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Distinction between primary and secondary mitochondrial disease

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Review Article

Distinction between primary and secondary mitochondrial disease

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Abstract

Mitochondrial disease refers to a large spectrum of disorders involving defective cellular energy production. Primary mitochondrial disease (PMD) is diagnosed clinically and by genetic testing of mitochondrial and nuclear DNA. However, many disorders have the 'mitochondrial' phenotype without an identifiable genetic defect. Even muscle biopsy can be markedly abnormal either due to a PMD or a non-mitochondrial disorder (NMD) affecting mitochondria and giving rise to secondary mitochondrial dysfunction (SMD). Many patients can have clinical signs of mitochondrial dysfunction based on their phenotype, biomarkers, imaging, and muscle biopsy. In these cases, it is often tempting to assign a patient's phenotype to 'mitochondrial disease' but many times it is actually SMD while primary process may be distinct from mitochondria. Fortunately, rapid advances in molecular testing, made possible by next generation sequencing, have been effective in establishing accurate diagnoses to distinguish between PMD and SMD. This is important, since their treatments and prognoses can be quite different. In the absence of the ability to distinguish between PMD and SMD, treating SMD with standard treatments used to treat PMD, can be effective. In this article, the latest research regarding mitochondrial disease/dysfunction is reviewed. In addition, representative examples are illustrated where the distinction between PMD and SMD is crucial for diagnosis and treatment.

Keywords: Mitochondria, mitochondrial DNA, non-mitochondrial disorder, nuclear DNA, primary mitochondrial disease, secondary mitochondrial dysfunction

Introduction

Mitochondria are complex cellular organelles governing many metabolic processes including fatty acid oxidation, Krebs cycle, oxidative phosphorylation (oxphos) in the electron transport chain (ETC) and many others. Mitochondrial disease refers to a group of disorders that primarily affect ETC and therefore production of cellular energy in a form of adenosine-triphosphate or ATP.[1] However, with significant recent advances in genetic testing, mitochondrial dysfunction is increasingly recognized to be even more complex than originally thought. Therefore, the terms primary mitochondrial disease (PMD) and secondary mitochondrial dysfunction (SMD) have been used to describe mitochondrial pathophysiology and are important to distinguish.

Distinction between PMD and SMD

PMD occurs due to germline mutations in mitochondrial DNA (mtDNA) and/or nuclear DNA (nDNA) genes encoding ETC proteins. Point mutations can occur in any of the mtDNA's 37 genes encoding 13 proteins, 22 transfer RNAs (tRNA) and 2 ribosomal RNAs (rRNA), which are essential for optimal ETC function.[1] Approximately 250--300 genes are estimated to govern ETC from the nucleus[2] and about 1500 nuclear genes in total are believed to involve mitochondrial processes, including non-ETC functions such as fatty acid oxidation and Krebs cycle. The ETC is organized in five complexes including complex I (NADH: Ubiquinone oxidoreductase), complex II (succinate

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dehydrogenase), complex III (CoQ-cytochrome c reductase), complex IV (cytochrome c oxidase), and complex V (ATP synthase). For example, mtDNA genes (such as *MT-ND1*) encode seven subunits of the complex I, while nDNA genes (such as *NDUFS1*) encode additional 38 subunits of complex I.[3] Complex II is encoded entirely by nDNA genes, while complexes III, IV and V, similar to complex I, have contribution from both mtDNA and nDNA.

PMD occurs not only due to defective genes encoding ETC proteins, but also due to germline mutations in other nDNA genes that affect oxphos function by impacting production of the complex machinery needed for the ETC to perform optimally. Some of the examples include *POLG* encoding mtDNA polymerase gamma, which replicates mtDNA, and *C10ORF2* encoding the twinkle protein, which catalyzes mtDNA unwinding. Even though *POLG* and *C10ORF2* do not encode ETC proteins directly, without these genes ETC function is impaired. When they are mutated they can cause PMD. Deletions in mtDNA may be germline (inherited from the mother), or secondary to nDNA mutations (inherited from the mother and/or father) such as *POLG* gene mutations, which can cause mtDNA deletions or depletion resulting in PMD. Table 1 gives some examples of mtDNA and nDNA genes implicated in PMD.

SMD in contrast, can accompany many pathologic processes not involving oxphos, including inherited diseases with germline mutations in non-oxphos genes. SMD can also be acquired secondary to adverse environmental effects, which can cause oxidative stress. The latter can result in mtDNA alterations as seen in a variety of other processes adversely impacting mitochondria such as aging, inflammatory response, mitotoxic drugs, etc.[4–6] Thus, SMD can be inherited or acquired which is an important distinction from PMD, which can only be inherited. Distinguishing whether a *de novo* mutation is secondary to another genetic defect or acquired is extremely challenging and, at present, the best method for making this distinction is still poorly understood. One of the most reliable (but not all-encompassing) tools in this daunting task is comprehensive molecular testing of both mtDNA and nDNA which, at least in some cases, can ultimately distinguish between PMD and SMD.

