Mitochondrial Respiratory Disorders: A Perspective on their Metabolite Biomarkers and Implications for Clinical Diagnosis and Therapeutic Intervention

Abstract

Mitochondrial respiratory disorders are incurable progressive degenerative diseases with multi-organ system manifestations. These orphan diseases are caused by mutations in the nuclear or mitochondrial genome affecting the oxidative phosphorylation (OXPHOS) system responsible for ATP synthesis. Currently, therapeutic treatments are not available to patients, resulting in significant disability and a poor prognosis. Patients exhibit a constellation of complex neurological and multisystem phenotypic symptoms. The hallmark of these diseases is their clinical heterogeneity and high variability among patients. Consequently, establishing an accurate diagnosis remains a challenging, invasive, and time-consuming process due to the limited sensitivity, specificity and reliability of the current serum biomarkers used in clinical settings. Recent mouse model-based research combined with patient studies led to the identification of fibroblast growth factor 21 (FGF-21) as a promising serum biomarker. With its high specificity and sensitivity, FGF-21 is a promising diagnostic tool for muscle-affecting mitochondrial respiratory disorders, which might be a useful first-line diagnostic tool instead of the invasive muscle biopsy currently performed in clinical settings. Discovering additional diagnostic biomarkers is critical for establishing an accurate diagnosis given the high clinical heterogeneity of these mitochondrial respiratory diseases. Ultimately, these novel biomarkers might be instrumental to monitor the progression of these diseases and the efficacy of novel therapeutic interventions.

Key Words: Biomarkers, Creatine; Fibroblast-growth factor 21; Lactate; Mitochondrial respiratory disorders; Pyruvate; Oxidative phosphorylation.

Abbreviations: CNS: Central nervous system; CSF: Cerebrospinal fluid; FGF-21: Fibroblast growth factor 21; LHON: Leber Hereditary optic neuropathy; MDS: Mitochondrial DNA depletion syndrome; MELAS: Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes; MIRAS: Mitochondrial recessive ataxia syndrome; MRD, Mitochondrial respiratory disorder; MRRF: Myoclonus epilepsy and ragged-red fibers; mtDNA: mitochondrial DNA; NGS: Next-generation sequencing; OXPHOS: oxidative phosphorylation; WT: Wild type.

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Key Concepts and Clinical Features of Mitochondrial Respiratory Disorders

Mitochondrial Respiratory Disorders (MRDs) are a group of rare and incurable diseases defined by insufficient ATP levels due to impaired oxidative phosphorylation (OXPHOS) [1-3]. The OXPHOS system, which is embedded in the inner mitochondrial membrane, is responsible to produce ATP upon electron transfer through a...
series of four OXPHOS respiratory complexes, with complexes I and II being the two points of entry for electrons and ATP synthesis occurring at complex V, also called ATP synthase (Figure 1). Except for complex II that solely contains nuclear-encoded subunits, the OXPHOS complexes are composed of subunits encoded by both the nuclear and mitochondrial genomes [4, 5].

MRD patients harbor inherited mutations that map in their mitochondrial or nuclear genome thereby impairing OXPHOS activity and ATP production [6]. Thus, the retrograde signaling pathway is impaired due to an accumulation of NAD+ and AMP, a direct consequence of a defective OXPHOS system (Figure 2). This affects the activity of the key regulators, Sirt1 and AMPK, and their main substrate, PGC-1α, required to activate the anterograde signaling for a full mitobiogenic response, thereby failing to rectify the chronic ATP deficit (Figure 2). Currently, no therapeutic options are available to these patients to mitigate their ATP insufficiency, resulting in significant disability, a poor prognosis and premature death [7,8]. MRDs can manifest at any age, ranging from the neonatal phase to adulthood, with variable severity. In the case of severe defects in the OXPHOS system, organogenesis is affected at the onset since it coincides with a metabolic shift from glycolytic to oxidative respiration to ensure optimal ATP production during high-energy embryonic developmental stages [9]. Embryonic differentiation of neural stem cells into neurons, astrocytes, or oligodendrocytes consumes about 50% of cellular ATP to execute key differentiation processes [10]. Nevertheless, MRDs always worsen over time due to their progressive degenerative characteristics. Most MRD patients display heterogeneous clinical symptoms affecting several organs with high-energy demand, such as the central nervous system (CNS), peripheral nerves, skeletal and cardiac muscles, kidneys, and endocrine organs [11,12]. MRD patients exhibit symptoms with variable intensity that fall into two clinical groups: 1) central neurodegenerative phenotypes, including encephalopathy, stroke-like episodes, migraines, seizure, ataxia and dementia; and 2) peripheral neuronal and muscular phenotypes, such as myopathy, cardiomyopathy, peripheral neuropathy, sensorineural deafness, and optic atrophy [13].

