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Large duplication in *MTM1* associated with myotubular myopathy

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Abstract

Myotubular myopathy is a subtype of centronuclear myopathy with X-linked inheritance and distinctive clinical and pathologic features. Most boys with myotubular myopathy have *MTM1* mutations. In remaining individuals, it is not clear if disease is due to an undetected alteration in *MTM1* or mutation of another gene. We describe a boy with myotubular myopathy but without mutation in *MTM1* by conventional sequencing. Array-CGH analysis of *MTM1* uncovered a large *MTM1* duplication. This finding suggests that at least some unresolved cases of myotubular myopathy are due to duplications in *MTM1*, and that array-CGH should be considered when *MTM1* sequencing is unrevealing.

Keywords

Myotubular myopathy; Congenital myopathy; *MTM1*; Gene duplication; Diagnostic testing

1. Introduction

Centronuclear myopathies (CNMs) are a group of congenital myopathies characterized by hypotonia, proximal weakness, ophthalmoparesis, ptosis, and respiratory insufficiency [1,2]. Onset is variable from birth to adulthood. Several inheritance patterns have been described including autosomal dominant, autosomal recessive and X-linked. Diagnosis is typically based on pathological features and molecular genetic testing. The most prominent pathologic feature is centrally placed nuclei [3,4]. Other, more variable, pathologic features typically include type I fiber predominance, myofiber hypotrophy, increased connective

tissue and fibro-adipose replacement. Currently, mutations in four genes are associated with CNM: dynamin-2 (*DNM2*) [5], ryanodine receptor (*RYR1*) [6], amphiphysin 2 (*BINI*) [7] and myotubularin (*MTM1*) [8]. Mutations in *MTM1* are specifically associated with myotubular myopathy (XLMTM), a subtype of centronuclear myopathy with X-linked inheritance and some distinctive clinical features.

Typically, the most severe cases of CNM have been associated with *MTM1* mutations [1] or occasional rare mutations of *RYR1* or *BINI* [6,7]. Over 200 different disease-causing mutations have been identified in *MTM1* including missense, nonsense, intronic, small deletions/ duplications and large rearrangements [8–22]. While *MTM1* mutations have been identified in the vast majority of patients with a consistent clinical and histopathologic history, 10% of males with a suspected diagnosis of myotubular myopathy remain genetically unresolved [23], suggesting either additional genetic causes for myotubular myopathy or that *MTM1* mutations in some individuals are not recognized by conventional diagnostic techniques. A recent case report by Trump and colleagues [9], described a patient with a severe presentation of CNM. Routine sequencing did not identify a pathogenic mutation in *MTM1*. MLPA analysis was performed and identified a duplication of exon 10. This report emphasizes the importance of screening for complex rearrangements in *MTM1*. In the current study, we report a patient with a severe presentation of CNM due to a large duplication in *MTM1*, suggesting that analysis for *MTM1* deletions and duplications should be carried out in cases of suspected myotubular myopathy where conventional sequencing does not detect a mutation.

2. Case report

This male patient was born at 33 4/7 weeks by C-section for a breech position. Birth weight was 1890 grams (10th – 25th percentile) and length was 49.5 cm (97th percentile). During the pregnancy, the mother reported reduced fetal movements, though all prenatal ultrasounds were normal. At birth, the patient was hypotonic and was in respiratory distress. CPAP was used initially, but at 4 days of age the patient was intubated.

In terms of motor function, at one day of age the patient was able to move his hands; at one week, he was able to move all limbs. Weakness of the neck and extra-ocular muscles was noted on exam. Difficulties swallowing and excessive secretions led to the placement of a naso-gastric tube. The patient passed away at 8 weeks of age due to acute respiratory failure.

No family history of neuromuscular diseases was reported. The initial diagnostic workup consisted of chromosomal analysis and molecular testing for myotonic dystrophy type I, both of which were negative. These tests were followed by a muscle biopsy and additional molecular testing.