PMD and SMD are examined in detail below with a caveat that distinction between them is often blurred and contributes to incomplete penetrance and variable expressivity, which make diagnosis and treatment of mitochondrial diseases quite challenging. Table 2 gives some examples of non-PMD disorders that can result in SMD and causative nDNA genes.

Primary Mitochondrial Disease

PMD is caused by germline mutations that are passed through nDNA of both the egg and sperm and through mtDNA of the egg only. Although transmission of mtDNA from sperm to zygote can occur, paternal mtDNA is eliminated (Stewart and Larsson, 2014). Unless there is reversion or other restoration of the wild-type gene, germline mutations will be present in nDNA of all cells. Mitochondria are unique, being the only cellular organelles carrying their own DNA outside of the nucleus. Thousands of mitochondria may be present in one cell depending on its function (higher numbers in more metabolically active tissues). Each mitochondrion may have roughly 10 copies of mtDNA. Therefore, even within the same cell, some mtDNA copies can have germline mutations, while the other mtDNA copies are wild type. This is one aspect of heteroplasmy. As the zygote divides, germline mutations in mtDNA from the mother may end up in all cells or only in specific cell lines, with other cell lines having wild type genetic material.[7–8] Repair of mutations in mtDNA is less efficient than that of nDNA, which results in an mtDNA mutation rate 10–20 times higher than nDNA.[9]

Mitochondria in the oocytes can have mutated mtDNA. Heteroplasmy can originate when wild type and mutant mtDNA are both present in one egg; this results in both wild type and mutant mtDNA present at a zygotic level; this then propagates to different embryonic cell lines. Unequal distribution of mutant mitochondria among various organs and tissues can take place during fetal development. How the proportion of mutant mtDNA in the egg that ends up distributed in the tissues is apparently a random process.[10] Throughout life, there may be selective pressures in cells and tissues leading to changes in the proportion of mutant mitochondria and the worsening or amelioration of symptoms.

Recent findings suggested that some mtDNA heteroplasmic mutations are associated with chronic diseases such as atherosclerosis and cancer.[11] Undoubtedly, the impact of environmental stressors can contribute to changes of both egg and zygote mtDNA. Epigenetics refers to processes which alter the expression of genes, independent of changes in the genetic code. Epigenetic processes, particularly methylation, can be influenced by environmental factors and the metabolic state of the cell.[12] Considerable research shows that epigenetic alterations may contribute to both PMD and SMD with modifier genes and retrograde signaling as some of the few examples.[13-14]

PMDs due to germline mtDNA mutations are caused by a variety of maternally inherited mtDNA defects in both heteroplasmic and homoplasmic states. Over 250 different mtDNA mutations have been reported with an incidence of pathogenic variants in as high as 1 in 200 live births. Most of individuals with these mutations do not have any clinical manifestations.[15-16] Indeed, only 1 in 5000 individuals is estimated to phenotypically express clinical manifestations of these mutations.[17-18] Aberrations in mtDNA include point mutations and deletions and in rare cases, duplications.[19]

Mitochondrial DNA-related PMD

These disorders are strictly maternally inherited. Commonly the mother has an mtDNA low-level heteroplasmic mutation, which may or may not cause mild symptoms in her, depending on a threshold at which heteroplasmic level results in symptoms. This so-called 'threshold effect' reflects the proportion of normal to mutant mtDNA within a cell, where mitochondrial dysfunction occurs.[20] This threshold can be different depending on the organ involved. Less often, the mother has an mtDNA mutation only in her oocytes (germline *de novo*), in which case she would be asymptomatic. Some of the representative PMDs caused by mtDNA mutations include diseases involving protein-encoding genes such as Leber hereditary optic neuropathy (LHON) and neurogenic weakness with ataxia and retinitis pigmentosa (NARP). Mutations in mitochondrial tRNA encoding genes can result in diseases like mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) and myoclonic epilepsy with ragged-red fibers (MERRF). MtDNA deletions and rarely duplications are seen in Kearns-Sayre (retinopathy, cardiac conduction defects, and ragged red fibers) and Pearson (anemia, pancytopenia, and pancreatic insufficiency) syndromes. Aminoglycoside-induced nonsyndromic deafness occurs due to a mutation in the gene encoding ribosomal RNA; this mitochondrial gene is frequently included in genetic panels for hearing loss. The above mentioned mtDNA related diseases may occur due to mutated mtDNA in germline before conception (inherited), which would lead to PMDs. These mtDNA mutations can also occur post-conception (acquired) secondary to a high level of oxidative stress or environmental insults, thereby causing SMD rather than PMD. In other words, PMD only originates from the germline mtDNA mutated before conception, while wild type germline mtDNA can be mutated secondary to some process post-conception, giving rise to SMD.

