Published in final edited form as:

J Clin Neuromuscul Dis. 2018 March ; 19(3): 117–123. doi:10.1097/CND.0000000000000200.

Mitochondrial DNA deletions with low-level heteroplasmy in adult-onset myopathy

Doris G. Leung, MD, PhD^{1,2}, Julie S. Cohen, ScM¹, E. Harlan Michelle, MD², Renkui Bai, MD, PhD³, Andrew L. Mammen, MD, PhD^{2,4,5}, and Lisa Christopher-Stine, MD, MPH⁵

¹Center for Genetic Muscle Disorders at Kennedy Krieger Institute, Baltimore, Maryland

²Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland

³Genetic Testing for Mitochondrial Disorders, GeneDx, Gaithersburg, Maryland ⁴Muscle Disease Unit, National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, Maryland ⁵Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland

Abstract

We report the cases of two patients who presented to our Myositis Center with myalgias and elevated creatine kinase levels. Muscle biopsy showed pathologic features consistent with mitochondrial myopathy. In both cases, a single large deletion in mitochondrial DNA at low-level heteroplasmy was identified by next generation sequencing in muscle tissue. In one case, the deletion was identified in muscle tissue but not blood. In both cases, the deletion was only identified on next generation sequencing of muscle mitochondrial DNA and missed on array comparative genome hybridization testing. These cases demonstrate that next generation sequencing of mitochondrial DNA in muscle tissue is the most sensitive method of molecular diagnosis for mitochondrial myopathy due to mitochondrial DNA deletions.

Keywords

mitochondrial myopathy; necrotizing myopathy; metabolic myopathy; next-generation sequencing; mtDNA

Introduction

The mitochondrial myopathies are unique among the genetic muscle diseases, with distinctive clinical and pathological features that result from impaired oxidative respiration. However, the diagnosis of these conditions is complicated by the numerous genes (approximately 1500 genes encoded in both mitochondrial and nuclear DNA) that are associated with mitochondrial function.[1] The presentation of mitochondrial disorders, even within the same family, can also vary considerably. In diseases caused by mutations in

Corresponding Author: Doris G. Leung, MD, PhD, Center for Genetic Muscle Disorders, Hugo W. Moser Research Institute at Kennedy Krieger Institute, 716 North Broadway, Room 411, Tel: 443-923-9521; Fax: 443-923-9515, leungd@kennedykrieger.org.

Conflicts of Interest: JSC is a consultant for Invitae Corp. RB is an employee of GeneDx.

mitochondrial DNA (mtDNA), the variability is attributable in part to varying levels of heteroplasmy between family members and even within different tissue types in a single person. The accurate diagnosis of these conditions benefits patients by prompting physicians to screen for potential disease complications, providing accurate estimates of inheritance risk to other family members, and guiding appropriate disease-specific treatment and medical management.

We describe two patients with late-onset mitochondrial myopathy enrolled in a clinical cohort study of patients presenting to our Myositis Center. The study protocol was reviewed and approved by the Johns Hopkins Institutional Review Board, and written consent to use clinical data for research was obtained from both patients. These patients were subsequently referred to the Center for Genetic Muscle Disorders at the Kennedy Krieger Institute for genetic evaluation.

Case 1

Clinical presentation

Patient 1 presented to the Myositis Clinic at the age of 62. Two years prior to her first visit, she developed pain in the lower legs that she described as a deep feeling of pressure. There was no accompanying edema or tenderness to palpation, and she did not observe any weakness or sensory disturbance. Her initial evaluation at an outside institution showed a creatine kinase (CK) level of >900 U/L. On the basis of this finding, she was instructed to stop her statin medication (simvastatin), which she had been taking for six years. The pain improved substantially, but subsequent CK levels never completely normalized and remained between 300-700 U/L.

Prior to symptom onset, Patient 1 had met all of her early developmental milestones and played sports throughout her childhood and early adulthood. In her mid-30's, she began to develop pain in the calves and thighs when walking up an incline. These symptoms always resolved spontaneously and occurred much less frequently in late adulthood. In addition to high cholesterol, she had infrequent migraine headaches with auras. Her family history revealed a history of Parkinson's disease and lifelong ptosis in her mother. She had two children, one of whom had experienced an episode of exertional myalgia with elevated CK levels.

Diagnostic evaluation

Neurological examination of the patient demonstrated mild weakness in the hip flexors and mildly reduced vibratory sensation in the toes. No extraocular weakness was observed. In addition to the elevated CK levels, the blood lactate level was mildly elevated at 2.5mmol/L (reference range is 0.4 to 2.0mmol/L). EMG and nerve conduction studies were unremarkable, and MRI of the bilateral thighs did not show evidence of fat infiltration or intramuscular edema (Figure 1a, 1c).

