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Primer on mitochondrial disease: Biochemistry, genetics, and epidemiology

Primary diseases of the mitochondria are caused by mutations in both mitochondrial DNA and nuclear DNA, and can affect members of the same family in unexpectedly diverse ways.

ABSTRACT: Mitochondria are intracellular organelles that provide energy for cellular activities through a series of chemical reactions known as oxidative phosphorylation. The DNA inside mitochondria is inherited from the mother and is distinct from DNA inside the nucleus in its genetic code. The central role of mitochondria in cellular function means that dysfunction of mitochondria in any organ system can lead to widely disparate clinical presentations, as illustrated by Family A, a large family in British Columbia. Primary mitochondrial diseases are relatively common and affect up to 1 in 5000 people. Mitochondrial dysfunction secondary to other diseases is even more common and plays a role in Alzheimer disease and Parkinson disease and is also involved in the aging process. Treatment strategies are based on understanding the biochemistry of mitochondrial activity and can be applied in cases of both primary and secondary mitochondrial dysfunction.

This article has been peer reviewed.

Mitochondria are intracellular organelles that make energy through processes requiring oxygen. Primordial eukaryotic cells were initially anaerobic before they developed a symbiotic relationship with bacteria that could use oxygen. These bacteria eventually evolved into the mitochondria of human cells. Today, the genetic control of mitochondria comes from both nuclear DNA (inherited in a mendelian fashion) and separate DNA within the mitochondria (inherited from the mother). An understanding of the genetic control of mitochondria as well as the basic structure and biochemistry of aerobic metabolism (known as oxidative phosphorylation) can help clinicians understand the presentation of patients with mitochondrial diseases and their treatment. The study of Family A (Figure 1), discussed here and elsewhere in the theme issue, shows that very diverse presentations of disease are possible.

Mitochondrial structure and biochemistry

An excellent review of mitochondrial structure and biochemistry is provided by DiMauro and Schon,[1] who explain that mitochondria are bound by two membranes and perform numer-ous tasks in three different areas of the organelle:

• The inner mitochondrial membrane.
• The area between the two membranes.
• The matrix or mitochondrial "cytoplasm."

A series of protein complexes (designated as complex I to V and collectively called the electron transport chain or ETC or respiratory chain) are bound to the inner mitochondrial membrane and play a central role in the mitochondrial respiratory chain. These complexes produce adenosine triphosphate (ATP) through electron transfer using reduced nicotinamide adenine dinucleotide (NADH) and

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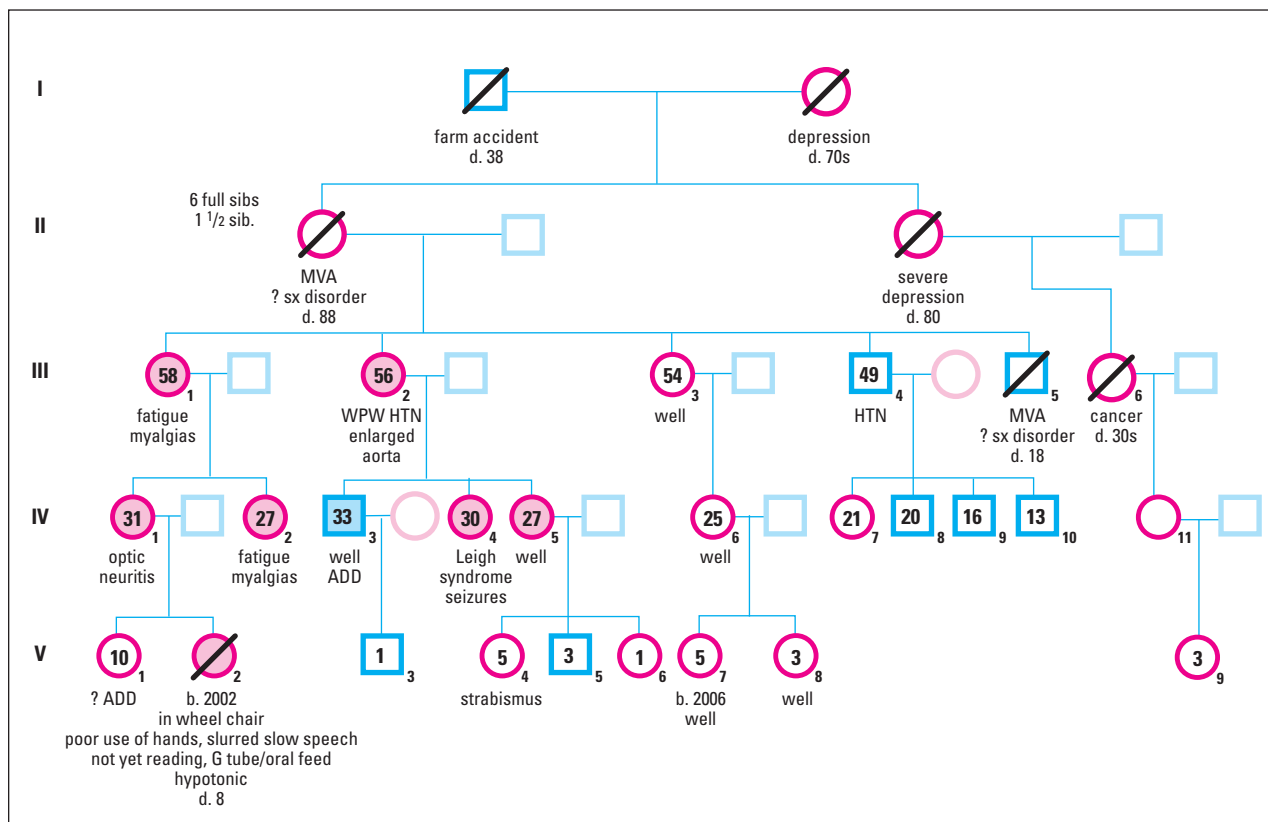
Figure 1. Family A pedigree. A familial pathogenic mutation in mitochondrial DNA was first identified in patient IV-4. Affected members of this large BC family exhibit symptoms ranging from mild to severe.

