

Metabolomics in the Study of Human Mitochondrial Diseases



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Abstract Mitochondria are dynamic cellular organelles playing many biological roles that are fundamentally required for cellular functions. The primary role of mitochondria is ATP production through oxidative phosphorylation (OXPHOS). Mitochondria are found in nearly all cell types, and their number within cells varies in a tissue-/organ-dependent manner. Tissues/organs characterized by high-energy demands contain abundant mitochondria, and these tissues/organs are most frequently affected when their mitochondria are dysfunctional. The resulting pathologies can be generally referred to as mitochondrial diseases (MDs). MDs can be caused by nuclear or mitochondrial DNA mutations in genes encoding mitochondrial proteins, including OXPHOS proteins. Also, MDs can be developed through nongenetic mechanisms such as those involving environmental factors, mitotoxicity drugs, oxidative stress, and aging. MDs can appear over the entire life span. Patients with particular MDs present a wide range of heterogeneous phenotypes with differ-

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ent levels of disease severity. The wide variety of leading causes and heterogeneous phenotypes of MDs make diagnosing MDs notoriously challenging. Despite these challenges, multiple diagnostic examinations and tools, including family history, phenotypic examinations, neurological imaging, biochemical tests, and genetic analyses, have collectively enhanced the diagnosis of MDs. As a result of the diagnostic limitations and drawbacks, there have been demands for developing new diagnostic approaches capable of detecting metabolic perturbations of MDs used as metabolic biosignatures. For that reason, the metabolomic approach, the study of small metabolites ≤ 1500 daltons, has recently garnered attention. While metabolomics offers significant advances, it is recommended that data sets be integrated with other diagnostic approaches. This chapter reviews the application of metabolomic analyses in studying human MDs.

Keywords Mitochondria · Mitochondrial diseases · Mass spectrometry · Nuclear Magnetic Resonance (NMR) Spectroscopy · Untargeted metabolomics · Targeted metabolomics · Metabolic biosignatures

Abbreviations

ANT	Adenine nucleotide translocase
ATP	Adenosine triphosphate
BAT	Brown adipose tissue
CAT	Catalase
CE-MS	Capillary electrophoresis-coupled mass spectrometry
CIL	Chemical isotope labeling
CPEO	Chronic progressive external ophthalmoplegia
DRP1	Dynamin-related protein-1
ETF	Electron transfer flavoprotein
FIS1	Fission protein-1
GC-MS	Gas chromatography-coupled mass spectrometry
GPxs	Glutathione peroxidases
GSH	Glutathione
IMM	Inner mitochondrial membrane
IMS	Intermembrane space
KSS	Kearns-Sayre syndrome
LC-MS	Liquid chromatography-coupled mass spectrometry
LHON	Leber hereditary optic neuropathy
MDs	Mitochondrial diseases
MELAS	Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
MERRF	Myoclonic epilepsy with ragged-red fibers
MFF	Mitochondrial fission factor
MFN1	Mitofusins-1
MFN2	Mitofusins-2
MiD49	Mitochondrial dynamic proteins of 49 kDa

MiD51	Mitochondrial dynamic proteins of 51 kDa
MNGIE	Mitochondrial neurogastrointestinal encephalopathy
MS	Mass spectrometry
mtDNA	Mitochondrial DNA
NAC	N-acetylcysteine
NARP	Neuropathy, ataxia, and retinitis pigmentosa
nDNA	Nuclear DNA
NMR	Nuclear magnetic resonance
OMM	Outer mitochondrial membrane
OPA1	Optic atrophy-1
OXPPOS	Oxidative phosphorylation
PMF	Proton motive force
PMS	Pearson marrow pancreas syndrome
ROS	Reactive oxygen species
rRNA	Ribosomal RNA
SOD	Superoxide dismutase
SPG7	Hereditary spastic paraplegia 7
TCA	Tricarboxylic acid cycle
TFAM	Transcription factor A of mitochondria
TFB2M	Mitochondrial transcription factor B2
tRNA	Transfer RNA
Trx	Thioredoxin
UCP1	Uncoupling protein-1
VUS	Variants of uncertain significance
WES	Whole exome sequencing
WGS	Whole genome sequencing

1 Introduction

Mitochondria are organelles most commonly referred to as the powerhouses of cells due to their fundamental function of ATP production to fuel the energy-demanding process in cells. Mitochondria transduce energy substrates into adenosine triphosphate (ATP) in key metabolic tissues [1]. Mitochondria are found in nearly all cell types, although their numbers vary in a tissue-/organ-dependent manner. Tissues characterized by high energy demands have many mitochondria, but those with less energy demand have fewer mitochondria to match their cellular energy requirements [2]. Despite the variations in mitochondrial content across different tissues, certain factors can increase or decrease the mitochondrial number in adaptive processes when cells undergo physiological changes such as exercise, dietary energy surfeit or deficit, tissue growth or atrophy, and aging [3, 4]. The role of mitochondria in energy transduction is widely understood. However, mitochondria are also involved in other vital biological functions, including reactive oxygen species (ROS) production, redox signaling, Ca^{+2} hemostasis, and cellular apoptosis [5, 6].

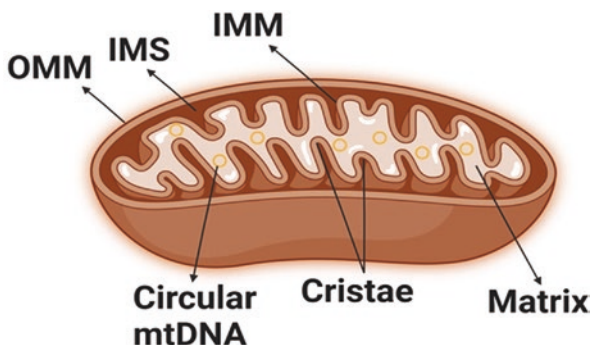
Moreover, mitochondrial functions are influenced by certain properties such as mitochondrial morphology, ultrastructure, and dynamics [7, 8].

Depending on cell type and physiological and energetic status, mitochondria present diverse lengths from 0.5 to 10 μm and shapes, but they share the main structural components [9]. Mitochondria have five main compartments, including the outer mitochondrial membrane (OMM), inner mitochondrial membrane (IMM), intermembrane space (IMS), cristae, and matrix. The OMM and IMM are mainly composed of phospholipids and proteins to support the function of these membranes, with the IMM having a far greater membrane protein density than the OMM. The specific compositions of phospholipids and proteins in the OMM and IMM determine the degree of the integrity and permeability of these membranes. The OMM is relatively permeable, transporting low molecular metabolites, solutes, and ions from the cytoplasm into the IMS. The IMM is highly selectively impermeable to most solutes and metabolites as this structural feature of the IMM is important to allow OXPHOS to occur.

Furthermore, the IMM is associated with a wide range of transporters and protein shuttles to support the many mitochondrial metabolic and bioenergetic pathways [10, 11]. Aqueous regions defined by the membranes are the IMS and matrix. While IMS is present between OMM and IMM, the matrix is defined and enveloped by the IMM. Both the IMS and matrix are important for metabolic events and pathways. The matrix contains many enzymes involved in metabolic pathways such as the tricarboxylic acid cycle, fatty acid β -oxidation, ketogenesis, amino acid metabolism, urea cycle, hormone synthesis, etc. Also, the matrix houses the circular mitochondrial DNA (mtDNA), which exclusively encodes mitochondrial components and its genetic machinery elements such as ribosomes and RNA (more details mentioned below). The fifth mitochondrial compartment is the cristae, defined as the folds of IMM into the matrix to increase the surface area of IMM for enhancing mitochondrial metabolic activity and ATP production and to allow important protein-protein interactions [12]. The mitochondrial structure is illustrated in (Fig. 1).

Concerning mitochondrial morphology, mitochondria exist in many morphologies, such as short oval or spherical tubules, long elongated tubules, or reticular networks. Mitochondrial morphology is distinctively different across cell/tissue types. Moreover, multiple morphologies of mitochondria can be seen with one cell

Fig. 1 Mitochondrial structure consists of outer mitochondrial membrane (OMM), inner mitochondrial membrane (IMM), intermembrane space (IMS), matrix, cristae, and circular mitochondrial genome (mtDNA)



type [13–15]. Under various physiological and pathological conditions, mitochondria continuously remodel their morphology to allow the cells to adapt [16, 17].

Mitochondria are highly dynamic and continuously undergo events of fission and fusion. Mitochondrial dynamics maintain mitochondrial health, morphology, size, and numbers [18, 19]. The morphological changes are induced when cells are triggered by certain stressors or undergo energetic changes and are required during cellular adaptation [9, 19]. Mitochondrial dynamics is under the control of GTPase proteins that mediate the events of fission and fusion. Mitochondrial fission is the fragmentation of one mitochondrion into two or more mitochondria. It requires the recruitment of a cytosolic protein called dynamin-related protein-1 (DRP1) through the action of dynamic proteins, including mitochondrial fission protein-1 (FIS1), mitochondrial fission factor (MFF), and mitochondrial dynamic proteins of 49 and 51 kDa (MiD49 and MiD51). Mitochondrial fission is important for quality control as the fragmented, damaged mitochondria are removed. This is referred to as mitophagy, the selective removal of damaged mitochondria by autophagy [20–22]. In contrast to mitochondrial fission, mitochondrial fusion promotes the joining of two separate mitochondria by merging OMM, IMM, and matrix to make one elongated organelle. Mitochondrial fusion needs the action of several GTPase proteins, including mitofusin (MFN1) and mitofusin-2 (MFN2), to promote OMM fusion and optic atrophy-1 (OPA1) protein for IMM fusion [23, 24]. Fission and fusion should be balanced to maintain a healthy mitochondrial reticulum in cells; otherwise, mitochondrial dysfunction occurs and contributes to the development of mitochondrial diseases (MDs).