Given the persistently elevated CK levels, the patient was referred for a muscle biopsy of the right biceps brachii (Figure 2a, 2c). Staining with cytochrome oxidase (COX) and succinate dehydrogenase (SDH) revealed excessive numbers of COX-negative fibers. Gomori

trichome staining showed scattered ragged red fibers; these fibers were also noted to be dark on SDH stain and had disrupted intermyofibrillar architecture.

Based on the findings of the muscle biopsy, the patient was referred to the Center for Genetic Muscle Disorders for evaluation of mitochondrial myopathy. Next-generation sequencing (NGS) of mtDNA in blood was normal. An NGS panel of 504 nuclear-encoded genes that cause mitochondrial disorders was performed (Fulgent Diagnostics, Temple City, CA) and identified a heterozygous variant of unknown significance, c.443G>A (p.Gly148Asp), in *SLC25A4*, which encodes a mitochondrial ADP/ATP carrier.[2] A sample of frozen muscle biopsy tissue was also submitted for mtDNA testing (GeneDx Laboratory, Gaithersburg, MD).

Whole mitochondrial genome sequencing and deletion testing was performed using whole mitochondrial genome amplification followed by sequencing on the Illumina MiSeq sequencing system. The resultant mtDNA sequence was assembled and analyzed in comparison with the revised Cambridge Reference Sequence (rCRS) and MITOMAP database (<http://www.mitomap.org>).[3] Identified single nucleotide variants or small insertions/deletions were confirmed using Sanger sequencing (for heteroplasmy >15%) or real-time ARMS qPCR (for heteroplasmy <15%).[4] For large deletions, junction PCR followed by Sanger sequencing was used to confirm the presence of the deletions and their break points. Mitochondrial genome array comparative genomic hybridization (CGH), which can detect and quantify deletion heteroplasmy as low as 14%, was used to confirm and quantify the heteroplasmy of the deletions.[5] For this patient, deletion/duplication testing by CGH array was normal; however, genome sequencing identified a 7.2 kb deletion in the mtDNA (m.7111-14363). The level of heteroplasmy for this deletion was estimated to be less than 15%.

Case 2

Clinical presentation

Patient 2 presented to the Myositis Center at the age of 74. Three years prior to presentation, he began to experience myalgias in both legs and difficulty standing from a seated position. He was found to have elevated CK levels (ranging from 800-3000 U/L). At the time of symptom onset, he had been using statin medications for more than 10 years; discontinuation of the statin did not result in improvement of his symptoms. Empiric trials of prednisone, mycophenolate mofetil, and intravenous immunoglobulin for presumed polymyositis did not result in any improvement and his weakness progressed to the point that he required a cane for ambulation. It was at this point that he was referred to the Myositis Center for further evaluation.

Past medical history for Patient 2 was significant for high cholesterol, obstructive sleep apnea, and diabetes. In childhood, infancy, and early adulthood, Patient 2 had been very active and had performed manual labor throughout most of his adult life. There was no family history of neuromuscular impairment.

Diagnostic evaluation

Neurological examination of Patient 2 showed mild weakness in knee extension only (also without extraocular weakness). EMG studies showed small-amplitude, short-duration motor unit action potentials with early recruitment in multiple proximal muscles in the upper and lower extremities. Abnormal spontaneous activity (such as fibrillations and positive sharp waves) was not observed, and these findings were felt to be consistent with a non-irritable myopathy. MRI of the bilateral thighs showed marked hyperintensity on short-tau inversion recovery (STIR) images and fatty replacement in the quadriceps and adductor muscles on T1-weighted images (Figure 1b, 1d). Laboratory evaluation for myositis-specific and myositis-associated antibodies (including antibodies against HMGCoA reductase and NT5C1A) was negative. Plasma lactate levels were not obtained as part of this patient's evaluation.

A muscle biopsy was performed in the left vastus medialis (Figure 2b, 2d). This biopsy showed myofiber degeneration and necrosis and was interpreted as showing a moderately severe necrotizing myopathy. Innumerable COX-negative fibers were seen on oxidative staining.

Based on the results of the muscle biopsy, the patient was referred to the Center for Genetic Muscle Disorders for genetic evaluation. A sample of frozen muscle tissue was submitted for mtDNA testing. NGS identified a 12.8 kb deletion (m.3264_16070) with less than 15% heteroplasmy. As with the prior case, the deletion was not detected by array CGH of mtDNA.

