# A recurrent ACTA1 amino acid change: mosaic form causes milder asymmetric myopathy

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## 1 INTRODUCTION

We describe three cases in which the same amino acid change p.(Gly247Arg) in *ACTA1* in mosaic form gives rise to asymmetric weakness and a relatively mild course of nemaline myopathy (NM). We also revisit a published case of severe NM caused by a heterozygous variant in the same position [1].

ACTA1 variants are the most common cause of severe NM [2]. Their mosaic state may explain the more favorable outcome in Patients 1, 2, and 3.

## 2 METHODS

Sequencing of lymphocyte-derived DNA of patients 1 & 2 was carried out using a NextSeq 500 (Illumina Nextera, San Diego, CA, USA) with variant annotation in Alissa (Agilent Technologies, Santa Clara, CA, USA). The analysis of Patient 3 was performed at Invitae Corp as previously described [3]. All variants were verified by Sanger sequencing.

The grade of mosaicism was verified using the QX200 droplet digital PCR System (Bio-Rad Laboratories, Hercules, CA, USA) and FAM/HEX labeled probes targeting the ACTA1 variants. Analysis was performed using the QuantaSoft Analysis Pro software (v.1.0, Bio-Rad)

## 3 RESULTS

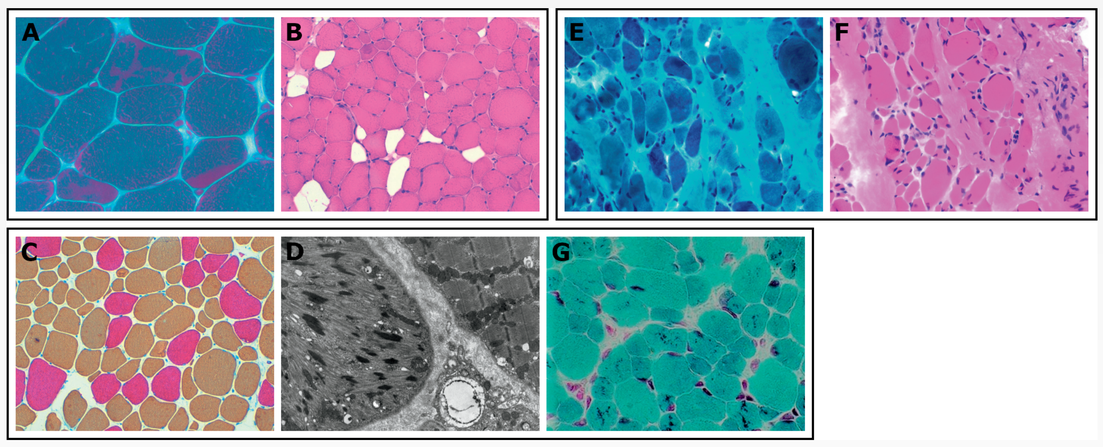


Figure 1. In Patient 1, red-staining clusters of nemaline rods were suspected in the modified GTC staining (A). H&E and immunohistochemical MyHC slow and MyHC fast double staining showed variability of fibre size for both main fibre types and normal fibre type distribution (B-C). EM showed small rods and a few nemaline bodies of the usual size (D). In Patient 2, GTC staining showed scattered small fibres containing possible rods (E) and wide variation in fibre size with a proportion of hypotrophic fibres in the H&E staining (F). In Patient 4, GTC staining of a biopsy from 1999 confirmed the presence of nemaline rods, thus establishing the diagnosis of NM (G).

Table 1. The four patients share the same amino acid change, p.(Gly247Arg). Patient 4 is a previously described heterozygote with severe NM caused by a de novo mutation. Patients 1, 2, and 3 are mosaics for the variants. The variant allele frequencies as per sequencing results (MPS VAF) and droplet digital PCR (ddPCR VAF) are shown in the table.

| Patient | Variant | MPS VAF | ddPCR VAF |
|---|---|---|---|
| Patient 1 | c.739G>A | 19% (134/720) | 15% |
| Patient 2 | c.739G>C | 35% (62/175) | 32% |
| Patient 3 | c.739G>A | 28% (95/339) | 24% |
| Patient 4 | c.739G>C | N/A | N/A |

