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NOVEL INTRONIC MUTATION IN *MTM1* DETECTED BY RNA ANALYSIS IN A CASE OF X-LINKED MYOTUBULAR MYOPATHY

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X-linked myotubular myopathy (XLMTM) is a rare neuromuscular condition that presents with neonatal hypotonia and weakness and is associated with severe morbidities (including wheelchair, feeding tube, and ventilator dependence) and early death.[1] It is defined by muscle biopsy features, including central nuclei, abnormal oxidative stain distribution, and type I fiber hypotrophy.[2] Mutations in myotubularin (*MTM1*) account for all genetically solved cases of XLMTM, but have not been discovered in all individuals with characteristic clinical and biopsy features.[3] Of note, there are some forms of autosomal centronuclear myopathy that can resemble XLMTM, such as those associated with mutations in *BIN1*, *DNM2*, *RYR1*, and *SPEG*, although rarely are such cases a complete phenocopy of XLMTM.[4] In this study, we present a case that illustrates the importance of considering noncoding mutations as a cause of XLMTM and illustrate the utility of RNA analysis in individuals with a phenotype suggestive of a particular genetic diagnosis.

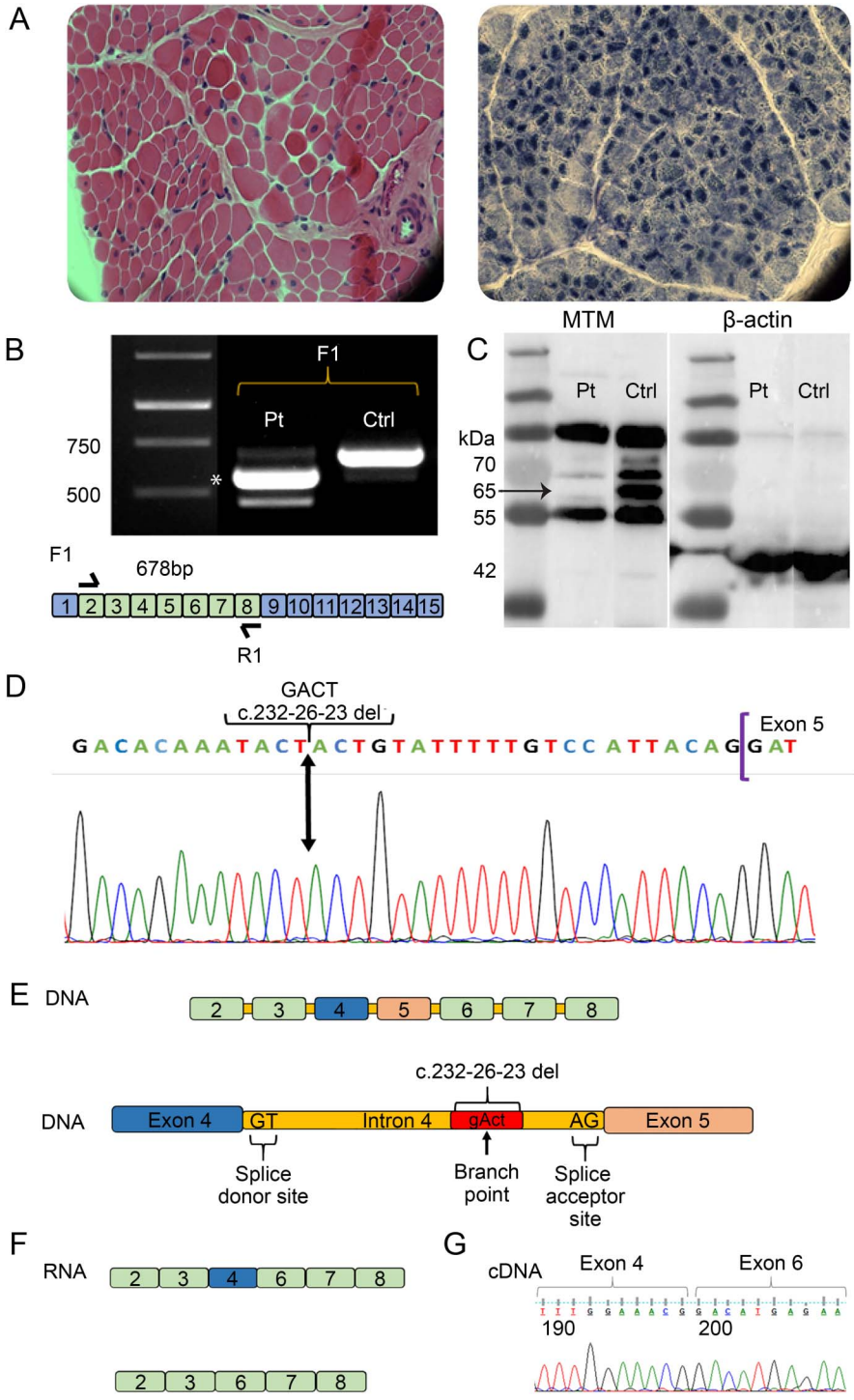
The proband is a 27-year-old man with severe generalized weakness and prominent disease morbidities, including wheelchair, feeding tube, and ventilator dependence. The family history was unremarkable for neuromuscular disorders. He was born at term from a healthy mother after a pregnancy complicated by diminished fetal movements. He was noted at birth to be weak and hypotonic, although he did not require invasive respiratory support as a neonate. He had multiple episodes of respiratory failure that resulted in repeated hospitalizations, and at age 6.5 years underwent tracheostomy and thereafter required continuous ventilatory support. He achieved the ability to walk independently at age 30 months and lost the ability at 48 months after prolonged hospitalization for a respiratory infection. Since that time, he has been supported by wheelchair. He is able to eat independently, although he has difficulties with chewing and swallowing. Physical examination is notable for myopathic facies, ptosis, ophthalmoparesis, head circumference greater than the 90th percentile, diffuse extremity weakness, and long fingers, a combination of clinical features highly suggestive of a mutation in *MTM1*.[1]

