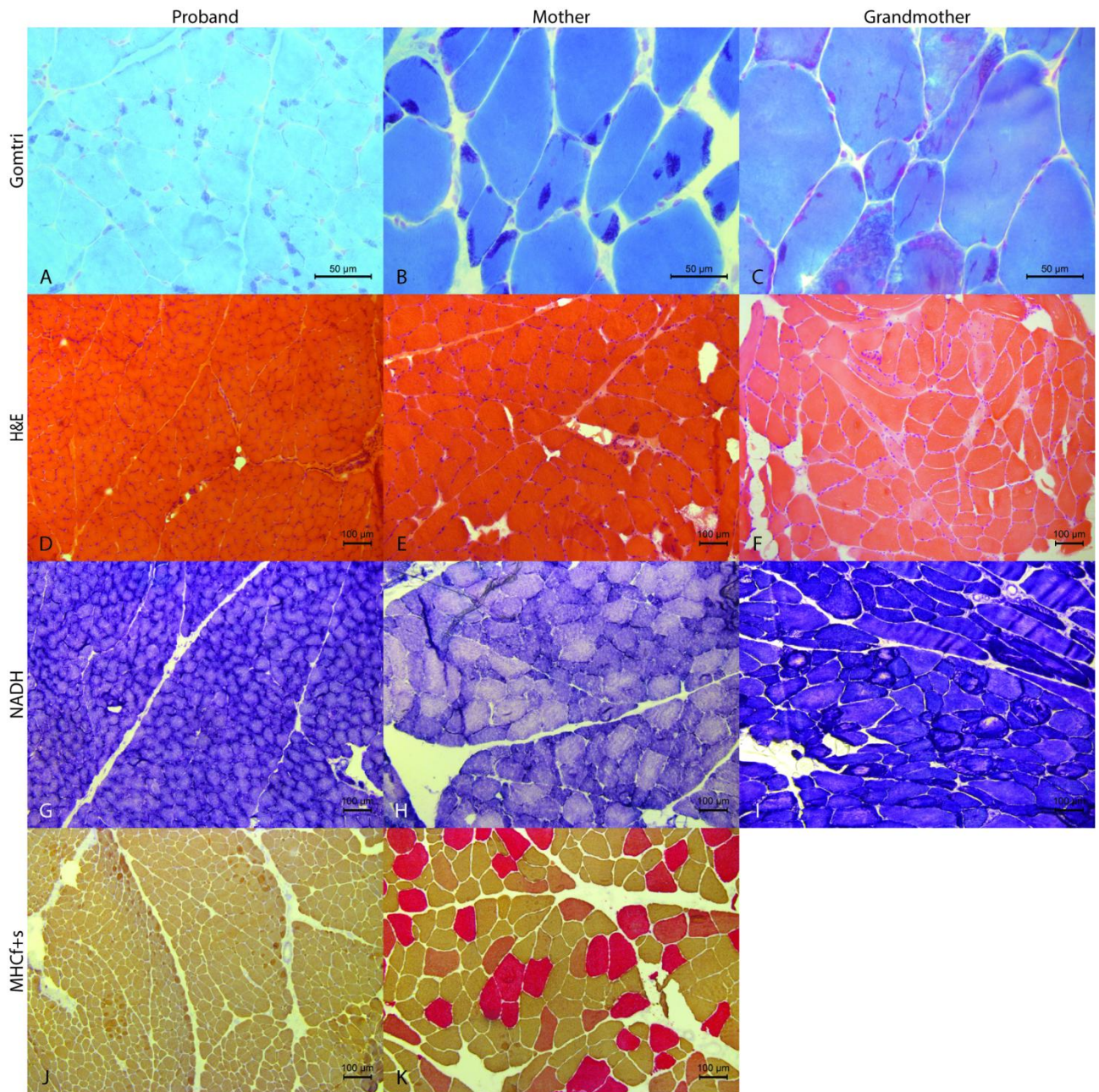
Fig. 1. The proband (A-D): a 5-year-old boy, with myopathic facies, slender build and hyperlordosis. His mother (E) has slight hypomimia and mild weakness of the neck flexors, grip strength and quadriceps muscles.

Fig. 2. Histological findings in muscle biopsies from the proband, the mother and the grandmother. Gömöri Trichrome staining (A-C): in all three biopsies, aggregations of nemaline bodies are seen as dark-staining areas. H&E staining (D-F): fibre size variability is most apparent in the mother's (E) and grandmother's (F) biopsies. NADH staining (G-I) is unremarkable in the proband's (G) and the mother's (H) biopsy, but reveals a few core-like centrally unstained fibres in the grandmother's biopsy (I). MHC double staining (J, K): fast (red) and slow (brown) demonstrates fibre type 1 uniformity in the proband's biopsy (J). The mother's biopsy (K) shows slight predominance and atrophy of type 1 fibres. Some hypertrophic type 2 fibers are seen, as well as red-brown immature hybrid fibers.



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