From the genetic aspect, mitochondria have the exceptional feature of being the only cellular organelle possessing DNA beyond that found in the nucleus. mtDNA is maternally inherited since paternal sperm mitochondria are targeted and destroyed after egg fertilization during embryogenesis. mtDNA is circular, and there are 100–10,000 copies per cell. mtDNA contains 37 genes encoding only 13 mitochondrial protein subunits involved in OXPHOS, 2 ribosomal RNA (rRNA), and 22 transfer RNA (tRNA) for intra-mitochondrial protein synthesis [25, 26]. In contrast, approximately 1500 mitochondrial proteins are encoded by nuclear DNA (nDNA), translated into the cytoplasm, and imported to the mitochondria [27]. As mitochondria have their transcriptional machinery, mtDNA is colocalized with transcriptional factors, which are nuclear-encoded, such as transcription factor A of mitochondria (TFAM) and mitochondrial transcription factor B2 (TFB2M), to initiate and regulate mtDNA transcription and eventually synthesize OXPHOS subunits [28]. mtDNA is particularly susceptible to mutations compared to nDNA due to the limited repair mechanisms and the high exposure to ROS emission in mitochondria. Mutations in mitochondrial protein-encoding genes lead to dysfunctional mitochondria and various MDs (mentioned below).

2 Mitochondrial Metabolic Pathways

Mitochondria are hubs of cellular metabolism. They are involved in many crucial metabolic pathways, including the tricarboxylic acid cycle (TCA), fatty acid β -oxidation, acyl-carnitine metabolism, urea cycle, amino acid degradation, ketogenesis/ketolysis, steroidogenesis, and OXPHOS as shown in (Fig. 2). These pathways produce small molecular weight intermediate molecules called metabolites, which are also important for cellular proliferation, signaling, survival, and function. Defects in mitochondrial metabolism are associated with alterations in the level of metabolites, which can directly or indirectly affect the physiology of tissues/organs in the body [12, 29].

The OXPHOS system in the IMM consists of NADH dehydrogenase (complex I), succinate dehydrogenase (complex II), cytochrome *c* reductase (complex III), cytochrome *c* oxidase (complex IV), and ATP synthase (complex V) [30]. In detail, in the presence of oxygen, mitochondria convert chemical energy stored in energy substrates, such as pyruvate, acyl-CoA, ketone bodies, etc., into ATP through coupled OXPHOS systems mediated via complex V. These energetic metabolites undergo oxidative reactions producing reducing agents such as nicotinamide adenine dinucleotide hydrogen (NADH + H) and flavin adenine dinucleotide dihydrogen (FADH₂). These reduced coenzymes then provide electrons either to certain OXPHOS complexes, including complex I and complex II or other IMM-bounded

Cytosol

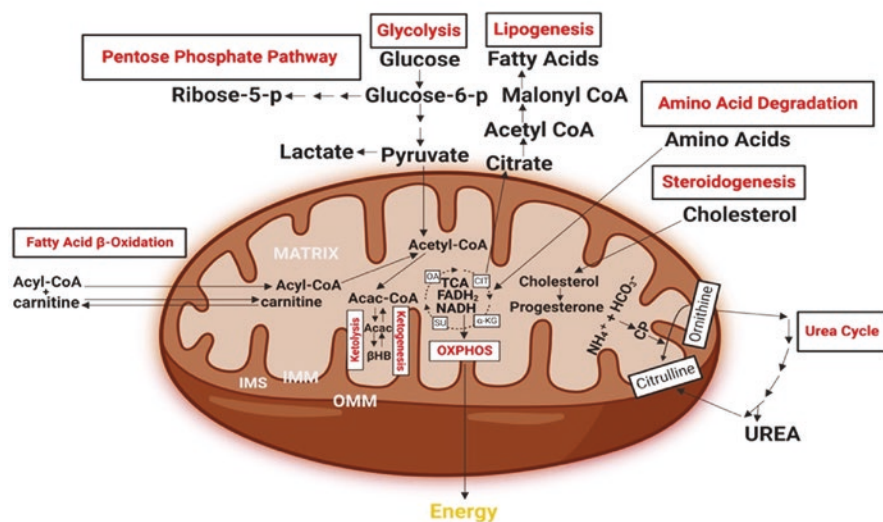


Fig. 2 Simplified illustration of various metabolic pathways taking place in mitochondria

proteins in conjunction with OXPHOS, such as electron transfer flavoprotein (ETF). When electrons are accepted by complex I, complex II, or ETF, they are subsequently passed to coenzyme Q, a mobile fat-soluble electron carrier found in the IMM. Then, coenzyme Q transfers the electrons to complex III, which are then transferred to complex IV through cytochrome C. Ultimately, the electrons reduce oxygen to produce water. While electron flow happens along complexes I–IV, protons are pumped from the matrix to IMS through complex I, complex III, and complex IV, contributing to the generation of an electrochemical gradient called proton motive force (PMF) located across the IMM. Subsequently, free energy stored in PMF drives protons back to the mitochondria matrix through complex V, resulting in the production of ATP, as shown in (Fig. 3) [30–33].

The coupling of electron flow (and oxygen consumption) from ATP production is far from perfect. A certain amount of uncoupling occurs through proton leaks in all cell types [34]. However, one highly metabolic tissue called brown adipose tissue (BAT) that uncoupled OXPHOS has the physiological function of heat production (thermogenesis) instead of ATP production. BAT has low levels of ATP synthase but high amounts of uncoupling protein 1 (UCP1). UCP1 belongs to a gene family of UCPs, and in BAT, it mediates proton leak for thermoregulatory functions. When UCP1 is activated, oxidative reactions upstream of UCP1 support a high influx of reducing equivalents (i.e., electrons) into the electron transfer system in complexes I–IV. This thereby results in proton pumping from the matrix into IMS, causing the formation of PMF, which is rapidly dissipated through UCP1-mediated proton leak

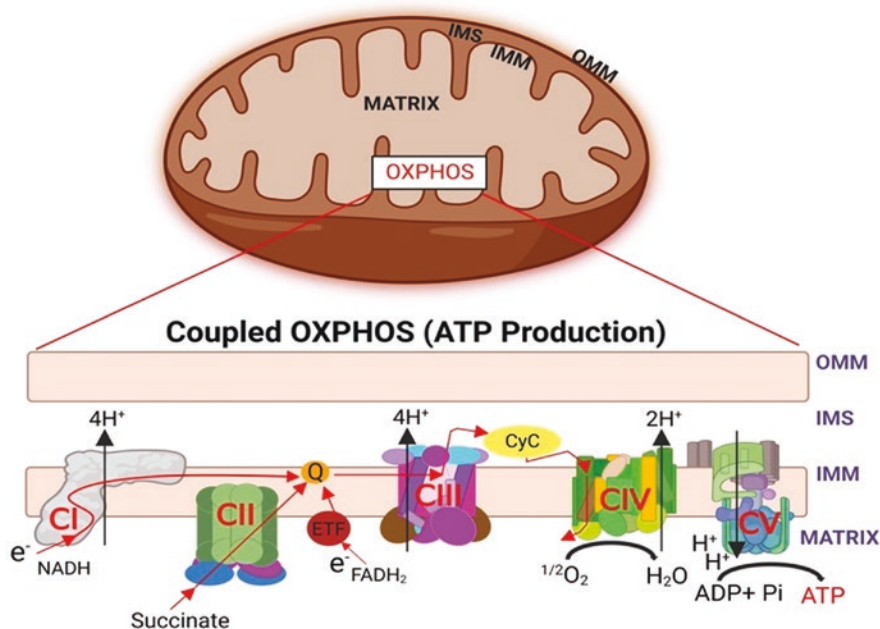


Fig. 3 Mitochondrial-coupled OXPHOS pathways

activity. Thermogenesis results from the upstream oxidative reactions [35]. This is depicted in (Fig. 4). Interestingly, there are other uncoupling proteins named UCP2 and UCP3, which protect against oxidative damage and facilitate fatty acid oxidation in the tissues/cells [36]. UCP2 is found in most tissue types, while UCP3 is predominantly found in the skeletal muscle and BAT, but their levels of expression are approximately two orders of magnitude lower than the expression of UCP1 in BAT [37, 38]. Adenine nucleotide translocase (ANT), which normally exchanges cytosolic ADP for mitochondrial ATP, has been shown to cause proton leak across the IMM and thereby possibly protect against oxidative damage [38–42].

During coupled or uncoupled OXPHOS pathways, ROS, which are byproducts of aerobic metabolism, can be formed. ROS include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH\cdot$). The last are reactive molecules and free radicals derived from molecular oxygen produced in mitochondria. Although they are needed at certain physiological levels to support cellular signaling and transduction, increased and uncontrolled levels of ROS are detrimental, causing pathological effects on cells/tissues such as proteins, lipids, and DNA [43–45]. Therefore, cells/tissues have antioxidant systems that neutralize excessive amounts of ROS. The antioxidant systems can be enzymatic and nonenzymatic based. The enzymatic antioxidant system contains several enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidases (GPxs), and

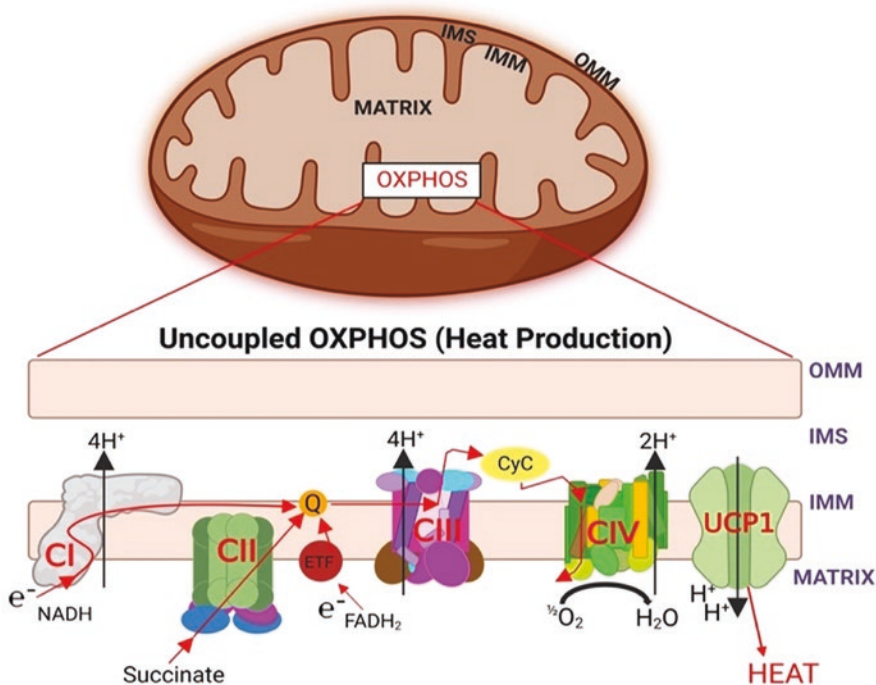


Fig. 4 Mitochondrial uncoupled OXPHOS pathways

thioredoxin (Trx). Nonenzymatic-based antioxidant systems include reduced glutathione (GSH), GSH precursors and reducers, such as N-acetylcysteine (NAC), and vitamins such as vitamins C, A, and E and their derivatives. Accordingly, the antioxidant systems are fundamental as a protective cellular mechanism against oxidative damage. Thus, impairments in the antioxidant mechanisms can lead to oxidative stress due to the imbalance between ROS and antioxidant systems. Oxidative stress can lead to pathological conditions, including MDs [46–49].