Nuclear DNA-related PMD

These disorders are more complex and often inherited in an autosomal recessive pattern, although dominant and X-linked have also been described.[21] Some of the representative PMDs caused by nDNA point mutations include Leigh syndrome, which has multigenic causes (*NDUFS1*, *SURF1*, etc.). PMDs such as Alpers-Huttenlocher (*POLG*) and mitochondrial neurogastrointestinal encephalomyopathy or MNGIE (*TYMP*) are associated with mtDNA deletions and depletion. Again, nDNA mutations that induce mtDNA defects are different from those environmentally induced due to factors such as oxidative stress. The PMD genes either encode oxphos proteins directly or affect oxphos function by impacting production of the complex machinery needed to run the oxphos process.

PMDs have variable clinical and biochemical characteristics. This is due to the fact that their clinical effects rarely occur in isolation. Indeed, in addition to the innate damage to mitochondrial function caused by these mutations before conception, additional factors modulate the clinical severity of these PMDs. Such factors include secondary damage induced by the environment to modifier genes, which can either exacerbate or compensate for the primary mutation. Therefore, there is a fine line between PMD and SMD, with a significant overlap which makes accurate diagnosis quite challenging. Just like mtDNA-related PMD, germline nDNA defects causing PMDs occur before conception, while post-conceptual environmentally induced epigenetic alterations or somatic nDNA mutations could result in SMD.

Secondary Mitochondrial Dysfunction

SMD essentially refers to any abnormal mitochondrial function other than PMD, i.e. the process caused by the genes encoding the ETC proteins directly or impacting the production of the machinery needed for ETC. Although the primary mitochondrial function is ATP production, nonbioenergetic capabilities of these complex organelles can be affected by mitochondrial dynamics.[22] SMD does not involve inborn defects of genes controlling oxphos and usually presents after conception. Impact of another pathologic process on mitochondria as seen in many inherited and acquired disorders (non-PMD disorders) can secondarily attenuate mitochondrial ability to generate ATP and alter mitochondrial dynamics, which can impact the non-ATP producing capabilities as well. For instance, mitochondrial fission and fusion, as examples of mitochondrial dynamics, are often implicated in multifactorial disorders such as diabetes,[23] heart disease,[24] cancer,[25] kidney diseases[26] and neurodegenerative disorders.[27] Therefore, these complex disorders are frequently accompanied by SMD.

Diagnosis of both PMD and SMD often relies on one or several mitochondrial disease criteria (MDC) scoring systems designed to diagnose the energy producing mitochondrial function such as Nijmegen,[28] modified Walker,[29-30] Morava[31] criteria and others. These criteria take into account biochemical, clinical, tissue and molecular characteristics and assign points based on the importance or specificity of certain clinical or laboratory findings. These criteria typically rate the significance of the clinical and biochemical findings as consistent with a definite, probable or possible diagnosis of mitochondrial disease or that mitochondrial disease is unlikely. In some cases, patients meet enough criteria to be diagnosed with a mitochondrial disorder despite absence of molecular confirmation of an mtDNA or nDNA mutation. This raises the question of whether this diagnosed mitochondrial disorder is a PMD or SMD.

MDC systems use some symptoms and signs that are non-specific and can be part of non-PMD conditions. However, there are currently no consensus guidelines, universally used, for diagnosis of mitochondrial disease (whether primary or secondary) because of the significant variable expressivity and incomplete penetrance. Therefore, no MDC system can be perfect because, PMD and SMD can have overlapping symptoms and signs. A recent publication by Parikh et al.[32] makes an attempt to provide some consensus recommendations based on the literature and the authors' experience and many diagnostic parameters are discussed. However, since no minimal criteria are given and no single patient would have all the mentioned variables, diagnosis heavily relies on subjective analysis and experience, which vary widely between clinicians. Therefore, in our opinion (in absence of consensus guidelines,) the MDC scoring systems, while imperfect, currently represent the best guidance on the minimal diagnostic criteria sufficient to diagnose PMD or SMD. In other words, certain minimal clinical phenotype and/or genotype (combination of symptoms, signs and tests) should be considered to diagnose a patient with PMD or SMD for clinical purposes, given that a vast majority of patients do not perfectly fit all the MDC components. In a practical sense, no diagnostic criteria for any disorder can capture all patients with that disorder simply due to the variable expressivity and incomplete penetrance characteristic to virtually all diseases. For instance, diagnosis of tuberous sclerosis complex (TSC) is based on the diagnostic criteria similar to MDC. TSC criteria indicate definite, probable and possible TSC and genetic testing is used to support the clinical diagnosis.[33] However, testing for detection of a definitive mutation in the *TSC1* or *TSC2* genes is only 80% sensitive. Therefore, TSC remains to be primarily a clinical diagnosis based on the diagnostic criteria, which are neither sensitive nor specific for every TSC patient and sometimes without a genetic confirmation.