2.1. Pathologic findings

The patient underwent muscle biopsy of the right thigh at 6 weeks of age. Hematoxylin and eosin (H&E)-stained sections of muscle biopsy tissue revealed uniformly small myofibers and a numerous fibers containing large, centrally located nuclei (Fig. 1). Some fibers contained central areas with either excessive basophilic staining or clearing of the cytoplasm, which corresponded to aggregates of organelles on histochemical stains for reduced nicotinamide adenine dinucleotide (NADH) and cytochrome oxidase (COX). No spoked-wheel structures were seen on NADH stain. Central nuclei were found in both fiber types, and there was no evidence of grouping or other fiber type-specific changes on ATPases at pH 4.3 and 9.8. Dystrophin antibodies showed no abnormalities. Overall, the presence of small myofibers, markedly increased numbers of fibers with large, central

nuclei, and central accumulations of organelles was highly consistent with a pathological diagnosis of XLMTM or severe CNM.

2.2. Molecular analyses

Molecular genetic testing for several causes of CNM was performed. This included conventional sequencing from genomic DNA (i.e. Sanger sequencing of all coding exons as well as all intron–exon boundaries) of *MTM1*, *DNM2* and *RYR1*. No pathogenic mutations were detected in any of the 3 genes. Research based whole exome sequencing also failed to detect any mutations in these three genes as well as in *BINI*. Since the patient's symptoms and biopsy findings were strongly suggestive of XLMTM, we pursued additional testing of *MTM1*.

Deletion and duplication testing of the *MTM1* gene was performed on the patient and his mother using a custom high resolution, targeted 8 × 60 K array-CGH platform containing the *MTM1* gene and an additional 44 genes (Agilent Technologies). A total of 965 probes spanning the *MTM1* gene and flanking regions were present on this custom array. All experimental procedures were conducted using the manufacture recommended protocol. Microarray data was analyzed using the Nexus software (BioDiscovery, El Segundo, CA) for copy number analysis. This analysis identified a previously unreported duplication of *MTM1* in the patient and his mother. The duplication appeared to be complex with a large duplicated region involving *MTM1* exons 1 (chrX: g.(?_149,736,384)_(149,743,600_149,744,456)dup [hg19]), 3–13 (chrX:g.(149,764,500_149,764,537)_(149,828,980_149,830,745)dup [hg19]) interrupted by a non-duplicated region involving exon 2 (Fig. 2). The last two coding exons of the *MTM1* gene (exons 14 and 15) were also not involved in this rearrangement. This duplication is predicted to affect the production of a normal *MTM1* transcript.

To better define the proximal end of the duplication upstream of *MTM1* exon 1, SNP array analysis of the mother's sample was performed using the CytoScan HD Array (Affymetrix, Santa Clara, CA). The CytoScan HD Array is a whole-genome array containing over 2.6 million markers, including 750,000 SNPs. SNP array analysis was performed according to the manufacturer's recommended protocol. Results were processed and visualized using Chromosome Analysis Suite (ChAS) 1.2.3 software (Affymetrix, Santa Clara, CA). An interstitial duplication of the long arm of chromosome X in the cytogenetic band position Xq28 was observed (arrXq28(149,474,705–149,830,745)x3 [hg19]). The duplication spanned approximately 356 kb and was interrupted by a non-duplicated region, confirming the finding on the *MTM1* targeted array-CGH. The duplication involved the *MTM1* gene, excluding its terminal end, and the entire proximal *MAMLD1* gene (MIM 300120) (Fig. 3). Of note, the duplication was not identified in the whole exome sequencing data.

To further investigate the potential effect of the duplication on *MTM1*, we examined RNA isolated from skin fibroblasts from the proband's mother. RT-PCR analysis was performed using different combinations of primers designed at the distal end of the duplication under the assumption that the repeated copy was arranged in tandem. This included multiple forward primers in exon 13 and reverse primers in exons 2 and 3. This analysis failed to identify the position of this breakpoint under the above assumption. This is consistent with either further complexity of this rearrangement or instability of the duplicated transcript.