3 Mitochondrial Diseases

As described above, MDs can be developed because of defects in mitochondrial properties, including mitochondrial genome, metabolism, structure, and dynamics. MDs are heterogeneous and complex diseases sharing the common feature of mitochondrial dysfunction. MDs are mainly caused directly by inherited mutations in genes encoding mitochondrial OXPHOS proteins, regardless of whether genes in nDNA or mtDNA encode the proteins. MDs can also develop because of inherited alterations in non-OXPHOS mitochondrial proteins, which are essentially required for mitochondrial function. In addition, nongenetic factors such as environmental stressors, myotoxicity drugs, oxidative stress, and aging can progressively cause dysfunctional mitochondria leading to eventually MDs during a lifetime [50].

Examples of MDs developed by either genetic or nongenetic causes are mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), chronic progressive external ophthalmoplegia (CPEO), neuropathy, ataxia, and retinitis pigmentosa (NARP), myoclonic epilepsy with ragged-red fibers (MERRF), Leber hereditary optic neuropathy (LHON), Kern-Sayre syndrome (KSS), Pearson marrow pancreas syndrome (PMS), mitochondrial neurogastrointestinal encephalopathy (MNGIE), Leigh syndrome, Alpers-Huttenlocher Syndrome, Barth Syndrome, Parkinson's disease, Alzheimer's disease, autism, Huntington's disease, amyotrophic lateral sclerosis, Wilson's disease, Charcot-Marie-Tooth type 2 K, hereditary spastic paraplegia 7 (SPG7), Friedreich's ataxia, schizophrenia, sepsis, cardiovascular diseases, cancers, diabetes, and metabolic syndromes [51–55]. Expectedly, new MDs are about to develop and continue to be discovered. The prevalence of MDs is not accurately recorded. It has been challenging to precisely determine the prevalence of MDs due to the following reasons: First, the identification of MDs accounted only for diagnostic patients who have undergone molecular testing for the monogenic MDs, which does not account for other unidentified and/or suspected individuals of MDs, especially for those who had mitochondrial defect without developing obvious symptoms of MD during their lifetimes. Second, one particular MD can show heterogeneous phenotypes and symptoms in different patients, which makes the symptoms of that particular MD not firm. Third, MDs can develop from different factors, including genetic and nongenetic factors, and for nongenetic factors, there is a possibility that new factors might arise with time.

Thus, the current prevalence of MDs is probably underestimated. A better estimate of the true prevalence of MDs will lead to increased efforts to diagnose and effectively treat the diseases accurately. To do so, there have been increasing global efforts from the research and clinical community to develop clinical approaches for diagnosing patients with MDs, contributing to the correction of underestimated MD prevalence.

Currently, studies have reported that MDs observed in children account for approximately 5 individuals per 100,000 population [56]. Another study reported that the prevalence of MDs in adults is estimated to be one individual per 5000 [57]. MDs are currently more common than previously thought, requiring an early diagnosis to enhance the health outcomes of patients at an early stage and provide them with proper therapeutic interventions.

4 Diagnostic Tools for MDs

Various clinical approaches are utilized for diagnosing MDs, ranging from simple to sophisticated methods. Suspected MD patients undergo a series of examinations and tests. Examinations are extensive and include investigations of a patient's family medical history, clinical phenotypes, biochemical parameters, molecular genetic tests, tissue biopsies, and neurological abnormalities. All these examinations have usually been considered in diagnosing MDs because they give a comprehensive picture of pathological status at distinct levels. Consensus-based recommendations written by the Mitochondrial Medicine Society mention the optimal diagnostic tools that can be used for MDs involving biochemical tests, genetic analyses, tissue biopsy examinations, and neuroimaging as they explained the purposes of each approach to verify the diagnosis of MDs [58].

Following the initial investigation of the medical family history and clinical phenotypes of the suspected MD patients, biochemical tests are usually performed on biological fluids such as blood, urine, and CFS samples to measure the levels of certain metabolites commonly disrupted in MDs, including pyruvate, lactate, acyl-carnitines, ketone bodies, amino acids, and organic acids [58]. Lactate levels and the ratio of lactate/pyruvate are commonly increased when mitochondria are defective as pyruvate utilization by the mitochondria decreases. Moreover, ketone bodies are often perturbed in MD patients. In addition, there are alterations in the level of amino acids and organic acids in MD patients acting as an indication of mitochondria dysfunction [59]. All the biochemical analyses mentioned above are not specific and exclusive to MDs because these alterations are a common feature in most MDs and also could be seen in non-MDs.

Thus, supportive and specific analyses are needed to perform in addition to the biochemical tests to improve the diagnostic investigation of MDs, involving neurological imaging of the central nervous system and collecting tissue biopsies from MD patients for enzymatic analyses of OXPHOS and other mitochondrial proteins. These methods show their great ability to inform about alterations in mitochondria

in MDs cases; however, these methods have certain pitfalls as neurological imaging detect abnormalities in the brain and nerve system developed only in particular, but not all, MD patients [58]. In addition, enzymatic assays provide valuable functional analyses of mitochondria, although they require an invasive tissue biopsy procedure.

In addition, genetic testing has been extensively included in the diagnostic criteria of MDs since most MDs are inherited diseases. Genetic testing discovers the genetic mutations leading to MDs. The implication of genetic testing has been done for MDs through testing for common pathogenic variants in nDNA or mtDNA genes associated with MDs by sequencing the whole mtDNA or nDNA via whole genome sequencing (WGS) and/or whole exome sequencing (WES). MDs that were identified by multiple genetic testing approaches include those MDs developed by various pathogenic variants in genes coding for the following proteins, NADH dehydrogenase, pyruvate dehydrogenase complex component X (PDHX), ethylmalonic encephalopathy 1 protein (ETHE1), mitochondrial inner membrane protein (MPV17), mitochondrial carnitine/acylcarnitine carrier protein (SLC25A20), mitochondrial fission factor (MFF), F-Box and leucine-rich repeat protein 4 (FBXL4), *elaC* ribonuclease Z 2 (ELAC2), protein PET100 (PET100), iron-sulfur cluster assembly 2 (ISCA2), mitochondrial-processing peptidase subunit alpha (PMPCA), metal cation symporter ZIP8 (SLC39A8), mitochondrial coenzyme A transporter (SLC25A42), ATP-dependent zinc metalloprotease (YME1L1), mitochondrial intermediate peptidase (MIPEP), mitochondrial calcium uptake protein 2 (MICU2), cytochrome c oxidase subunit 5A (COX5A), ubiquinone biosynthesis methyltransferase COQ5 (COQ5), and nundid hydrolase 2 (NUDT2) [60–74].

Regardless of the substantial implications of genetic approaches in identifying MDs, the genetic approaches have certain diagnostic downsides. Specifically, these genetic tests require reading out an unlimited number of genes, and the roles of many genes are still not fully understood. Finding mutations in metabolism-related genes that are functionally unknown, called variants of uncertain significance (VUS), could be misleading in the context of MDs because it is not fully understood if these unknown mutations affect health. Furthermore, mutations identified by genetic tests might be secondary findings of other diseases not discovered or developed yet and unrelated to MDs of interest, which is considered a false-positive discovery. In addition, genetic testing cannot be used for those MDs that are developed by nongenetic factors over a patient's lifetime [75, 76].

Consequently, based on the previous issues associated with the genetic approaches, there is still a definitive need to perform alternative functional analyses of suspected VUS found in metabolism-related genes to ensure whether these genetic mutations truly cause MDs. Also, these alternative functional analyses could help examine the pathogenic effects of nongenetic factors leading to MDs. Since mitochondria work as a hub of metabolism and as metabolite producers, metabolomics, which detect small metabolites ≤ 1500 Daltons through high-throughput mass spectrometry, can predict mitochondrial status in health and disease. Metabolomics has recently gained great attention because of its high potential to be used as a diagnostic tool for all types of MDs, either genetic or nongenetic MDs. The strengths of

using metabolomics as a diagnostic tool are as follows: First, metabolomics measures intermediates or products of ongoing metabolic pathways, and their measurements directly reflect the snapshot readouts of physiological conditions, which make metabolomics more real functional analyses of metabolism compared to other omic approaches. Thus, if there are any metabolic perturbations, as seen in MDs, they could be detected by the metabolomic approach. Second, metabolomics shows high sensitivity with extraordinary capabilities for identifying distinct metabolic biosignatures/profiles of MDs, reflecting the disrupted metabolic pathways in MD conditions. Third, metabolomic data analysis is easier than other omic data because of the limited number of metabolites that currently can be accurately measured compared to the huge numbers of genes or proteins that can be accurately measured. Fourth, compared to other approaches, metabolomics is not expensive, requires relatively little time, and provides comprehensive biological measurements. All these reasons encourage researchers and scientists to focus on applying metabolomics to MDs.