Discussion

These cases illustrate several challenges and controversies in the diagnosis of mitochondrial myopathies. Unlike mutations in nuclear-encoded genes, the proportion of mtDNA copies containing a mutation can vary widely between different cells in the same individual, and the degree of heteroplasmy needed to produce symptomatic disease is not clearly established. Studies of patients with mitochondrial disorders have found that a larger proportion of mutated mtDNA is associated with a more severe phenotype (e.g. earlier age of onset and greater numbers of COX-negative fibers on muscle biopsy), and symptomatic disease with <15% heteroplasmy is relatively uncommon.[6, 7] Studies of control populations have also identified low-level heteroplasmy (0.2 to 2%) in asymptomatic individuals that increases with normal aging.[8, 9] However, large mtDNA deletions with low-level heteroplasmy have been identified in patients with myopathic disease, and the clinical and biopsy findings seen in our patients support the conclusion that their mtDNA deletions were pathogenic.[10]

Another important aspect of these cases is the fact that the large deletions were identified on NGS of mtDNA, but not on deletion/duplication testing using array CGH. For nuclear-encoded genes, NGS is considered to be less sensitive to deletions and duplications compared to array CGH. The opposite occurs in the case of mtDNA deletions with low heteroplasmy because of differences in the depth of coverage, or the number of times a base pair is read. When NGS is performed in mtDNA, more copies of each base pair (typically

5,000 to 10,000×) are read, which increases the likelihood of detecting mtDNA deletions with low-levels of heteroplasmy.[11]

These cases also illustrate the importance of muscle biopsy in the diagnostic evaluation of myopathies. Muscle biopsy was crucial to the diagnosis for two reasons. First, the histopathology offered convincing evidence of mitochondrial dysfunction in muscle, which prompted further molecular testing even after initial mtDNA sequencing in blood and a panel of nuclear-encoded mitochondrial disease genes were unrevealing in Patient 1. Second, the mtDNA deletion in at least one of these patients was only detectable in muscle tissue (given the gene testing results for Patient 1, mtDNA testing was only performed in muscle and not blood for Patient 2). Although muscle biopsy is more invasive than blood or saliva collection, obtaining muscle tissue may be necessary to confirm a molecular diagnosis in mitochondrial myopathies.[12]

Another point of interest is the finding of necrotizing myopathy in Patient 2. While myofiber necrosis without inflammatory infiltration is most frequently associated with autoimmune myopathies (particularly those with anti-signal recognition particle and anti-HMGCoA-reductase antibodies), these findings can also be seen in other muscle diseases.[13] Necrotizing features have been reported in several types of hereditary muscle disease, including mitochondrial disease.[14-16] Patient 2's lack of response to immunosuppression further supports a hereditary etiology for the muscle fiber necrosis seen on his muscle biopsy rather than a concomitant autoimmune process.

Although neuromuscular symptoms are a known side effect of statin medications, the role that these medications played in the cases of these patients is unclear. Because both patients had taken statins for years prior to the development of symptoms, it is difficult to argue that there is a direct temporal association. However, research by our group has shown that HMG-CoA reductase antibody-associated myopathies can present more than three years after statin exposure.[17] Furthermore, Patient 1 did note improvement in her myalgias following statin discontinuation, which would not be expected had her symptoms been entirely caused by mitochondrial myopathy. Studies have demonstrated changes in mitochondrial function associated with statin use, and we are unable to exclude the possibility that statins contributed to the presentation of these patients.[18] It is possible that the statins unmasked the myopathy by placing additional oxidative stress on an existing, potentially asymptomatic, mitochondrial myopathy.

The diagnosis of mtDNA deletions in these two cases allows for better informed genetic counseling as to the inheritance risk to other family members. While mtDNA is only inherited through maternal transmission, there are several additional factors that should be considered when counseling patients. First, not all mtDNA mutations are transmitted with equal frequency. Point mutations in the mtDNA sequence are often maternally transmitted; however, large deletions (particularly those found in only one tissue type) are often the result of de novo somatic mutations and are far less likely to be inherited or transmitted.[19, 20] Second, the penetrance and phenotypic expression of mtDNA disorders can vary within families, and the symptoms of one proband may not be predictive of disease manifestations in other relatives.[21] Third, the presence of mtDNA deletions may be secondary to

mutations in nuclear-encoded genes that regulate replication or maintenance of the mitochondrial genome, such as *SLC25A4*, which can follow autosomal dominant or recessive pattern of inheritance. The significance of the novel heterozygous *SLC25A4* variant in Patient 1 is uncertain. Although it is not a common benign variant and the corresponding amino acid is highly evolutionarily conserved, computational predictions of the impact of this variant on the protein were indeterminate. Segregation analysis also did not identify this variant in Patient 1's child with elevated CK levels. Furthermore, dominant *SLC25A4* mutations have been shown to produce multiple mtDNA deletions and/or mtDNA depletion, while single large-scale mtDNA deletions (such as those seen in our patients) have not been reported.[22]