3. Discussion

This report reinforces the importance of complete testing of *MTM1* as previously suggested by Trump and colleagues [9]. For those patients where sequencing of *MTM1* is negative, but the diagnosis is strongly suspected, it is important to pursue testing for intronic mutations

(via RT-PCR and/or Western blot analysis) and for large rearrangements (deletions/duplications, tested via array-CGH or MPLA) of the gene. The value of confirming this specific diagnosis is many fold. Myotubular myopathy due to *MTM1* mutation carries a risk of non-muscle related phenotypes not reported in other CNMs that include pyloric stenosis, spherocytosis, gallstones, kidney stones or nephrocalcinosis, a vitamin K-responsive bleeding diathesis, hepatic peliosis, and liver dysfunction manifested by pruritus and elevated serum transaminases [22]. Additionally, the implications for familial inheritance and genetic counseling are important since this is an X-linked condition whereas all other CNMs have been found to have an autosomal inheritance pattern.

The duplication identified in this report includes *MTM1* and *MAMLD1*. A previous report described a patient with a deletion of these genes with a phenotype consistent with XLMTM and genital abnormalities [24], though not all patients with deletions of these genes have the genital phenotype (potentially as a result of an *MTMR1*–*MAMLD1* fusion transcript) [15]. While the patient in this report did not have any genital abnormalities, this is perhaps not surprising, as a duplication of the *MAMLD1* gene would not necessarily be predicted to result in genital abnormalities, and to date only loss function mutations/ deletions of the *MAMLD1* gene have been associated with hypospadias – OMIM #300758 [25]). Additionally, the mechanism leading to the duplication and how it affects gene function are unclear at this time.

In terms of the *MTM1* duplication itself, our prediction is that it results in a non-functional *MTM1* transcript. The fact that exon 2 is not duplicated would indicate that the resulting transcript should produce an out-of-frame coding sequence and a premature stop codon. Such a transcript is likely to be degraded by nonsense-mediated decay mechanisms and not translated into *MTM1* protein. This assertion is supported by our data, where we were unable to detect any of the abnormal transcript from total RNA isolated from fibroblasts from the mother. However, there was insufficient remaining sample from the proband's biopsy to definitely demonstrate the absence of either *MTM1* transcript or *MTM1* protein.

In summary, we present a case study of a male infant with a clinical history consistent with myotubular myopathy and with a large duplication at the *MTM1* gene locus. This study emphasizes the utility of duplication testing by MPLA or array-CGH in situations where *MTM1* mutation is suspected by not detected by conventional gene sequencing.

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Fig. 1. Pathological findings on muscle biopsy. (A) Hematoxylin and eosin staining of frozen muscle tissue reveals small round myofibers and numerous fibers containing large, centrally placed nuclei. Central areas with clear or basophilic staining are present within many fibers, which contain aggregates of mitochondria and other organelles, and are best visualized on NADH staining (B). Bar = 50 mm.

Fig. 2.

Array comparative genomic hybridization (aCGH) results for MTM1. Deletion and duplication testing of the MTM1 gene locus was performed using a custom designed 8×60 K array-CGH platform (Agilent Technologies) that contained probes for 45 genes including MTM1. Analysis of genomic DNA of the affected male patient and his mother revealed a duplication of the MTM1 gene involving exons 1, 3–13 and no duplication of exons 2, 14 and 15. Regions of MTM1 copy number gains are highlighted in purple and evidenced by the elevated log2 fluorescence ratios. Each dot shows a log2 ratio of the hybridization signals of patient versus gender-match control for each probe in the MTM1 gene. The positions of the MTM1 exons are indicated in red above each aCGH chart. The x and y axes represent genomic coordinates and the log2 hybridization signals, respectively.

Fig. 3.

SNP array results for MTM1 duplication. SNP array was performed to define the proximal end of the MTM1 gene duplication using the Affymetrix CytoScan HD Array (Affymetrix, Santa Clara, CA). Analysis using the ChAS software showed a 356 kb duplication that includes the MTM1 and MAMLD1 genes in the patient's mother, and was detected as an increase in the weighted log2 ratio and copy number state. Duplicated probes are shown within the green box, while probes in this region with a normal copy number are shown within the blue box.