5 Metabolomics of MDs

Several metabolomic studies have been conducted to ultimately identify diagnostic biomarkers/biosignatures of MDs using different biological samples with various mass spectrometry (MS) machines. Herein, we mentioned examples of these metabolomic studies conducted for MDs (Table 1). For instance, a study performed gas chromatography-coupled mass spectrometry (GC-MS)-based metabolomic analyses on plasma samples and skeletal muscle fibers collected from patients diagnosed with mitochondrial myopathy/progressive external ophthalmoplegia disease showing elevated levels of certain metabolites such as cystathionine, glutamic acid, serine, and arachidyl carnitine compared to the samples collected from the controls [77]. Also, GC-MS-based metabolomic analyses of urine samples collected from children diagnosed with deficiencies in OXPHOS proteins revealed increased levels of organic acids, including fumaric acid, glutaric acid, lactic acid, malic acid, and others, compared to the control children [78]. Another study used a metabolomic approach as a pre-screening tool to identify metabolic biosignatures of MD patients who need to undergo tissue biopsies. In this way, MD patients who do not need a tissue biopsy do not need to undergo the invasive procedure [79]. Their study would help to select the proper MD patient who needs to undergo tissue biopsies. Smuts et al. identified biosignatures in urine samples collected from patients with deficient OXPHOS to be used as indicators before the tissue biopsies. They performed untargeted nuclear magnetic resonance (NMR). They targeted GC-MS on OXPHOS-deficient patients' urine samples. They found six organic acids (lactic, succinic, 2-hydroxybutyric, 3-hydroxybutyric, 3-hydroxyisovaleric, and 3-hydroxy-3-methylglutaric acids), six amino acids (alanine, glycine, glutamic acid, serine, tyrosine, and α -amino adipic acid), and creatine as biosignatures of these patients that need to undergo tissue biopsies for the validation of MDs [79].

Table 1 Examples of metabolomic studies done to screen metabolomic biosignatures of MDs by using various biological samples and metabolomic approaches

MD name	Biological specimen	Major findings	Technique	Ref.
Mitochondrial myopathy/ progressive external ophthalmoplegia disease	Plasma samples and skeletal muscle fibers	Elevated levels of certain metabolites such as cystathionine, glutamic acid, serine, and arachidoyl-carnitine	GC-MS	Nikkanen et al. [77]
Deficiencies in OXPHOS proteins	Urine samples	Increased levels of organic acids, including fumaric acid, glutaric acid, lactic acid, malic acid, and others	GC-MS	Reinecke et al. [78]
Deficient OXPHOS in muscles	Urine samples	Altered six organic acids (lactic, succinic, 2-hydroxybutyric, 3-hydroxyisobutyric, 3-hydroxyisovaleric and 3-hydroxy-3-methylglutaric acids), six amino acids (alanine, glycine, glutamic acid, serine, tyrosine, and α -aminoadipic acid), and creatine	Untargeted NMR and targeted GC-MS	Smuts et al. [79]
French-Canadian Leigh syndrome	Urine and plasma samples	Altered 45 metabolite markers, including alanine, asparagine, ketones, acylcarnitines, succinate, kynurenine, lactate, and pyruvates	Targeted GC-MS and LC-MS	Thompson Legault et al. [80]
MELAS	Plasma samples	Elevated levels of pyruvate, lactate, malate, alanine, α -hydroxybutyrate, N-lactoyl-amino acids, β -hydroxy acylcarnitines, and β -hydroxy fatty acids	Targeted and untargeted MS	Sharma et al. [81]
MELAS and MIDD with renal dysfunction	Urine samples	Lower levels of 4-cresyl sulfate, S-methyl-cysteine-sulfoxide, N-methylnicotinamide, and hippuric acid	NMR spectroscopy	Hall et al. [82]
LHON	Fibroblasts	Decreases in amino acids, spermidine, putrescine, isovaleryl-carnitine, propionyl-carnitine, and five sphingomyelin species but increases in ten phosphatidylcholine species	Targeted LC-MS	Chao de la Barca et al. [83]

(continued)

Table 1 (continued)

MD name	Biological specimen	Major findings	Technique	Ref.
Barth syndrome	Plasma samples	Perturbations in creatinine, fatty acids, methionine, and proline	NMR spectroscopy	Sandlers et al. [84]
		Alterations in acylcarnitines, bigeneric amines, PC/IysoPC, and amino acids	Targeted LC-MS	
KSS	Urine samples	Increased levels of pyruvate, fumarate, and 3-hydroxybutyrate	Targeted GC-MS	Semeraro et al. [85]
PMS	Urine samples	Increased levels of lactate, 3-hydroxybutyrate, 3-hydroxyisobutyrate, fumarate, pyruvate, 2-hydroxybutyrate, 2-methyl-2,3-dihydroxybutyrate, 3-methylglutarate, 2-ethyl-3-hydroxypropionate, 3-methylglutaconate, malate, and tiglylglycine		
MELAS	Urine samples	Increases in caproic/caprylic acid, 2-hydroxyglutaric acid, butyric/valeric/2-hydroxybutyric/3-methyl-2-oxovaleric acid, 4-pentenoic acid, acetylcarnitine, propionylcarnitine, taurine, acetic acid but decreased metabolites pyruvic acid, glycerol, carbamate, 2,5-furandicarboxylic acid, fumaric acid, pseudouridine, glycolic acid, and arabinose	Targeted LC-MS/MS and untargeted GC-MS and NMR spectroscopy	Esterhuizen et al. [86]
MIDD	Urine samples	Increases in myoinositol, 2-hydroxyglutaric acid, 4-pentenoic acid, glucuronic acid, 2-hydroxyisovaleric acid, glucose, 2-ethylhydracrylic acid, and 3-hydroxyisobutyric acid. Metabolites' decrease includes glycolic acid, sarcosine, 1,2-ethandiol, 3-methylphenol, 2,5-furandicarboxylic acid, homocysteine, arabinose, and pseudouridine		
Myopathy	Urine samples	Increases in 2-hydroxyglutaric acid, 4-pentenoic acid, 3-methylphenol, 2-ethylhydracrylic acid, and creatine but decreased glycolic acid		

Table 1 (continued)

MD name	Biological specimen	Major findings	Technique	Ref.
Parkinson's disease	Plasma and CSF samples	Altered pattern of metabolites involved in the metabolism of glycerophospholipid, sphingolipid, acylcarnitine, and amino acids	Untargeted LC-MS and MS/MS	Stoessel et al. [87]
Parkinson's disease	Urine samples	Altered metabolites related to tryptophan and tyrosine metabolism	Untargeted LC-MS	Luan et al. [88]
Alzheimer's disease	CSF samples	Altered patterns of choline, dimethylarginine, arginine, valine, proline, serine, histidine, creatine, carnitine, and suberylglycine	Untargeted capillary electrophoresis mass spectrometry (CE-MS)	Ibáñez et al. [89]
Medium-chain acyl-coenzyme A dehydrogenase deficiency disease	Dried blood spots	Altered levels of certain amino acids and acylcarnitines	Targeted LC-MS	Scolamiero et al. [90]
Long-chain acyl-coenzyme A dehydrogenase deficiency disease	Dried blood spots and serum samples	Decreases in lysine, valine, glycerol, and niacinamide but increases in glutamine succinic acid and guanosine	Targeted LC-MS/MS	Jacob et al. [91]
Type 2 diabetes (T2D)	Urine samples	Decreases in t3-hydroxyundecanoyl-carnitine	Targeted LC-MS	Salihovic et al. [92]
Insulin resistance	Serum samples	Insulin resistance altered amino acids such as amino acids (Asn, Gln, and his), methionine (met) sulfoxide, 2-methyl-3-hydroxy-5--formylpyridine-4-carboxylate, serotonin, L-2-amino-3-oxobutanoic acid, and 4,6-dihydroxyquinoline	Chemical isotope labeling (CIL) liquid chromatography-mass spectrometry (LC-MS)	Gu et al. [93]
Type 2 diabetes		Distinct amino acids, amino acid metabolites, and dipeptides for T2D		

A cohort of French-Canadian Leigh syndrome patients with mutations in the LRPPRC gene causing defects in one of the OXPHOS proteins was involved in a metabolomic study. Urine and plasma samples were collected from the Leigh syndrome patients and were analyzed using targeted GC-MS and liquid chromatography-coupled mass spectrometry (LC-MS). As a result, they found 45 outstanding metabolic biomarkers, including alanine, asparagine, ketones, acylcarnitines, succinate, kynurenine, lactate, and pyruvates, altered compared to controls (Thompson [80]). Additionally, another research group recruited mitochondrial encephalomyopathy lactic acidosis and stroke-like episode (MELAS) syndrome and maternally inherited diabetes and deafness (MIDD) patients with renal dysfunction for identification of urinary metabolic markers via NMR spectroscopy-based metabolomics and found that these patients distinguished from controls by having lower levels of 4-cresyl sulfate, S-methyl-cysteine-sulfoxide, N-methylnicotinamide, and hippuric acid [82]. Recently, Sharma et al. performed metabolomic analyses on plasma samples collected from MELAS and controls by using targeted and untargeted MS, revealing that elevated levels of pyruvate, lactate, malate, alanine, α -hydroxybutyrate, N-lactoyl-amino acids, β -hydroxy acylcarnitines, and β -hydroxy fatty acids [81]. Another example of metabolomic study is those conducted for Barth syndrome. They identified dysregulated metabolic markers and pathways underlying Barth syndrome using human plasma samples showing perturbations in creatinine, fatty acids, methionine, and proline detected by NMR. At the same time, LC-MS revealed metabolic alterations found in acylcarnitines, bigeneric amines, PC/lysoPC, and amino acids in patients compared to controls [84].

Furthermore, a targeted LC-MS-based metabolomic approach was applied to fibroblasts taken from LHON patients to uncover metabolites affected in this disease used as biomarkers, including decreases in amino acids, spermidine, putrescine, isovaleryl-carnitine, propionyl-carnitine, and five sphingomyelin species but increases in ten phosphatidylcholine species [83]. Also, metabolomic studies of PMS and KSS were performed recently using a targeted GC-MS approach on urine samples collected from PMS and KSS patients. Semeraro et al.'s study revealed that abnormal alterations in urinary organic acids were detected in both PMS and KSS. Still, the alterations are more pronounced in PMS patients than in KSS. They found that urine samples from KSS had increased levels of pyruvate, fumarate, and 3-hydroxybutyrate, while urinary metabolites detected in PMS patients indicated elevated levels in lactate, 3-hydroxybutyrate, 3-hydroxyisobutyrate, fumarate, pyruvate, 2-hydroxybutyrate, 2-methyl-2,3-dihydroxybutyrate, 3-methylglutarate, 2-ethyl-3-hydroxypropionate, 3-methylglutaconate, malate, and tiglylglycine [85].

