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

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1. \[2\] Nowak et al. 2007, PMID: 17187373
2. \[3\] Dellefave-Castillo et al. 2022, PMID: PMC9366660

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 same position [1].

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 QUANTOS Software (v.1.0, Bio-Rad)

3 RESULTS

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 table shows variant allele frequencies as per sequencing results (MPS VAF) and droplet digital PCR (ddPCR VAF) are shown in the table.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Variant</th>
<th>MPS VAF</th>
<th>ddPCR VAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>c.739G&gt;A</td>
<td>19% (134/720)</td>
<td>15%</td>
</tr>
<tr>
<td>Patient 2</td>
<td>c.739G&gt;C</td>
<td>35% (62/175)</td>
<td>32%</td>
</tr>
<tr>
<td>Patient 3</td>
<td>c.739G&gt;A</td>
<td>28% (55/195)</td>
<td>24%</td>
</tr>
<tr>
<td>Patient 4</td>
<td>c.739G&gt;C</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

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 thresholds 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 disease.

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REFERENCES

1 Laing et al. 2009, PMID: PMC2784950
2 Nowak et al. 2007, PMID: 17187373
3 Dellefave-Castillo et al. 2022, PMID: PMC9366660