The MDC systems strive to balance diagnostic specificity and sensitivity to achieve a high enough specificity without too many patients being undiagnosed and a high enough sensitivity without too many patients being over-diagnosed. This is a daunting but essential task to accomplish in the field of mitochondrial medicine, which also applies to any other medical field. In a recent report, the MDC scoring system had a high sensitivity for diagnosis of mitochondrial encephalomyopathy. The authors stated that the simplified MDC might distinguish between mitochondrial and non-mitochondrial disorders and aid in early diagnosis before invasive muscle biopsy is undertaken.[34] In some cases, the minimum MDC can be used to decide whether a muscle biopsy is warranted.[31] In our practice, we use muscle biopsy as a last resort due to its invasiveness and anesthesia requirement, while we heavily use other non-invasive methods including advanced molecular DNA testing. Figure 1 delineates a pathway to help distinguish between PMD and SMD.

With the advent of the next generation sequencing (NGS), powerful genetic tests such as whole mitochondrial DNA sequencing, panels of multiple nDNA genes and whole exome/genome sequencing (WES/WGS) for the entire coding and noncoding DNA, can be performed in a time and cost-effective manner compared to the standard Sanger sequencing.[35] In a recent study assessing the yield of WES in mitochondrial disease, a molecular diagnosis was achieved in 39% of the patients suspected for mitochondrial disease and the yield was even higher (57%) in the subgroup of patients highly suspected for mitochondrial disease.[36] The authors used the MDC scoring system[31] to assign their patients into the different severity groups. The WES yield was the highest in those meeting more criteria, thus strengthening support for the use of the MDC.[31] In several patients in our practices, molecular confirmation was found after a long 'diagnostic odyssey' which either confirmed PMD or diagnosed a non-PMD disorder which caused SMD, with significant therapeutic implications (see examples below). Even though genetic testing can be quite useful, it is important to perform it in conjunction with thorough genetic counseling before and after the test. Medical geneticists or genetic counselors are the most qualified in selecting the appropriate test, counsel on possible variants of unknown significance and provide post-test counseling including result interpretation and further steps affected by genetic results.

Disorders with SMD

The list of non-PMD disorders and pathologic processes involving SMD is constantly growing as scientists increasingly recognize that many diseases impact mitochondria in ways that have been under appreciated. With the advent and progress of NGS, it may become increasingly evident that SMD commonly accompanies many non-PMD disorders supporting evidence that SMD is likely to be much more common than PMD. This knowledge is crucial for therapeutic management including investigation of potential new therapeutic agents and assignment into potentially life-saving clinical trials. Discussion of all diseases involving SMD is beyond the scope of this review, but we highlight some of the most representative examples commonly seen in patients in our practice.

Neuromuscular disorders

A wide variety of myopathies and muscular dystrophies have solid evidence of SMD. Examples include spinal muscular atrophy (SMA), limb-girdle muscular dystrophy, Bethlem myopathy, inflammatory myopathies, Charcot-Marie-Tooth (CMT) neuropathy, drug-induced peripheral neuropathies, etc.[37] Some proposed mechanisms include dysregulation of the mitochondrial permeability and defective autophagy.[38] Recent molecular studies of muscle samples in various neuromuscular diseases showed mtDNA depletion.[39]

SMA is one of the representative disorders associated with SMD. SMN1 gene mutation in

SMA results in defective spinal neurons leading to denervation and disuse muscular atrophy, which in turn brings about decreased mitochondrial function in myocytes. Carnitine level in muscle is decreased and fatty acids are elevated due to an abnormal fatty acid metabolism.[40] SMA patients also have decreased complex I-IV activities in muscle tissue.[41] In our practice, we routinely offer our patients with SMA ubiquinol and levocarnitine, which improve strength as evidenced by the reports of physical therapists, who are blinded to the administration of these agents. Previous investigators indicated that CoQ10 can be beneficial for physical performance of SMA patients.[42-43]

Chromosomal Defects

Chromosomal derangements can result in SMD by disrupting nuclear genes governing mitochondria. Similarly, abnormal copy number variation can disrupt a particular gene or group of genes involved in mitochondrial function.[44] Down syndrome (DS) or trisomy 21 is one of the representative chromosomal disorders associated with mitochondrial dysfunction. Chromosome 21 contains the SOD1 gene encoding Cu, Zn-superoxide dismutase. Gain of an extra SOD1 copy results in a redox imbalance due to an overexpression of Cu, Zn-superoxide dismutase resulting in an excessive oxidative stress in DS due to the overproduction of reactive oxygen species (ROS) by mitochondria, which can contribute to SMD.[45-46] Interestingly, deleterious SOD1 gene mutations, which have a dominant negative effect in the pathogenesis of amyotrophic lateral sclerosis (ALS), can also cause SMD.[47] Altered mitochondrial function and oxidative stress have been established to play a central role in the pathogenesis of neurodevelopmental impairment in DS[48-49] and ALS. Furthermore, there is a potential role of Coenzyme Q10 in modulating DNA repair mechanisms in DS.[50] In our practice, we routinely offer CoQ10 to our patients with trisomy 21. By optimizing the dosing based on CoQ10 level in leukocytes and clinical response, we achieve a faster progress primarily in gross motor skills as evidenced by physical therapy reports while therapists are blinded to the CoQ10 administration. Large scale studies are needed to assess the role of CoQ10 or other antioxidants in motor function of DS patients.