Very recently, Esterhuizen et al. attempted to clinically distinguish between three types of MDs, including MELAS, MIDD, and myopathy, by identifying disease-specific metabolic profiles. They used combined multi-metabolomic approaches of targeted LC-MS/MS, untargeted GC-MS, and NMR spectroscopy to comprehensively investigate metabolites in urine samples collected from MELAS, MIDD, and myopathy patients compared to individual disease-matched controls. Although they found some metabolic similarities in identified metabolites shared in the three diseases, their metabolomic data shows that each of the three diseases has certain

unique metabolites compared to their controls. Compared to controls, MELAS patients showed increased caproic/caprylic acid, 2-hydroxyglutaric acid, butyric/valeric/2-hydroxybutyric/3-methyl-2-oxovaleric acid, 4-pentenoic acid, acetylcarnitine, propionylcarnitine, taurine, and acetic acid but decreased metabolites pyruvic acid, glycerol, carbamate, 2,5-furandicarboxylic acid, fumaric acid, pseudouridine, glycolic acid, and arabinose. Furthermore, MIDD patients showed increased myoinositol, 2-hydroxyglutaric acid, 4-pentenoic acid, glucuronic acid, 2-hydroxyisovaleric acid, glucose, 2-ethylhydracrylic acid, 3-hydroxyisobutyric acid although decreased metabolites were shown including glycolic acid, sarcosine, 1,2-ethandiol, 3-methylphenol, 2,5-furandicarboxylic acid, homocysteine, arabinose, pseudouridine. Lastly, they showed that myopathy patients revealed increased 2-hydroxyglutaric acid, 4-pentenoic acid, 3-methylphenol, 2-ethylhydracrylic acid, and creatine but decreased glycolic acid [86].

In addition to the previous studies, Stoessel et al. focused on the metabolic changes in Parkinson's disease, in which they performed untargeted LC/MS and MS/MS analysis to profile metabolic changes in plasma and CSF samples collected from Parkinson's disease patients. They uncovered a perturbed pattern of metabolites involved in the metabolism of glycerophospholipid, sphingolipid, acylcarnitine, and amino acids as Parkinson's disease biosignatures [87]. In concordance with the previous Parkinson's disease study, another research group studied Parkinson's disease and discovered alterations in metabolites related to tryptophan and tyrosine metabolism and other pathways using untargeted LC-MS approaches applied to urine samples from Parkinson's disease patients [88]. Moreover, Ibáñez et al. used untargeted metabolomic profiling based on capillary electrophoresis-coupled mass spectrometry (CE-MS) to identify Alzheimer's disease biosignatures in CSF samples collected from Alzheimer's disease patients for the prediction of the disease and its progression at distinct stages. They found choline, dimethylarginine, arginine, valine, proline, serine, histidine, creatine, carnitine, and suberylglycine as potential Alzheimer's disease progression markers [89]. Also, Scolamiero et al. analyzed dried blood spots (DBS) from patients diagnosed with medium chain acyl-coenzyme A dehydrogenase deficiency disease via targeted LC-MS to identify metabolic profiling associated with the disease condition. Their results showed that the level of certain acylcarnitine (C2, C6, C8, and C10) was perturbed distinctively in these affected patients compared to the controls, potentially used as diagnostic markers [90]. In addition, another research group performed a metabolomic study on patients diagnosed with long-chain acyl-coenzyme A dehydrogenase deficiency disease by performing targeted LC-MS analyses on dried blood spots and serum samples collected from the patients showing that there were alterations in metabolites in patients' samples compared to the controls. Examples of altered metabolites are decreases in lysine, valine, glycerol, and niacinamide but increases in glutamine succinic acid and guanosine [91].

Furthermore, diabetes was extensively studied in different biological samples with different MS approaches; thus, example studies are mentioned. A recent study conducted nontargeted LC-MS-based metabolomics to profile urine metabolites in patients diagnosed with type 2 diabetes (T2D), resulting in the discovery of a

promising biomarker t3-hydroxyundecanoyl-carnitine, which was decreased with T2D [92]. In addition, another study done by another research group revealed metabolite biosignatures related to insulin resistance and T2D. This study collected serum samples from normal, obese T2D participants for metabolic profiling using chemical isotope labeling (CIL) and LC-MS. Their results showed that certain biomarkers associated with insulin resistance, such as amino acids (asparagine, glutamine, histidine), methionine sulfoxide, 2-methyl-3-hydroxy-5-formylpyridine-4-carboxylate, serotonin, L-2-amino-3-oxobutanoic acid, and 4,6-dihydroxyquinoline and other biomarkers for T2D, including amino acids, amino acid metabolites, and dipeptides [93].

Last but not least, there is a growing body of recent studies demonstrating the application of metabolomic technologies in sepsis [94–96], cardiovascular diseases [97, 98], and cancers [99–102].

6 Conclusion

The application of metabolomics can be promising for the diagnosis and prognosis of MDs in humans. However, it has been reported that metabolomic analyses have certain challenges and limitations that need to be considered. For instance, metabolomic analyses can be conducted by multiple mass spectrometry approaches, including NMR, GC-MS, and LC-MS. All of them are used for various purposes due to their different functionalities, metabolite coverage, and experimental design. Each approach has advantages and disadvantages. For example, NMR is suitable for significant volume samples, is nondestructive to samples, does not require sample preparations, and gives reproducible data. However, NMR is not as sensitive as other techniques, such as GC-MS and LC-MS, which can detect a large range of metabolites with very tiny amounts of sample. GC-MS and LC-MS function based on the polarity of metabolites, as GC-MS is mainly used more for nonpolar metabolites. Thus LC-MS is the preferable approach for polar metabolites.

Nevertheless, MS approaches have certain drawbacks, such as lower reproducibility, higher-cost expensive techniques, and time-consuming sample preparation steps. Thus, no single approach can comprehensively detect all metabolites with various chemical and physical properties in biological samples within one analytical run. Other limitations are related to metabolomic data preprocessing and spectral library databases. Examples of issues during metabolomic data processing missing data can be seen, and that is due to several reasons, such as the concentration of metabolites being either lower or higher than the detection limit of the machine. Another limitation of metabolomics is the availability of metabolite spectral libraries for data analyses and interpretation. NMR and LC-MS approaches have limited libraries compared to GC-MS libraries.

Many of the challenges associated with metabolomics can be overcome by combining various approaches to detect and cover a comprehensive range of metabolites. Furthermore, metabolomics shows the powerful capability and potential in

diagnosing MDs as it represents real perturbations associated with MDs. Nonetheless, it is best to integrate metabolomic diagnostic data with other diagnostic approaches (i.e., patient and family histories, physical examinations, clinical biochemical and histological tests, and enzymatic assays). Finally, research in this area and the corresponding field development will improve with the involvement of larger patient cohorts. Increased global collaborations between many clinical communities will strengthen and validate the finding of metabolic biosignatures of human MDs and improve diagnostic platforms.

References

1. Elston T, Wang H, Oster G. Energy transduction in ATP synthase. *Nature*. 1998;391(6666):510–3. <https://doi.org/10.1038/35185>.
2. Veltri KL, Espiritu M, Singh G. Distinct genomic copy number in mitochondria of different mammalian organs. *J Cell Physiol*. 1990;143(1):160–4. <https://doi.org/10.1002/jcp.1041430122>.
3. Spiegelman BM. Transcriptional control of mitochondrial energy metabolism through the PGC1 coactivators. In: *Mitochondrial biology: new perspectives*. Chichester: John Wiley & Sons; 2007. p. 60–9. <https://doi.org/10.1002/9780470725207.ch5>.
4. Tzamelis I. The evolving role of mitochondria in metabolism. *Trends Endocrinol Metab*. 2012;23(9):417–9. <https://doi.org/10.1016/j.tem.2012.07.008>.
5. Friedman JR, Nunnari J. Mitochondrial form and function. *Nature*. 2014;505(7483):335–43. <https://doi.org/10.1038/nature12985>.
6. Herst PM, Rowe MR, Carson GM, Berridge MV. Functional mitochondria in health and disease. *Front Endocrinol*. 2017;8:296. <https://doi.org/10.3389/fendo.2017.00296>.
7. McBride HM, Neuspiel M, Wasiak S. Mitochondria: more than just a powerhouse. *Curr Biol*. 2006;16(14):R551–60. <https://doi.org/10.1016/j.cub.2006.06.054>.
8. Shaw JM, Nunnari J. Mitochondrial dynamics and division in budding yeast. *Trends Cell Biol*. 2002;12(4):178–84. [https://doi.org/10.1016/S0962-8924\(01\)02246-2](https://doi.org/10.1016/S0962-8924(01)02246-2).
9. Trushina E. A shape shifting organelle: unusual mitochondrial phenotype determined with three-dimensional electron microscopy reconstruction. *Neural Regen Res*. 2016;11(6):900–1. <https://doi.org/10.4103/1673-5374.184477>.
10. Gohil VM, Greenberg ML. Mitochondrial membrane biogenesis: phospholipids and proteins go hand in hand. *J Cell Biol*. 2009;184(4):469–72. <https://doi.org/10.1083/jcb.200901127>.
11. Kühlbrandt W. Structure and function of mitochondrial membrane protein complexes. *BMC Biol*. 2015;13(1):89. <https://doi.org/10.1186/s12915-015-0201-x>.
12. Bowsher CG, Tobin AK. Compartmentation of metabolism within mitochondria and plastids. *J Exp Bot*. 2001;52(356):513–27. <https://doi.org/10.1093/jexbot/52.356.513>.
13. Glancy B, Hartnell LM, Malide D, Yu Z-X, Combs CA, Connelly PS, Subramaniam S, Balaban RS. Mitochondrial reticulum for cellular energy distribution in muscle. *Nature*. 2015;523(7562):617–20. <https://doi.org/10.1038/nature14614>.
14. Glancy B, Kim Y, Katti P, Willingham TB. The functional impact of mitochondrial structure across subcellular scales. *Front Physiol*. 2020;11:541040. <https://doi.org/10.3389/fphys.2020.541040>.
15. Yoon Y. Sharpening the scissors. *Cell Biochem Biophys*. 2004;41(2):193–205. <https://doi.org/10.1385/CBB:41:2:193>.
16. Ding W-X, Li M, Biazik JM, Morgan DG, Guo F, Ni H-M, Goheen M, Eskelinen E-L, Yin X-M. Electron microscopic analysis of a spherical mitochondrial structure*. *J Biol Chem*. 2012;287(50):42373–8. <https://doi.org/10.1074/jbc.M112.413674>.