As a result of their diagnoses, both patients were started on a “mitochondrial cocktail” of antioxidants and vitamins that are prone to depletion in mitochondrial disorders. It is arguable that this could have been done based on the muscle biopsy findings alone and did not require identification of the specific mutation. However, when discussing the results of their testing, both patients stated that they preferred knowing the genetic cause even if it did not change their management. One of the primary motivating factors for these patients in seeking the causative genetic mutation was to determine the inheritance risk to other family members. In the case of Patient 2, we were able to confirm that his children would not be at risk for inheritance. In the case of Patient 1, all of her children would be at risk for inheritance, but the risk of transmission for a large deletion would be much lower than that of a point mutation.

Similar patients could benefit from identifying causative mutations in other ways. Had Patient 2 not already stopped immunotherapy prior to being seen, confirmation of a genetic diagnosis may have provided sufficient evidence for us to confidently discontinue immunotherapy. The immune-mediated necrotizing myopathies can be highly refractory to treatment, and it is possible that other physicians would have opted to advance to more aggressive immunotherapies in the absence of a genetic diagnosis. Another potential benefit to identifying a specific mutation would be better anticipatory guidance. Mitochondrial diseases can be associated with multi-systemic complications, and consensus guidelines for screening for these complications have been developed to improve and standardize patient care.[23] Specific mitochondrial mutations can be associated with unique syndromes or a higher risk for specific complications (such as hearing loss and cardiomyopathy) and knowing these risks may motivate more aggressive screening for these conditions.[24] We have also found in our clinical experience that identifying causative mutations can empower patients to reach out to other patients with the same condition (through social media and other platforms) and establish networks for mutual support, education, and advocacy.

Acknowledgments

The authors thank the patients and their families for their participation and support.

Funding: DGL receives support from a grant from the National Institutes of Health (5 K23 NS091379-03). LC-S's work is funded by the Huayi and Siuling Zhang Discovery Fund.