Chromosomal imbalances that cause contiguous gene syndromes can affect mitochondrial genes and therefore secondarily affect mitochondrial function. Examples include 22q11.2 deletion or DiGeorge syndrome,[51] 22q13 duplication[52] and deletion[53] syndromes and 15q11--13 duplication syndrome.[54] Chromosomal rearrangements can cause autosomal dominant PMDs or unmask recessive PMDs (sporadic mutation on one chromosome and point mutation on the other inherited from a parent). We recently described a female with a *de novo* 8q21.11 deletion and intellectual disability, failure to thrive, short stature, multiple congenital anomalies and dysmorphic features.[55] See the **case report** below.

Immunity and Autoimmunity

Mitochondria are considered to be critical components of the innate immune response.[56] The immune system requires significant energy, which varies depending on the specific activity. ATP is needed for neutrophil and T-cell function as well as antigen presentation and processing.[57] During systemic infections, the immune system expends a large amount of ATP to mount an inflammatory response.[58] Inflammatory responses result in catabolism and high rates of ATP consumption from massive release of pro-inflammatory cytokines, such as IL-1β, TNF-α and IL-6, which may cause fatigue and malaise.[59]

At a certain point, if ATP consumption is high enough, SMD can ensue, especially if infection is severe and immune response is strong. However, if an individual already has pre-existing SMD, the mitochondria may not be able to support the extra demand required by immune response, resulting in an inability to provide adequate ATP for basic cellular function, giving rise to neurologic regression. Neuroregression in the context of infection is a hallmark of mitochondrial diseases (both PMD and SMD) and it is not uncommon to have severe and even life-threatening events with inter-current infection.[60]

Autoimmunity consumes ATP to mount an immune response to the body's own tissues. SMD has been documented in a variety of auto-immune processes including multiple sclerosis and lupus.[59,61] Auto-

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immune diseases of the skin such as pemphigus vulgaris and lupus are associated with increased levels of anti-mitochondrial antibodies, which can result in alterations of mitochondrial energy metabolism.[62] Moreover, defective mitochondria can result in autoimmune activation from generated neopeptides.[63] This results in an unnecessary ATP consumption and expenditure of immune response, which can weaken the immune system and increase susceptibility to infections.[64] The latter consume more ATP by triggering an immune response and this vicious cycle can result in ATP depletion and neuro-degeneration.

Fever is a product of immune response that consumes ATP. Immune responses can cause oxidative stress.[65-66] If ATP is already deficient due to PMD or SMD prior to immune response and fever, neurologic regression could ensue after an immune response is triggered, when a certain 'threshold effect' is achieved. This threshold is higher in normal cells and lower in cells with PMD or SMD. A variety of cumulative factors including the proportion of mitochondrial heteroplasmy in different organs and tissues can contribute to the level of this threshold.

Autism

Autism spectrum disorder (ASD) is a multifactorial neurodevelopmental disorder in which genetic and environmental factors appear to contribute equally.[67-68] Genetic etiology may account for 30--40% of cases[69] with advanced testing techniques such as WES/WGS suggesting that a genetic component may be present in up to 50% of cases.[70-71] A meta-analysis suggests that classic PMD affects 5% of children with ASD, with over 30% exhibiting biomarkers consistent with PMD.[72] Several studies using immune cells demonstrate higher rates of functional mitochondrial abnormalities in ASD.[73-75] The much higher rate of abnormal biomarker levels is probably an indication of many types of SMD in ASD. Indeed, mitochondrial dysfunction has been implicated in immune dysregulation in ASD.[76] Autoimmune diseases are diagnosed significantly more often among children with ASD than among controls and their family members.[77]

Environment

There is evidence to suggest that mitochondria serve as potent epigenetic regulators of the cellular processes in part due to antero and retrograde signaling pathways.[13-14] Environmental influences of diet and exercise can have a significant impact on mitochondrial function. Diet is known to affect the epigenetic regulation of human mitochondrial superoxide dismutase (the Cu-Zn SOD discussed above).[78] Both overexertion and lack of physical activity can strain mitochondria, but exercise is one of the standard effective treatment modalities for PMD and SMD.[79-81] The catabolism associated with psychological stress causes a substantial change in mitochondrial function.[82] In addition, acute and chronic infections are among the most potent environmental forces exerting their effects on mitochondria via inflammatory processes.[83]

Pharmacologic Agents

Multiple studies in the past 50 years have demonstrated that many medications can induce mitochondrial damage by adversely impacting mitochondrial metabolic pathways and components.[84-85] Mitotoxicity has been shown for several drugs including propofol, statins, doxoribucin, risperidone, acetaminophen, etc. The most metabolically active organs, including the liver,[86] heart[87] and skeletal muscle[88] are usually affected. Statin medications used for hypercholesterolemia are well known to induce a specific ('statin-induced') myopathy by depleting CoQ10 in the tissues.[89] CoQ10 supplementation reduces severity of myalgia and muscle weakness in statin-induced myopathy.[90] As more drugs come to market, research has to be done to explore their impact on mitochondria.