17. Navaratnarajah T, Anand R, Reichert AS, Distelmaier F. The relevance of mitochondrial morphology for human disease. *Int J Biochem Cell Biol.* 2021;134:105951. <https://doi.org/10.1016/j.biocel.2021.105951>.
18. Busch KB, Kowald A, Spelbrink JN. Quality matters: how does mitochondrial network dynamics and quality control impact on mtDNA integrity? *Philos Trans R Soc Lond B Biol Sci.* 2014;369(1646):20130442. <https://doi.org/10.1098/rstb.2013.0442>.
19. Mishra P, Chan DC. Metabolic regulation of mitochondrial dynamics. *J Cell Biol.* 2016;212(4):379–87. <https://doi.org/10.1083/jcb.201511036>.
20. Ashrafi G, Schwarz TL. The pathways of mitophagy for quality control and clearance of mitochondria. *Cell Death Differ.* 2013;20(1):31–42. <https://doi.org/10.1038/cdd.2012.81>.
21. Losón OC, Song Z, Chen H, Chan DC. Fis1, Mff, MiD49, and MiD51 mediate Drp1 recruitment in mitochondrial fission. *Mol Biol Cell.* 2013;24(5):659–67. <https://doi.org/10.1091/mbc.E12-10-0721>.
22. Otsuga D, Keegan BR, Brisch E, Thatcher JW, Hermann GJ, Bleazard W, Shaw JM. The dynamin-related GTPase, Dnm1p, controls mitochondrial morphology in yeast. *J Cell Biol.* 1998;143(2):333–49.
23. Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE, Chan DC. Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *J Cell Biol.* 2003;160(2):189–200. <https://doi.org/10.1083/jcb.200211046>.
24. Song Z, Ghochani M, McCaffery JM, Frey TG, Chan DC. Mitofusins and OPA1 mediate sequential steps in mitochondrial membrane fusion. *Mol Biol Cell.* 2009;20(15):3525–32. <https://doi.org/10.1091/mbc.e09-03-0252>.
25. Gustafsson CM, Falkenberg M, Larsson N-G. Maintenance and expression of mammalian mitochondrial DNA. *Annu Rev Biochem.* 2016;85:133. <https://doi.org/10.1146/annurev-biochem-060815-014402>.
26. Wai T, Ao A, Zhang X, Cyr D, Dufort D, Shoubridge EA. The role of mitochondrial DNA copy number in mammalian Fertility1. *Biol Reprod.* 2010;83(1):52–62. <https://doi.org/10.1095/biolreprod.109.080887>.
27. Rath S, Sharma R, Gupta R, Ast T, Chan C, Durham TJ, Goodman RP, Grabarek Z, Haas ME, Hung WHW, Joshi PR, Jourdain AA, Kim SH, Kotryns AV, Lam SS, McCoy JG, Meisel JD, Miranda M, Panda A, Mootha VK. MitoCarta3.0: an updated mitochondrial proteome now with sub-organelle localization and pathway annotations. *Nucleic Acids Res.* 2021;49(D1):D1541–7. <https://doi.org/10.1093/nar/gkaa1011>.
28. D'Souza AR, Minczuk M. Mitochondrial transcription and translation: overview. *Essays Biochem.* 2018;62(3):309–20. <https://doi.org/10.1042/EBC20170102>.
29. Demine S, Reddy N, Renard P, Raes M, Arnould T. Unraveling biochemical pathways affected by mitochondrial dysfunctions using metabolomic approaches. *Meta.* 2014;4(3):831–78. <https://doi.org/10.3390/metabo4030831>.
30. Wallace DC, Fan W, Procaccio V. Mitochondrial energetics and therapeutics. *Annu Rev Pathol.* 2010;5:297–348. <https://doi.org/10.1146/annurev.pathol.4.110807.092314>.
31. Dimroth P, Kaim G, Matthey U. Crucial role of the membrane potential for ATP synthesis by F(1)F(o) ATP synthases. *J Exp Biol.* 2000;203(1):51–9. <https://doi.org/10.1242/jeb.203.1.51>.
32. Mitchell P, Moyle J. Chemiosmotic hypothesis of oxidative phosphorylation. *Nature.* 1967;213(5072):137. <https://doi.org/10.1038/213137a0>.
33. Watmough NJ, Frerman FE. The electron transfer flavoprotein: ubiquinone oxidoreductases. *Biochim Biophys Acta.* 2010;1797(12):1910–6. <https://doi.org/10.1016/j.bbabi.2010.10.007>.
34. Divakaruni AS, Brand MD. The regulation and physiology of mitochondrial proton leak. *Physiology (Bethesda).* 2011;26(3):192–205. <https://doi.org/10.1152/physiol.00046.2010>.
35. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev.* 2004;84(1):277–359. <https://doi.org/10.1152/physrev.00015.2003>.

36. Mailloux RJ, Harper M-E. Uncoupling proteins and the control of mitochondrial reactive oxygen species production. *Free Radic Biol Med.* 2011;51(6):1106–15. <https://doi.org/10.1016/j.freeradbiomed.2011.06.022>.
37. Brand MD, Esteves TC. Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. *Cell Metab.* 2005;2(2):85–93. <https://doi.org/10.1016/j.cmet.2005.06.002>.
38. Nicholls DG. Mitochondrial proton leaks and uncoupling proteins. *Biochim Biophys Acta.* 2021;1862(7):148428. <https://doi.org/10.1016/j.bbabo.2021.148428>.
39. Bertholet AM, Chouchani ET, Kazak L, Angelin A, Fedorenko A, Long JZ, Vidoni S, Garrity R, Cho J, Terada N, Wallace DC, Spiegelman BM, Kirichok Y. H⁺ transport is an integral function of the mitochondrial ADP/ATP carrier. *Nature.* 2019;571(7766):515–20. <https://doi.org/10.1038/s41586-019-1400-3>.
40. Bevilacqua L, Seifert EL, Estey C, Gerrits MF, Harper M-E. Absence of uncoupling protein-3 leads to greater activation of an adenine nucleotide translocase-mediated proton conductance in skeletal muscle mitochondria from calorie restricted mice. *Biochim Biophys Acta.* 2010;1797(8):1389–97. <https://doi.org/10.1016/j.bbabo.2010.02.018>.
41. Echtay KS, Esteves TC, Pakay JL, Jekabsons MB, Lambert AJ, Portero-Otín M, Pamplona R, Vidal-Puig AJ, Wang S, Roebuck SJ, Brand MD. A signalling role for 4-hydroxy-2-nonenal in regulation of mitochondrial uncoupling. *EMBO J.* 2003;22(16):4103–10. <https://doi.org/10.1093/emboj/cdg412>.
42. Klumpe I, Savvatis K, Westermann D, Tschöpe C, Rauch U, Landmesser U, Schultheiss H-P, Dörner A. Transgenic overexpression of adenine nucleotide translocase 1 protects ischemic hearts against oxidative stress. *J Mol Med (Berl).* 2016;94(6):645–53. <https://doi.org/10.1007/s00109-016-1413-4>.
43. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxidative Med Cell Longev.* 2014;2014:e360438. <https://doi.org/10.1155/2014/360438>.
44. Cadet J, Davies KJA, Medeiros MH, Di Mascio P, Wagner JR. Formation and repair of oxidatively generated damage in cellular DNA. *Free Radic Biol Med.* 2017;107:13–34. <https://doi.org/10.1016/j.freeradbiomed.2016.12.049>.
45. Davies MJ. Singlet oxygen-mediated damage to proteins and its consequences. *Biochem Biophys Res Commun.* 2003;305(3):761–70. [https://doi.org/10.1016/S0006-291X\(03\)00817-9](https://doi.org/10.1016/S0006-291X(03)00817-9).
46. Christofidou-Solomidou M, Muzykantov VR. Antioxidant strategies in respiratory medicine. *Treat Respir Med.* 2006;5(1):47–78. <https://doi.org/10.2165/00151829-200605010-00004>.
47. Holzerová E, Prokisch H. Mitochondria: much ado about nothing? How dangerous is reactive oxygen species production? *Int J Biochem Cell Biol.* 2015;63:16–20. <https://doi.org/10.1016/j.biocel.2015.01.021>.
48. Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J.* 2009;417(Pt 1):1–13. <https://doi.org/10.1042/BJ20081386>.
49. Shan Y, Schoenfeld RA, Hayashi G, Napoli E, Akiyama T, Iodi Carstens M, Carstens EE, Pook MA, Cortopassi GA. Frataxin deficiency leads to defects in expression of antioxidants and Nrf2 expression in dorsal root ganglia of the Friedreich's ataxia YG8R mouse model. *Antioxid Redox Signal.* 2013;19(13):1481–93. <https://doi.org/10.1089/ars.2012.4537>.
50. Sulaiman SA, Mohd Rani Z, Mohd Radin FZ, Abdul Murad NA. Advancement in the diagnosis of mitochondrial diseases. *J Transl Genet Genom.* 2020;4(3):159–87. <https://doi.org/10.20517/jtgg.2020.27>.
51. Chinnery PF. Primary mitochondrial disorders overview. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJ, Gripp KW, Amemiya A, editors. *GeneReviews*®. Seattle: University of Washington; 1993; <http://www.ncbi.nlm.nih.gov/books/NBK1224/>.
52. Khajuria K, Khajuria V, Sawhney V. SECONDARY MITOCHONDRIAL DYSFUNCTION. *Int J Pharm Pharm Sci.* 2021;14:14–9. <https://doi.org/10.22159/ijpps.2021v13i3.40335>.