Clinical heterogeneity is most acute in patients affected with a specific MRD due to mutations in the mitochondrial (mt) genome. These mutations, which are maternally inherited, alter either mitochondrial protein synthesis, when mapped in a mt- tRNA or mt-rRNA gene, or the OXPHOS system, when mapped in one of the 13 mt genes encoding for a subunit of an OXPHOS complex [4]. Most pathogenic mtDNA mutations only affect a subset of the multi-copy mt genome, causing heteroplasmy, which is defined as the presence of wild-type (WT) and mutated mtDNAs in a mitochondrion [14,15]. Heteroplasmy is dictated by the ratio of WT and mutated mtDNAs in mitochondria, which results in a mixed population of healthy and diseased mitochondria within a cell. A mitochondrion is considered diseased or dysfunctional if its mutant mtDNAs surpass a certain threshold, overwhelming its WT mtDNAs, and vice versa for healthy/functional mitochondria. Therefore, a diseased phenotype occurs when mitochondrial heteroplasmy reaches a certain threshold, which ranges from 60 to 90% depending of the tissue and mutation, leading to insufficient ATP levels [14,16]. Thus, the degree of heteroplasmy influences the severity of the diseased phenotype as well as the heterogeneity of clinical symptoms. This is best exemplified by the mitochondrial respiratory disorder MELAS (Mitochondrial Encephalopathy with Lactic Acidosis and Stroke-like episodes), which is due to a maternally inherited A to G substitution at position 3243 of the mitochondrial gene for tRNA\(^{Lys}\), known as the A3243G MELAS mutation [17,18]. This progressive neurodegenerative disease has an early onset of heterogeneous clinical symptoms that include encephalopathy, seizures, stroke-like episodes, and chronic lactic acidosis [19-21]. Among siblings, high clinical variability is often observed. Furthermore, the MELAS mutation can cause cardiomyopathy or myopathy in some families, while causing hearing loss and diabetes in others [22-24].

The prevalence of these rare MRDs at about 1 in 5000 live births in the United States is most likely underestimated based on recent epidemiological studies on newborn cord blood that revealed 1 in 200 newborn carrying a potentially pathogenic mtDNA mutation [25,26]. With improved clinical diagnosis and advances in next-generation sequencing (NGS) technology, more patients will be accurately diagnosed, resulting in increased prevalence of these orphan diseases [27,28].

Current Metabolite Biomarkers Used in Clinical Setting for Diagnosis of a Mitochondrial Respiratory Disorder

Establishing an accurate diagnosis of MRD is often challenging, time consuming, and costly in part due to the absence of sensitive and specific biomarkers [29,30]. Due to extreme phenotypic variability among patients, the diagnostic process requires a set of clinical assessment combined with complex biochemical, histological and genetic analyses [31,32]. In the case of a single syndromic phenotype with known causative genes, such as Leber hereditary optic neuropathy (LHON), DNA sequencing easily confirms its clinical diagnosis since primary LHON mitochondrial DNA mutations are responsible for about 95% of LHON cases by directly affecting the enzymatic activities of the OXPHOS system and subsequently ATP levels [33-35].

Several conventional metabolite biomarkers, such as lactate, pyruvate, amino acids, and creatine, are routinely measured in plasma and/or cerebrospinal fluid (CSF) and provide a minimally invasive evaluation in patients suspected of having an MRD [8,30]. Due to their limited specificity, sensitivity and consistency, altered levels are only suggestive of a specific MRD [29].