Aging

Aging negatively affects mitochondrial function.[56,91-92] Aging contributes to oxidative stress in virtually all organs and tissues in the body and increases risk for SMD. That is one reason an athlete's endurance is not the same at 20 and 65 years of age,[93] since mitochondria provide energy to virtually all processes in the body. Their changes are one of the most important factors that contribute to aging. With time and aging, dysfunctional mitochondria accumulate under the plasma membrane in skeletal

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and smooth muscle cells. This gives an appearance of red ragged fibers (RRF) on Gomori trichrome stain of histopathologic muscle sections. RRFs are present in healthy individuals and their numbers increase with age.[94] Point mutations and deletions in mtDNA also increase with age owing to a diminished precision of mitochondrial machinery responsible for mtDNA replication[95] and a number of other factors including environmental influences such as diet, exercise, infections, drugs and radiation. Neurodegenerative diseases such as Alzheimer's and Parkinson's are among the best examples of deterioration of mitochondrial function with aging.[96] In our practice, we routinely see multiple low-level mtDNA deletions in muscle specimens from adults, much more so than in children. This trend of accumulation of mtDNA deletions and ETC abnormalities in muscle with aging has been described in the literature.[97]

Neurodegenerative disorders

Altered mitochondrial fusion/fission dynamics have been found to be a recurring theme in neurodegeneration.[22] There's evidence of mitochondrial dysfunction in neurodegenerative diseases such as Alzheimer's and Parkinson's, but it's unclear whether it is secondary to the disease process or whether it is due to oxidative stress increased by aging and whether or not it is contributing to the pathogenesis of these diseases. For instance, mutations in the *PINK1* gene in Parkinson's disease cause accumulation of the fission protein DRP1. DRP1, being a positive regulator of fission, results in excessive fission which increases oxidative stress and reduces ATP production.[98] Reduced ATP production contributes to SMD as documented in the mouse model of Rett syndrome[99] and patient with *MECP2* mutation.[100]

SMD is well described in some CMT types. Some representative examples include mitofusin-2 protein (encoded by the *MFN2* gene), which is implicated in CMT type 2A[101] and ganglioside-induced differentiation-associated protein 1 (encoded by the *GDAP1*gene) implicated in CMT type 2K (55). See the **case report** below.

One of our patients presented at 12 years of age with progressive fatigue, weakness, abnormal gait, speech disturbance, drooling and foot eversion. She had high lactate and lactate to pyruvate ratio and low carnitine on two occasions, which along with her clinical phenotype and MDC provided evidence for SMD. Her whole mtDNA testing was negative, but nuclear mitochondrial panel showed a variant likely disease-causing in the *MFN2* gene responsible for CMT type 2A, which is known to cause SMD. However, her asymptomatic father and paternal grandmother had this variant as well which made it less likely to be a culprit, since CMT is almost completely penetrant. WES was ordered next and revealed a *de novo* mutation in the *THAP1* gene implicated in torsion dystonia type 6 (DYT6). Both CMT2A and DYT6 can involve SMD with important differences, especially for therapy. Therefore, the more advanced genetic testing (WES vs a limited nuclear mitochondrial panel) can provide a more accurate diagnosis with potential implications in prognosis and treatment such as enrollment into clinical trials utilizing deep brain stimulation and transcranial electrical polarization.

FXN gene in Friedreich's Ataxia (FA) encodes frataxin, a mitochondrial protein, which plays a role in mitochondrial iron homeostasis.[102] Mutation in a form of expanded trinucleotide repeats in the FXN causes deficiency of the respiratory chain due to free radical-mediated oxidative damage.[103] Idebenone was reported to achieve a dose-related neurologic benefit as measured by the International Cooperative Ataxia Rating Scale (ICARS).[104] Due to study duration, less than 12 months this study was excluded from the review by Kearney et al.[105] who concluded that no randomized trials with idebenone have shown significant benefit on neurological symptoms associated with FA. In our experience, one of our FA patients was started on idebenone at the age of 10 and now at 17 years of age her ICARS score has decreased by 4 points.