References

1. Wong LJ. Next generation molecular diagnosis of mitochondrial disorders. *Mitochondrion*. 2013; 13:379–87. [PubMed: 23473862]
2. Thompson K, Majd H, Dallabona C, et al. Recurrent De Novo Dominant Mutations in SLC25A4 Cause Severe Early-Onset Mitochondrial Disease and Loss of Mitochondrial DNA Copy Number. *American journal of human genetics*. 2016; 99:1405.
3. Retterer K, Juusola J, Cho MT, et al. Clinical application of whole-exome sequencing across clinical indications. *Genetics in medicine : official journal of the American College of Medical Genetics*. 2016; 18:696–704. [PubMed: 26633542]
4. Bai RK, Wong LJ. Simultaneous detection and quantification of mitochondrial DNA deletion(s), depletion, and over-replication in patients with mitochondrial disease. *The Journal of molecular diagnostics : JMD*. 2005; 7:613–22. [PubMed: 16258160]
5. Chinault AC, Shaw CA, Brundage EK, Tang LY, Wong LJ. Application of dual-genome oligonucleotide array-based comparative genomic hybridization to the molecular diagnosis of mitochondrial DNA deletion and depletion syndromes. *Genetics in medicine : official journal of the American College of Medical Genetics*. 2009; 11:518–26. [PubMed: 19546809]
6. Grady JP, Campbell G, Ratnaike T, et al. Disease progression in patients with single, large-scale mitochondrial DNA deletions. *Brain : a journal of neurology*. 2014; 137:323–34. [PubMed: 24277717]
7. Schroder R, Vielhaber S, Wiedemann FR, et al. New insights into the metabolic consequences of large-scale mtDNA deletions: a quantitative analysis of biochemical, morphological, and genetic findings in human skeletal muscle. *Journal of neuropathology and experimental neurology*. 2000; 59:353–60. [PubMed: 10888364]
8. Wallace DC, Chalkia D. Mitochondrial DNA genetics and the heteroplasmy conundrum in evolution and disease. *Cold Spring Harbor perspectives in biology*. 2013; 5:a021220. [PubMed: 24186072]
9. Bua E, Johnson J, Herbst A, et al. Mitochondrial DNA-deletion mutations accumulate intracellularly to detrimental levels in aged human skeletal muscle fibers. *American journal of human genetics*. 2006; 79:469–80. [PubMed: 16909385]
10. Manfredi G, Vu T, Bonilla E, et al. Association of myopathy with large-scale mitochondrial DNA duplications and deletions: which is pathogenic? *Annals of neurology*. 1997; 42:180–8. [PubMed: 9266727]
11. Palculict ME, Zhang VW, Wong LJ, Wang J. Comprehensive Mitochondrial Genome Analysis by Massively Parallel Sequencing. *Methods Mol Biol*. 2016; 1351:3–17. [PubMed: 26530670]
12. Naue J, Horer S, Sanger T, et al. Evidence for frequent and tissue-specific sequence heteroplasmy in human mitochondrial DNA. *Mitochondrion*. 2015; 20:82–94. [PubMed: 25526677]
13. Mammen AL. Necrotizing myopathies: beyond statins. *Current opinion in rheumatology*. 2014; 26:679–83. [PubMed: 25203117]
14. Mancuso M, Orsucci D, Ienco EC, et al. An “inflammatory” mitochondrial myopathy. A case report *Neuromuscular disorders : NMD*. 2013; 23:907–10. [PubMed: 24011700]
15. Haferkamp O, Scheuerle A, Schlenk R, Melzner I, Pavenstadt-Grupp I, Rodel G. Mitochondrial complex I and III mutations and neutral-lipid storage in activated mononuclear macrophages and neutrophils: a case presenting with necrotizing myopathy, poikiloderma atrophicum vasculare, and xanthogranulomatous bursitis. *Human pathology*. 1994; 25:419–23. [PubMed: 8163275]
16. Schneider I, Stoltenburg G, Deschauer M, Winterholler M, Hanisch F. Limb girdle muscular dystrophy type 2L presenting as necrotizing myopathy. *Acta myologica : myopathies and cardiomyopathies : official journal of the Mediterranean Society of Myology*. 2014; 33:19–21. [PubMed: 24843231]
17. Basharat P, Lahouti AH, Paik JJ, et al. Statin-Induced Anti-HMGCR-Associated Myopathy. *Journal of the American College of Cardiology*. 2016; 68:234–5. [PubMed: 27386780]
18. Apostolopoulou M, Corsini A, Roden M. The role of mitochondria in statin-induced myopathy. *European journal of clinical investigation*. 2015; 45:745–54. [PubMed: 25991405]
19. Chinnery PF, DiMauro S, Shanske S, et al. Risk of developing a mitochondrial DNA deletion disorder. *Lancet*. 2004; 364:592–6. [PubMed: 15313359]

20. Alston CL, Rocha MC, Lax NZ, Turnbull DM, Taylor RW. The genetics and pathology of mitochondrial disease. *The Journal of pathology*. 2017; 241:236–250. [PubMed: 27659608]
21. Wong LJ. Diagnostic challenges of mitochondrial DNA disorders. *Mitochondrion*. 2007; 7:45–52. [PubMed: 17276740]
22. Park KP, Kim HS, Kim ES, Park YE, Lee CH, Kim DS. SLC25A4 and C10ORF2 Mutations in Autosomal Dominant Progressive External Ophthalmoplegia. *J Clin Neurol*. 2011; 7:25–30. [PubMed: 21519523]
23. Parikh S, Goldstein A, Karaa A, et al. Patient care standards for primary mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. *Genetics in medicine : official journal of the American College of Medical Genetics*. 2017
24. Pfeffer G, Chinnery PF. Diagnosis and treatment of mitochondrial myopathies. *Annals of medicine*. 2013; 45:4–16. [PubMed: 21867371]

Figure 1.

Axial T1-weighted (T1W) (a) and short-tau inversion recovery (STIR) images (c) of the mid-thigh in Patient 1. These MRI scans are normal without evidence of fat infiltration or muscle edema. T1W (b) and STIR (d) images of the mid-thigh for Patient 2 shows extensive fat infiltration of the adductor and quadriceps muscles. There is mild STIR hyperintensity in the quadriceps.

Figure 2.

Cytochrome oxidase (COX)/succinate dehydrogenase (SDH) stains of biceps brachii muscle from Patient 1 (a) and vastus medialis muscle from Patient 2 (b) show excessive numbers of COX-negative fibers. Gomori trichrome stain of muscle from Patient 1 (c) shows ragged fibers with granular-appearing cytoplasm. H&E staining from Patient 2 (d) shows excessive numbers of internalized nuclei and degenerating muscle fibers with necrosis.