Lactate levels are not consistently elevated in blood or CSF of patients affected with MRD. For example, patients diagnosed with LHON, Leigh disease, Kearns-Sayre syndrome and complex I deficiency display normal lactate levels [36], while most of the MELAS patients exhibit elevated levels of lactate in blood and CSF [18,19]. More specifically, high lactate levels are common in patients affected with neurodegenerative MRDs, such as MELAS, MERRF (Myoclonus Epilepsy and Ragged-Red Fibers) and MDS (Mitochondrial DNA Depletion Syndrome) [40]. Since increased lactate levels are also observed in unrelated pathologies, such as CNS infection, seizures, and stroke, lactate by itself is not a
are not only found in MRD patients, but also in patients affected with pyruvate metabolism disorders characterized by an accumulation of cytosolic pyruvate converted into alanine (Figure 1) [41].

Recent studies investigated whether creatine could be used as a biomarker for MRDs given its known link with mitochondrial bioenergetics [42]. While plasma creatine levels were elevated in patients with specific MRDS, such as MELAS, MERFF and mtDNA deletion, when compared to healthy subjects [43,44], such correlation was not consistently observed among patients affected with different MRDs from another independent cohort [45]. Thus, additional studies are required to validate the specificity and reliability of creatine as a biomarker for a definitive diagnosis of MRD.

FGF-21: A promising Diagnostic Biomarker for a Group of MRDs

Since none of the metabolite biomarkers currently used in a clinical setting can definitively establish an MRD diagnosis [38]. Pyruvate is another plasma metabolite integrated into diagnostic chemistry profile currently performed in clinical settings for diagnosis [29]. The fact that pyruvate levels are prone to inaccurate measurement due to instability and susceptibility to inadequate specimen collection diminishes its reliability as a diagnostic biomarker. Finally, measurement of the ratio of lactate to pyruvate (L:P) in blood or CSF only provides valuable diagnostic information in MRDs affecting the CNS given the elevated lactate levels in blood and/or CSF [39,40].

Elevated blood or CSF levels of amino acids, such as alanine, glycine, and proline, have also been reported in MRD patients as a result of a defective OXPHOS system and subsequent changes in the NADH:NAD⁺ redox signature (Figure 2) [29]. However, their sensitivity and specificity remain uncertain. For example, elevated alanine levels are not only found in MRD patients, but also in patients affected with pyruvate metabolism disorders characterized by an accumulation of cytosolic pyruvate converted into alanine (Figure 1) [41].

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FGF-21: A promising Diagnostic Biomarker for a Group of MRDs

Since none of the metabolite biomarkers currently used in a clinical setting can definitively establish an MRD diagnosis [38].
The fact that FGF-21 is secreted from the liver in response to fasting [54]. FGF-21 levels are unaltered in patients with non-mitochondrial muscle diseases, implying that FGF-21 serum levels are a direct result of both OXPHOS defect skeletal muscle pathology [55].

Most importantly, the sensitivity and specificity of FGF-21 was estimated at 92% for muscle-manifesting MRDs, making FGF-21 a reliable first-line diagnostic tool for these diseases instead of the more invasive muscle biopsy, which is currently the gold standard diagnostic tool [8]. In addition, FGF-21 could be a diagnostic indicator of the progression of the disease since FGF-21 levels correlate with the severity of the symptoms. One of the siblings with MELAS exhibiting limited cardiomyopathy and myopathy had lower FGF-21 levels than his sibling with severe phenotypic manifestation of myopathy [55]. These clinical observations are in agreement with results from studies using the “deletor” mice showing increased FGF-21 levels upon progression of mitochondrial myopathy [53]. Ultimately, FGF-21 may be a promising biomarker to monitor efficacy of therapeutic intervention and therefore promote the design of novel therapeutic strategies for the currently intractable MRDs.

**Conclusion**

The fact that the FGF-21 biomarker is specific for MRDs affecting skeletal muscles emphasizes the urgent need for large-scale clinical analysis and identification of novel diagnostic biomarkers tailored to the complexity and clinical heterogeneity of those MRDs. Such discoveries will most likely establish a shift in the diagnostic pathway used in clinical settings for differential and accurate diagnosis for patients suspected of having an MRD. Such progress in translational research will result in a more precise assessment of the prevalence of those mitochondrial diseases than currently estimated. In fact, recent epidemiological studies on newborn cord bloods have revealed that 1 in 200 newborns...
harbor mutations affecting the OXPHOS system and therefore are at risk of developing MRD. In sum, the use of new biomarkers combined with the advent of NGS technique will bring the field of mitochondrial medicine forward and accelerate the discovery of novel therapeutic strategies.

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