Cancer

Mutations in genes encoding mitochondrial proteins have been linked to cancer.[106] For example, defects in succinate dehydrogenase (SDH), also known as Complex II of the ETC, may lead to increased oxygen production and increased sensitivity to oxidative stress.[107] SDH mutations have been linked to paraganglioma and pheochromocytoma.[108] Mitochondrial dysfunction may result in growth advantage for cancer cell during migration and invasion. Rapidly dividing cancer cells have extreme metabolic requirements and therefore utilize various metabolic pathways to support the high rate of proliferation. Increased requirement for ATP production is a basis of novel therapeutic interventions such the use of the enzyme L-asparaginase to treat acute lymphoblastic leukemia.[109-110]

Inborn Errors of Metabolism

A significant number of metabolic disorders include SMD as a part of their phenotypes. Representative examples include methylmalonic and propionic acidurias,[111-112] fatty acid oxidation disorders,[113] disorders of purine/pyrimidine synthesis,[114] urea cycle disorders such argininosuccinic aciduria due to lyase deficiency,[115] and many others. The importance of distinguishing between PMD and primary cause of clinical phenotype with or without SMD is emphasized by a clinical example of the following patient in our practice.

A boy was presented to our practice for the first time at the age of nine years with developmental delay, seizures, autism, periods of developmental regression and self-injurious behavior (finger biting). At three years of age he was diagnosed with PMD due to complex III deficiency, which was demonstrated on a fresh muscle sample. His lactic acid and lactate/pyruvate ratio were elevated. His treating physicians thought that most of his clinical phenotype could be explained by the PMD diagnosis and self-injurious behavior was attributed to autism. He was treated by CoQ10 and levocarnitine, which did result in improvement of his muscle tone (per physical therapy reports). However, his propensity to harm himself went beyond a typical autistic child. Even though his original plasma uric acid was normal, we tested his hypoxanthine phosphoribosyl-transferase (HPRT), which showed a very low activity (4% of control) consistent with a newly diagnosed Lesch-Nyhan syndrome (LNS) patient. His *HPRT1* gene sequencing confirmed a hemizygous mutation (IVS1 + 1G>T) which was also present in his healthy mother. His renal ultrasound demonstrated bilateral renal calculi (determined to be uric acid calculi) in the renal pelvis close to obstructing the ureters. Prompt therapy with allopurinol and lithotripsy avoided extreme pain of the stone obstruction. LNS is an X-linked inborn error of metabolism involving the HPRT deficiency, which results in abnormal purine synthesis. Defective nucleotide availability affects mtDNA production and causes SMD with ATP depletion.[114] To date, the child appears to benefit from his mitochondrial vitamin cocktail as well as allopurinol. The molecular diagnosis of the *HPRT1* mutation in the patient and his mother also provided important information on recurrence risk and reproductive options for the parents.

Treatment

Suspicion for mitochondrial dysfunction (whether PMD or SMD) is usually based on clinical and family history and laboratory findings including metabolic and molecular tests, which are frequently aided by the MDC scoring systems (Figure 1). However, failure to demonstrate a mitochondrial diagnosis does not rule it out; further testing must be pursued until it is either confirmed by a different testing method or disproven by finding a non-PMD diagnosis. In various clinical trials, including pharmacologic and gene therapy, it is crucial to have a molecular diagnosis. Nevertheless, even if a particular non-PMD disorder is not known to have a mitochondrial component, exclusion of SMD may be rather challenging if the clinical phenotype and biomarkers are present as illustrated in the cases presented in this review. Although it is tempting to treat only SMD, an undiscovered non-PMD etiology may require treatment different from that used in SMD alone. In our opinion, due to the relatively benign nature of treatment with antioxidants, regular caloric intake and exercise, we believe that potential SMD should be addressed in both PMD and non-PMD disorders. SMD should be treated as long as there are sufficient indicators of a probable or definite mitochondrial dysfunction as determined by the MDC.

Table 3 lists some of the most common agents used to treat PMD and SMD. These agents are frequently utilized due to their well-documented safety.[116] While discussion of each agent's detailed mechanism of action in PMD/SMD is beyond the scope of this review, we have highlighted the most commonly used ubiquinone (CoQ10) and provided examples of some combination therapies. Even though Pfeffer et al.[117] concluded that there is no clear evidence supporting any interventions in mitochondrial disorders, more promising trials have been conducted recently and are currently in process.[118-121]

Ubiquinone (CoQ10) or ubiquinol (reduced and more bioavailable form of CoQ10) is generally recommended, since it is an essential component of the ETC. It is an obligatory inner mitochondrial membrane cofactor and an antioxidant that reduces excess reactive species at the inner mitochondrial membrane. When mitochondrial dysfunction occurs, whether PMD or SMD, the ETC complexes can produce high levels of oxidative stress. Such oxidative stress can, in turn, cause dysfunction of the ETC. CoQ10, being a part of the ETC, becomes the major antioxidant of the ETC and if it becomes depleted by high levels of oxidative stress, the ETC will further dysfunction. CoQ10 is naturally produced as part of the cholesterol pathway and its exogenous form can be given as ubiquinone (the oxidized form) or ubiquinol (the reduced form). Many physicians prefer the ubiquinol form and studies have suggested that ubiquinol is more bioavailable than ubiquinone.[122] Several trials showed beneficial effects of CoQ10 on fatigue and muscle weakness.[123-125] However, other studies reported no significant improvement.[126] Ongoing large-scale studies would further assess efficacy of CoQ10 in improving mitochondrial function.