53. Niyazov DM, Kahler SG, Frye RE. Primary mitochondrial disease and secondary mitochondrial dysfunction: importance of distinction for diagnosis and treatment. *Mol Syndromol*. 2016;7(3):122–37. <https://doi.org/10.1159/000446586>.
54. Rötig A, Cormier V, Blanche S, Bonnefont JP, Ledesne F, Romero N, Schmitz J, Rustin P, Fischer A, Saudubray JM. Pearson's marrow-pancreas syndrome. A multisystem mitochondrial disorder in infancy. *J Clin Invest*. 1990;86(5):1601–8. <https://doi.org/10.1172/JCI114881>.
55. Schlieben LD, Prokisch H. The dimensions of primary mitochondrial disorders. *Front Cell Dev Biol*. 2020;8:1441. <https://doi.org/10.3389/fcell.2020.600079>.
56. Skladal D, Halliday J, Thorburn DR. Minimum birth prevalence of mitochondrial respiratory chain disorders in children. *Brain*. 2003;126(8):1905–12. <https://doi.org/10.1093/brain/awg170>.
57. Gorman GS, Schaefer AM, Ng Y, Gomez N, Blakely EL, Alston CL, Feeney C, Horvath R, Yu-Wai-Man P, Chinnery PF, Taylor RW, Turnbull DM, McFarland R. Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. *Ann Neurol*. 2015;77(5):753–9. <https://doi.org/10.1002/ana.24362>.
58. Parikh S, Goldstein A, Koenig MK, Scaglia F, Enns GM, Saneto R, Anselm I, Cohen BH, Falk MJ, Greene C, Gropman AL, Haas R, Hirano M, Morgan P, Sims K, Tarnopolsky M, Van Hove JLK, Wolfe L, DiMauro S. Diagnosis and management of mitochondrial disease: a consensus statement from the mitochondrial medicine society. *Genet Med*. 2015;17(9):689–701. <https://doi.org/10.1038/gim.2014.177>.
59. Rodenburg RJT. Biochemical diagnosis of mitochondrial disorders. *J Inher Metab Dis*. 2011;34(2):283–92. <https://doi.org/10.1007/s10545-010-9081-y>.
60. Al Aqeel AI, Rashid MS, Pn Ruiter J, Ijlst L, Ja Wanders R. A novel molecular defect of the carnitine acylcarnitine translocase gene in a Saudi patient. *Clin Genet*. 2003;64(2):163–5. <https://doi.org/10.1034/j.1399-0004.2003.00117.x>.
61. Al-Hassnan ZN, Al-Dosary M, Alfadhel M, Faqeh EA, Alsagob M, Kenana R, Almass R, Al-Harazi OS, Al-Hindi H, Malibari OI, Almutari FB, Tulbah S, Alhadeq F, Al-Sheddi T, Alamro R, AlAsmari A, Almutashri M, Alshaalan H, Al-Mohanna FA, Kaya N. ISCA2 mutation causes the infantile neurodegenerative mitochondrial disorder. *J Med Genet*. 2015;52(3):186–94. <https://doi.org/10.1136/jmedgenet-2014-102592>.
62. Baertling F, Al-Murshedi F, Sánchez-Caballero L, Al-Senaidi K, Joshi NP, Venselaar H, van den Brand MA, Nijtmans LG, Rodenburg RJ. Mutation in mitochondrial complex IV subunit COX5A causes pulmonary arterial hypertension, lactic acidemia, and failure to thrive. *Hum Mutat*. 2017;38(6):692–703. <https://doi.org/10.1002/humu.23210>.
63. Boycott KM, Beaulieu CL, Kernohan KD, Gebril OH, Mhanni A, Chudley AE, Redl D, Qin W, Hampson S, Küry S, Tetreault M, Puffenberger EG, Scott JN, Bezieau S, Reis A, Uebe S, Schumacher J, Hegele RA, McLeod DR, Abou Jamra R. Autosomal-recessive intellectual disability with cerebellar atrophy syndrome caused by mutation of the manganese and zinc transporter gene SLC39A8. *Am J Hum Genet*. 2015;6(97):886–93. <https://doi.org/10.1016/j.ajhg.2015.11.002>.
64. Eldomery MK, Akdemir ZC, Vögtle F-N, Charng W-L, Mulica P, Rosenfeld JA, Gambin T, Gu S, Burrage LC, Al Shamsi A, Penney S, Jhangiani SN, Zimmerman HH, Muzny DM, Wang X, Tang J, Medikonda R, Ramachandran PV, Wong L-J, Sutton VR. MIPEP recessive variants cause a syndrome of left ventricular non-compaction, hypotonia, and infantile death. *Genome Med*. 2016;8(1):106. <https://doi.org/10.1186/s13073-016-0360-6>.
65. Haack TB, Kopajtich R, Freisinger P, Wieland T, Rorbach J, Nicholls TJ, Baruffini E, Walther A, Danhauser K, Zimmermann FA, Husain RA, Schum J, Mundy H, Ferrero I, Strom TM, Meitinger T, Taylor RW, Minczuk M, Mayr JA, Prokisch H. ELAC2 mutations cause a mitochondrial RNA processing defect associated with hypertrophic cardiomyopathy. *Am J Hum Genet*. 2013;93(2):211–23. <https://doi.org/10.1016/j.ajhg.2013.06.006>.
66. Hartmann B, Wai T, Hu H, MacVicar T, Musante L, Fischer-Zirnsak B, Stenzel W, Gräf R, van den Heuvel L, Ropers H-H, Wienker TF, Hübner C, Langer T, Kaindl AM. Homozygous

- YME1L1 mutation causes mitochondriopathy with optic atrophy and mitochondrial network fragmentation. *elife*. n.d.;5:e16078. <https://doi.org/10.7554/eLife.16078>.
67. Heberle LC, Tawari AAA, Ramadan DG, Ibrahim JK. Ethylmalonic encephalopathy—report of two cases. *Brain Dev*. 2006;28(5):329–31. <https://doi.org/10.1016/j.braindev.2005.10.005>.
 68. Jobling RK, Assoum M, Gakh O, Blaser S, Raiman JA, Mignot C, Roze E, Dürr A, Brice A, Lévy N, Prasad C, Paton T, Paterson AD, Roslin NM, Marshall CR, Desvignes J-P, Roëckel-Trevisiol N, Scherer SW, Rouleau GA, Yoon G. PMPCA mutations cause abnormal mitochondrial protein processing in patients with non-progressive cerebellar ataxia. *Brain*. 2015;138(6):1505–17. <https://doi.org/10.1093/brain/awv057>.
 69. Malicdan MCV, Vilboux T, Ben-Zeev B, Guo J, Eliyahu A, Podesh-Shakked B, Dori A, Kakani S, Chandrasekharappa SC, Ferreira C, Shelestovich N, Marek-Yagel D, Pri-Chen H, Blatt I, Niederhuber JE, He L, Toro C, Taylor RW, Deeken J, Anikster Y. A novel inborn error of the coenzyme Q10 biosynthesis pathway: cerebellar ataxia and static encephalomyopathy due to COQ5 C-methyltransferase deficiency. *Hum Mutat*. 2018;39(1):69–79. <https://doi.org/10.1002/humu.23345>.
 70. Ramadan DG, Head RA, Al-Tawari A, Habeeb Y, Zaki M, Al-Ruqum F, Besley GTN, Wraith JE, Brown RM, Brown GK. Lactic acidosis and developmental delay due to deficiency of E3 binding protein (protein X) of the pyruvate dehydrogenase complex. *J Inher Metab Dis*. 2004;27(4):477–85. <https://doi.org/10.1023/B:BOLL.0000037336.91549.44>.
 71. Shamseldin HE, Alasmari A, Salih MA, Samman MM, Mian SA, Alshidi T, Ibrahim N, Hashem M, Faqeih E, Al-Mohanna F, Alkuraya FS. A null mutation in MICU2 causes abnormal mitochondrial calcium homeostasis and a severe neurodevelopmental disorder. *Brain*. 2017;140(11):2806–13. <https://doi.org/10.1093/brain/awx237>.
 72. Shamseldin HE, Smith LL, Kentab A, Alkhalidi H, Summers B, Alsedairy H, Xiong Y, Gupta VA, Alkuraya FS. Mutation of the mitochondrial carrier SLC25A42 causes a novel form of mitochondrial myopathy in humans. *Hum Genet*. 2016;135(1):21–30. <https://doi.org/10.1007/s00439-015-1608-8>.
 73. Spinazzola A, Santer R, Akman OH, Tsiakas K, Schaefer H, Ding X, Karadimas CL, Shanske S, Ganesh J, Di Mauro S, Zeviani M. Hepatocerebral form of mitochondrial DNA depletion syndrome: novel MPV17 mutations. *Arch Neurol*. 2008;65(8):1108–13. <https://doi.org/10.1001/archneur.65.8.1108>.
 74. Yavuz H, Bertoli-Avella AM, Alfadhel M, Al-Sannaa N, Kandaswamy KK, Al-Tuwaijri W, Rolfs A, Brandau O, Bauer P. A founder nonsense variant in NUDT2 causes a recessive neurodevelopmental disorder in Saudi Arab children. *Clin Genet*. 2018;94(3–4):393–5. <https://doi.org/10.1111/cge.13386>.
 75. Ferguson E. The strengths and weaknesses of whole-genome sequencing; 2020. p. 3.
 76. Petersen B-S, Fredrich B, Hoepfner MP, Ellinghaus D, Franke A. Opportunities and challenges of whole-genome and -exome sequencing. *BMC Genet*. 2017;18(1):14. <https://doi.org/10.1186/s12863-017-0479-5>.
 77. Nikkanen J, Forsström S, Euro L, Paetau I, Kohnz RA, Wang L, Chilov D, Viinamäki J, Roivainen A, Marjamäki P, Liljenbäck H, Ahola S, Buzkova J, Terzioglu M, Khan NA, Pirnes-Karhu S, Paetau A, Lönnqvist T, Sajantila A, Suomalainen A. Mitochondrial DNA replication defects disturb cellular dNTP pools and remodel one-carbon metabolism. *Cell Metab*. 2016;23(4):635–48. <https://doi.org/10.1016/j.cmet.2016.01.019>.
 78. Reinecke CJ, Koekemoer G, van der Westhuizen FH, Louw R, Lindeque JZ, Mienie LJ, Smuts I. Metabolomics of urinary organic acids in respiratory chain deficiencies in children. *Metabolomics*. 2012;8(2):264–83. <https://doi.org/10.1007/s11306-011-0309-0>.
 79. Smuts I, van der Westhuizen FH, Louw R, Mienie LJ, Engelke UFH, Wevers RA, Mason S, Koekemoer G, Reinecke CJ. Disclosure of a putative biosignature for respiratory chain disorders through a metabolomics approach. *Metabolomics*. 2013;9(2):379–91. <https://doi.org/10.1007/s11306-012-0455-z>.
 80. Legault JT, Strittmatter L, Tardif J, Sharma R, Tremblay-Vaillancourt V, Aubut C, Boucher G, Clish CB, Cyr D, Daneault C, Waters PJ. A metabolic signature of mitochondrial dysfunction