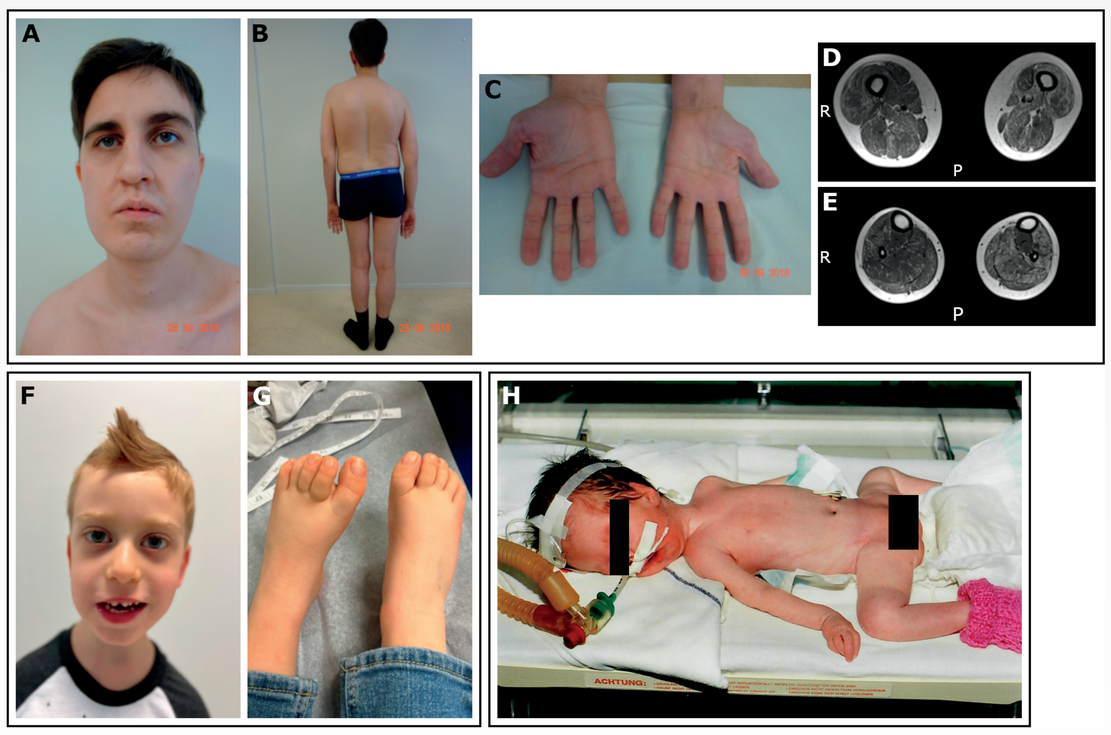


Figure 2. The asymmetric facial expression of Patient 1 (A). His left lower limb is shorter and thinner than his right (B). The hands showed a 1.5 cm difference in breadth (C). In MRI, smaller muscle bulk was seen on the left side both at thigh (D) and lower leg (E) level. Patient 2 had elongated, myopathic facies and slight asymmetry of the facial expressions (F). The right leg was 3 cm shorter than the left one (G). Patient 4 had a very severe clinical picture at birth with extreme hypotonia, areflexia, no spontaneous movements, no independent respiratory effort, persistent bradycardia and arthrogryposis affecting all large joints (H).

## 4 DISCUSSION

In the case of Patient 1, the variant was initially missed due to the low level of mosaicism. In contrast, Patients 2 and 3 had been reported as heterozygous. Retrospective genotype-phenotype correlation studies revealed all three patients to be mosaics for their respective variants.

Standard MPS library preparation protocols include enrichment steps which may introduce sequence/specific biases and hamper detection of mosaicism. Pipelines for constitutional mono- and oligogenic disease are optimised for detection of heterozygous and homozygous variants: VAF thresholds for heterozygosity often range between 25-35%. Provided that enrichment steps do not skew data, mosaic VAFs may be extracted from data and similar frequencies can be reproduced by ddPCR.

## 5 CONCLUSIONS

We suggest read depth should be at least 80x for higher likelihood of detecting lower-frequency variants. Knowledge of mosaicism for pathogenic variants is essential for the patient and genetic counselling, and possibly for the course of the disease.

This study underlines the importance of considering VAF tresholds and genotype-phenotype correlation in patients with asymmetry or milder phenotype, in whom no causative variant has been found, or in which the phenotype does not correlate with earlier descriptions of the same variant.

In the case of the ACTA1 c.739G position, VAF analysis by ddPCR seems to correspond well to the MPS-derived VAF; thus ddPCR may be used to verify the grade of mosaicism in case the variant is detected by Sanger sequencing or sequencing methods with inadequate read depth.

## FUNDING

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