There have been few trials examining the effectiveness of mitochondrial supplements in PMD and many of these have used combination therapy. A combination of creatine, CoQ10 and lipoic acid decreased resting plasma lactate and urinary 8-isoprostanes decreased a decline in peak ankle dorsiflexion strength and increased fat-free mass in several patients with PMDs.[127] The efficacy of L-arginine has been demonstrated in MELAS patients.[128-129] Mitoquinone (MitoQ) is a ubiquinone analogue and a potent antioxidant which specifically targets mitochondria and has demonstrated to provide neuroprotection against lipid peroxidation.[130] Besides antioxidants, these trials include gene therapy, allotopic expression of wild-type mitochondrial genes, mitochondria-targeted peptides and alteration of mitochondrial dynamics.[126]

In Figure 2, we propose a pathway to diagnose and treat PMD and SMD. In our experience, the treatment is beneficial and safe. However, since efficacy of some therapeutic agents may not be high in a particular patient, each individual vitamin composition has been regularly modified based on its clinical effects, safety and cost. The following case report illustrates our approach to a patient with SMD.[55]

Case Report

The female proband was seen by our practice for the first time at the age of 12 with a history of developmental delay, intellectual disability, failure to thrive, short stature, cleft palate, congenital heart disease, and dysmorphic features. At the age of 7, she was found to have a de novo 8q21.11 deletion of 11.8-Mb, which explained most of her symptoms until 11 years of age, when she experienced a regression in language, worsening gait and fatigue, which required intermittent use of a wheelchair.

Electromyography showed a mild axonal neuropathy. Brain MRI showed a global cerebral atrophy, which was more pronounced than five years prior. Additional new symptoms included tremor and hoarse voice.

Haploinsufficiency of more than 35 genes in the deleted region explained many of her symptoms. However, unbalanced chromosomal rearrangements in general do not result in developmental regression and neurologic deterioration. This prompted us to look for among the deleted genes for one that could be implicated in these symptoms. Analysis of the deleted genes revealed the *GDAP1* gene encoding ganglioside-induced differentiation-associated protein 1. Haploinsufficiency of the *GDAP1* is implicated in an autosomal dominant Charcot-Marie-Tooth type 2K (CMT2K), which likely contributed to her regression based on the GDAP1 involvement in mitochondrial function and a signal transduction

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pathway in neuronal development[131] In fact, we used one of the MDC scoring systems such as Modified Walker criteria[29] to show a probable or definite mitochondrial dysfunction or SMD. According to this MDC, she met 1 major criterion (pathogenic nDNA abnormality) and 3 minor criteria (incomplete mitochondrial clinical phenotype, antibody-based demonstration of a widespread defective complex I respiratory chain subunit expression, a decreased I+III complex activity in the muscle and abnormal metabolic studies such as high lactate and lactate/pyruvate ratio, low plasma and urine carnitine, high alanine and glutamine). This is a good example of SMD caused by a primary disorder, which, in this case, was a sporadic chromosomal deletion. Treatment of the patient's SMD included improved nutrition via a gastrostomy tube, using a high-fat regular caloric intake with no more than three hours fasting including at night. She also had a regular exercise program and physical therapy. Her vitamin cocktail included ubiquinol, creatine and lipoic acid and needed to be regularly modified based on efficacy, safety and cost. The girl experienced a stabilization of her neurological status and an improvement in several developmental parameters including motor and language skills as evidenced by reports of physical, occupational and speech therapists while they were blinded to the nutritional changes and antioxidant administration.

Conclusions

The many manifestations of mitochondrial dysfunction are extremely complex and at this time, still poorly understood. Nevertheless, some of these complexities are being unraveled with newly created diagnostic tools and therapeutic interventions. Mitochondrial dysfunction can be inherited and/or acquired, as well as continuously modified by environment factors through several mechanisms including epigenetics. There is an important distinction between PMD and SMD since; at times they require different therapeutic approaches. While we advocate treating both PMD and SMD using diet, exercise and antioxidants, it is crucial to identify a possible non-mitochondrial etiology, since it may require separate treatment to decrease morbidity and mortality. Even though there are no consensus guidelines on the diagnosis of mitochondrial disorders, the MDC scoring systems are available to aid the diagnosis and can be quite beneficial. While effective treatment remains to be elucidated in the current and upcoming trials, patients with mitochondrial disease and dysfunction desperately seek intervention. The standard interventions for PMD should be strongly considered while new more effective treatments such as gene therapy are being investigated. Successful clinical trials will eventually lead to FDA approval. In our opinion, standard treatment used in PMD can be effective for some individuals with non-PMD disorder and SMD and starting such treatments systematically even without a firm diagnosis is reasonable given their safety and availability.

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