- revealed through a monogenic form of Leigh syndrome. *Cell Rep.* 2015;13(5):981–9. <https://doi.org/10.1016/j.celrep.2015.09.054>.
81. Sharma R, Reinstadler B, Engelstad K, Skinner OS, Stackowitz E, Haller RG, Clish CB, Pierce K, Walker MA, Fryer R, Oglesbee D, Mao X, Shungu DC, Khatri A, Hirano M, De Vivo DC, Mootha VK. Circulating markers of NADH-reductive stress correlate with mitochondrial disease severity. *J Clin Invest.* 2021;131(2):136055. <https://doi.org/10.1172/JCI136055>.
 82. Hall AM, Vilasi A, Garcia-Perez I, Lapsley M, Alston CL, Pitceathly RDS, McFarland R, Schaefer AM, Turnbull DM, Beaumont NJ, Hsuan JJ, Cutillas PR, Lindon JC, Holmes E, Unwin RJ, Taylor RW, Gorman GS, Rahman S, Hanna MG. The urinary proteome and metabolome differ from normal in adults with mitochondrial disease. *Kidney Int.* 2015;87(3):610–22. <https://doi.org/10.1038/ki.2014.297>.
 83. Chao de la Barca JM, Simard G, Amati-Bonneau P, Safiedeen Z, Prunier-Mirebeau D, Chupin S, Gavras C, Tessier L, Gueguen N, Chevrollier A, Desquirit-Dumas V, Ferré M, Bris C, Kouassi Nzoughe J, Bocca C, Leruez S, Verny C, Miléa D, Bonneau D, Reynier P. The metabolomic signature of Leber’s hereditary optic neuropathy reveals endoplasmic reticulum stress. *Brain.* 2016;139(11):2864–76. <https://doi.org/10.1093/brain/aww222>.
 84. Sandler Y, Mercier K, Pathmasiri W, Carlson J, McRitchie S, Sumner S, Vernon HJ. Metabolomics reveals new mechanisms for pathogenesis in Barth syndrome and introduces novel roles for Cardiolipin in cellular function. *PLoS One.* 2016;11(3):e0151802. <https://doi.org/10.1371/journal.pone.0151802>.
 85. Semeraro M, Boenzi S, Carozzo R, Diodato D, Martinelli D, Olivieri G, Antonetti G, Sacchetti E, Catesini G, Rizzo C, Dionisi-Vici C. The urinary organic acids profile in single large-scale mitochondrial DNA deletion disorders. *Clin Chim Acta.* 2018;481:156–60. <https://doi.org/10.1016/j.cca.2018.03.002>.
 86. Esterhuizen K, Lindeque JZ, Mason S, van der Westhuizen FH, Rodenburg RJ, de Laat P, Smeitink JAM, Janssen MCH, Louw R. One mutation, three phenotypes: novel metabolic insights on MELAS, MIDD and myopathy caused by the m.3243A>G mutation. *Metabolomics.* 2021;17(1):10. <https://doi.org/10.1007/s11306-020-01769-w>.
 87. Stoessel D, Schulte C, Teixeira dos Santos MC, Scheller D, Rebollo-Mesa I, Deuschle C, Walther D, Schauer N, Berg D, Nogueira da Costa A, Maetzler W. Promising metabolite profiles in the plasma and CSF of early clinical Parkinson’s disease. *Front Aging Neurosci.* 2018;10:51. <https://doi.org/10.3389/fnagi.2018.00051>.
 88. Luan H, Liu L-F, Meng N, Tang Z, Chua K-K, Chen L-L, Song J-X, Mok VCT, Xie L-X, Li M, Cai Z. LC–MS-based urinary metabolite signatures in idiopathic Parkinson’s disease. *J Proteome Res.* 2015;14(1):467–78. <https://doi.org/10.1021/pr500807t>.
 89. Ibáñez C, Simó C, Martín-Álvarez PJ, Kivipelto M, Winblad B, Cedazo-Mínguez A, Cifuentes A. Toward a predictive model of Alzheimer’s disease progression using capillary electrophoresis–mass spectrometry metabolomics. *Anal Chem.* 2012;84(20):8532–40. <https://doi.org/10.1021/ac301243k>.
 90. Scolamiero E, Cozzolino C, Albano L, Ansalone A, Caterino M, Corbo G, di Girolamo MG, Di Stefano C, Durante A, Franzese G, Franzese I, Gallo G, Giliberti P, Ingenito L, Ippolito G, Malamisura B, Mazzeo P, Norma A, Ombrone D, Ruoppolo M. Targeted metabolomics in the expanded newborn screening for inborn errors of metabolism. *Mol Biosyst.* 2015;11(6):1525–35. <https://doi.org/10.1039/c4mb00729h>.
 91. Jacob M, Malkawi A, Albast N, Al Bougha S, Lopata A, Dasouki M, Abdel Rahman AM. A targeted metabolomics approach for clinical diagnosis of inborn errors of metabolism. *Anal Chim Acta.* 2018;1025:141–53. <https://doi.org/10.1016/j.aca.2018.03.058>.
 92. Salihovic S, Broeckling CD, Ganna A, Prenni JE, Sundström J, Berne C, Lind L, Ingelsson E, Fall T, Ärnlöv J, Nowak C. Non-targeted urine metabolomics and associations with prevalent and incident type 2 diabetes. *Sci Rep.* 2020;10(1):16474. <https://doi.org/10.1038/s41598-020-72456-y>.

93. Gu X, Al Dubayee M, Alshahrani A, Masood A, Benabdelkamel H, Zahra M, Li L, Abdel Rahman AM, Aljada A. Distinctive metabolomics patterns associated with insulin resistance and type 2 diabetes mellitus. *Front Mol Biosci.* 2020;7:411. <https://doi.org/10.3389/fmolb.2020.609806>.
94. Bu Y, Wang H, Ma X, Han C, Jia X, Zhang J, Liu Y, Peng Y, Yang M, Yu K, Wang C. Untargeted Metabolomic profiling of the correlation between prognosis differences and PD-1 expression in sepsis: a preliminary study. *Front Immunol.* 2021;12:740. <https://doi.org/10.3389/fimmu.2021.594270>.
95. Lin S-H, Fan J, Zhu J, Zhao Y-S, Wang C-J, Zhang M, Xu F. Exploring plasma metabolomic changes in sepsis: a clinical matching study based on gas chromatography–mass spectrometry. *Ann Transl Med.* 2020;8(23):1568. <https://doi.org/10.21037/atm-20-3562>.
96. Wang J, Sun Y, Teng S, Li K. Prediction of sepsis mortality using metabolite biomarkers in the blood: a meta-analysis of death-related pathways and prospective validation. *BMC Med.* 2020;18(1):83. <https://doi.org/10.1186/s12916-020-01546-5>.
97. Cavus E, Karakas M, Ojeda FM, Kontto J, Veronesi G, Ferrario MM, Linneberg A, Jørgensen T, Meisinger C, Thorand B, Iacoviello L, Börnigen D, Woodward M, Schnabel R, Costanzo S, Tunstall-Pedoe H, Koenig W, Kuulasmaa K, Salomaa V, BiomarCaRE consortium. Association of circulating metabolites with risk of coronary heart disease in a European population: results from the biomarkers for cardiovascular risk assessment in Europe (BiomarCaRE) consortium. *JAMA Cardiol.* 2019;4(12):1270–9. <https://doi.org/10.1001/jamacardio.2019.4130>.
98. Griffin JL, Atherton H, Shockcor J, Atzori L. Metabolomics as a tool for cardiac research. *Nat Rev Cardiol.* 2011;8(11):630–43. <https://doi.org/10.1038/nrcardio.2011.138>.
99. Debik J, Euceda LR, Lundgren S, Gythfeldt HVDL, Garred Ø, Borgen E, Engebraaten O, Bathen TF, Giskeødegård GF. Assessing treatment response and prognosis by serum and tissue metabolomics in breast cancer patients. *J Proteome Res.* 2019;18(10):3649–60. <https://doi.org/10.1021/acs.jproteome.9b00316>.
100. Feng LR, Barb JJ, Regan J, Saligan LN. Plasma metabolomic profile associated with fatigue in cancer patients. *Cancer Med.* 2021;10(5):1623–33. <https://doi.org/10.1002/cam4.3749>.
101. Guida F, Tan VY, Corbin LJ, Smith-Byrne K, Alcalá K, Langenberg C, Stewart ID, Butterworth AS, Surendran P, Achaintre D, Adamski J, Amiano P, Bergmann MM, Bull CJ, Dahm CC, Gicquiau A, Giles GG, Gunter MJ, Haller T, Johansson M. The blood metabolome of incident kidney cancer: a case–control study nested within the MetKid consortium. *PLoS Med.* 2021;18(9):e1003786. <https://doi.org/10.1371/journal.pmed.1003786>.
102. Wojakowska A, Chekan M, Widlak P, Pietrowska M. Application of metabolomics in thyroid cancer research. *Int J Endocrinol.* 2015;2015:e258763. <https://doi.org/10.1155/2015/258763>.