Diagnostic muscle biopsy performed at age 11 months showed the classic features of an XLMTM (figure, A); thus, he was presumptively diagnosed with the condition. However, genetic testing of *MTM1*, accomplished on a next-generation sequencing–based panel of 17 genes implicated in congenital myopathy (including all exons plus 10 base pairs of surrounding intronic sequence as performed at the University of Chicago), did not reveal a mutation, and multiplex ligation-dependent probe amplification (MPLA) testing for deletion/duplication of these genes was also normal.

Given the high suspicion of XLMTM, we investigated MTM1 transcript and protein from the proband's skin fibroblasts. Reverse transcription PCR (RT-PCR) analysis using sets of overlapping exonic primers identified abnormalities in the MTM1 transcript including primarily deletion of exon 5 (figure, B and C). Western blot analysis showed reduction of full-length MTM1 protein and the presence of a smaller molecular weight fragment (figure, D). Genomic DNA Sanger sequencing of intron 4 revealed a previously unreported 4 base pair deletion (c.232-26_232-23delGACT), 23 base pairs from the splice acceptor junction (figure, E). In silico analysis predicted that this mutation interrupts the splice branch point, the consequence of which is skipping of exon 5 (figure, F and G). In all, these data confirm this as a case of XLMTM due to a novel intronic mutation that was detected by RNA analysis in vitro.

This case is instructive for several reasons. First, it adds to the growing list of noncoding mutations identified as causes of rare mendelian disorders. Given the estimate that approximately 50% of all neurogenetic diseases are not solved by exome sequencing or gene panels, cases such as ours support a hypothesis that many of these unsolved cases are the result of intronic or regulatory sequence mutations.[5] Second, the case illustrates the utility of RNA analysis as a means of identifying such mutations. In settings like this one of a high index of suspicion for a specific genetic cause, RT-PCR is an easy modality for interrogating transcripts. However, when mutations in many genes may be responsible for a given phenotype, large-scale

Figure **Novel intronic mutation in MTM1 as a cause of X-linked myotubular myopathy**



(A) Hematoxylin & eosin and succinate dehydrogenase (SDH) staining of the patient's muscle biopsy showing the classic features of centronuclear myopathy, including myofiber hypotrophy, central nuclei, and aggregation of oxidative material; (B) RT-PCR of amplicon 1 (F1, spanning exons 2–8) on RNA from patient fibroblasts revealing an abnormal transcript of 567 bp (exon 5 skipping) and 472 bp (exons 4 and 5 skipping) compared with control 678 bp; (C) Western blot of myotubularin (MTM1) protein from patient (Pt) and control (Ctrl) fibroblasts (65 kDa normal band) revealing that MTM1 is almost not detectable in the patient (actin for loading control); (D) DNA chromatogram of the hemizygous c. 232-26_232-23 deletion in intron 4 of *MTM1* gene; (E) Schematic diagram of the *MTM1* mutation and its position within a branch point site. (F) Schematic depicting the consequences of the branch point mutation on RNA processing of MTM1 (i.e., skipping of exon 5 or of exons 4 and 5) as determined by RT-PCR (B). (G) Validation of exon 5 skipping as revealed in the chromatogram from Sanger sequencing of the middle band (*) identified by RT-PCR. Sequence analysis confirms the loss of exon 5 sequence. cDNA = complementary DNA; RT-PCR = reverse transcription PCR.

transcriptome analysis using RNA sequencing is an effective methodology.[5,6] Last, the case identifies an unexpected shortcoming of gene panels and exome sequencing; unlike Sanger sequencing, to enable cost-effective screening of many exons, these technologies use short primers that are typically within 10 base pairs of the exon, and thus miss out on noncoding mutations outside the immediate splice region. This is particularly relevant for splice lariat mutations (as in our situation), as the branch point is typically 20–40 base pairs from the intron boundary. Of note, the milder phenotype of our patient may be explained by our observation of a faint amount of full-length MTM1 transcript and protein, and/or by the fact that his *MTM1* mutation produces exon 5 skipping, the consequence of which is an in-frame deletion and predicted production of a truncated protein.

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