flavin adenine dinucleotide (FADH2) as sources of electrons. This process is shown in the top (A) portion of Figure 2 . These protein complexes require a number of cofactors to function, including ubiquinone (also known as coenzyme Q10 or CoQ) and cytochrome *c* (Cyto *c*). The generation of ATP through oxidative phosphorylation results in a high concentration of free oxygen radicals within the mitochondrion, making antioxidant compounds one potential therapeutic modality for mitochondrial diseases.[2]

Both NADH and FADH2 are needed for ATP production and are derived from two energy substrates, carbohydrate and fat. Enzymes involved in the transport of fatty acids through the two mitochondrial membranes include car-

nitine palmitoyltransferase types 1 and 2, which require carnitine as a cofactor. In the mitochondrial matrix, another group of enzymes involved in the beta oxidation of fatty acids generates FADH2. After carbohydrate is anaerobically metabolized to pyruvate, the pyruvate is transported into the mitochondrial matrix, where it is converted to acetyl-CoA in a reaction that requires thiamine as a cofactor. The acetyl-CoA is then metabolized through the enzymes of the tricarboxylic acid cycle, which generates NADH for the ETC.[1]

The following points about mitochondrial structure and biochemistry are key:
• The system is complex, so there are many areas where it can go wrong.

• Defects in one part of the system can cause different symptoms than defects in other parts.
• Therapeutic intervention might work at a variety of points in the biochemical process.

Mitochondrial genetics

Unlike nuclear DNA(nDNA), mitochondrial DNA (mtDNA) is a small, circular DNA molecule of 16 569 base pairs (Figure 3). Because mitochondria were originally derived from bacteria, the DNA inside the mitochondria differs from the DNA inside the nucleus[1,3] in several important ways:
• The genetic code by which nucleotide sequences are translated into amino acids in mtDNA is unlike the code in nDNA.
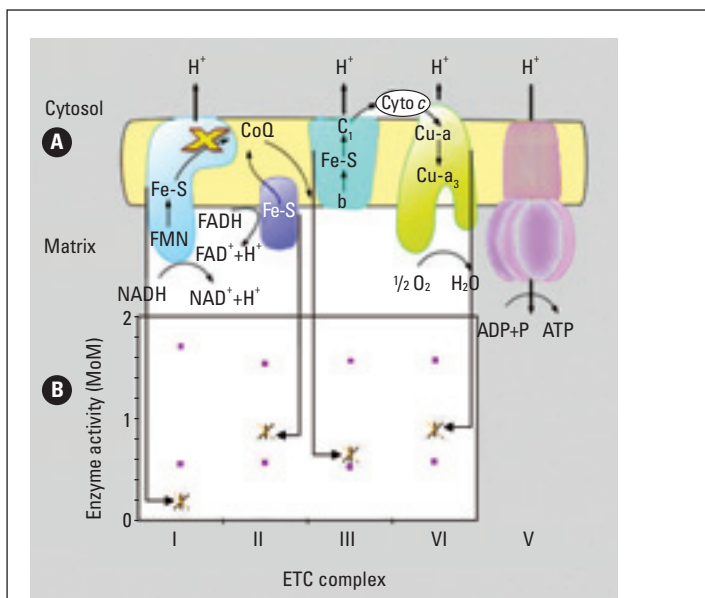
Figure 2. Mitochondrial respiratory chain showing dysfunction.
(A) The five complexes of the respiratory electron transport chain (ETC) are aligned from left to right along the inner mitochondrial membrane. The top portion of the figure depicts the normal flow of electrons from complex I to III to IV, or from complex II to III to IV. In either case, the transfer of electrons is facilitated by electron carriers CoQ and Cyto *c*. The transfer of electrons is down a redox gradient toward molecular oxygen associated with complex IV. The energy release associated with this electron transfer is utilized to transport hydrogen ions across the inner mitochondrial membrane (out of the mitochondrial matrix). The hydrogen ions then have a potential energy, which is subsequently released as the ions flow back into the mitochondrial matrix through complex V. Complex V utilizes this released energy to make ATP from ADP and inorganic phosphate. (B) The bottom portion of the figure illustrates how this process is deficient in the case of patient IV-4 from Family A. The enzyme activity, measured on a frozen skeletal muscle biopsy specimen, confirms the patient has a mitochondrial respiratory chain disorder. The normal enzyme activities are represented by the pink squares in terms of multiples of the normal median (MoM). The descending arrows highlight the patient's enzyme activity measurements (small Xs) for the representative complexes and show that only complex I is deficient, in keeping with the location of the mutant protein as depicted by an X in 2A.
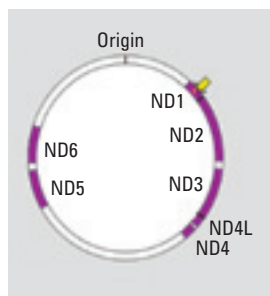

Figure 3. Mitochondrial DNA ring showing site of mutation affecting Family A. The seven mtDNA genes that encode select subunits of complex I are highlighted in purple. The arrow points to the site of the familial DNA mutation. Importantly, this mutation occurs at position 3697, which is within a critical sequence of the ND1 gene. The yellow X on the complex I subunit in Figure 2A marks the site of the mutant ND1 protein, which prevents the normal interaction of the CoQ electron acceptor with the complex I enzyme, resulting in abnormal complex I activity and, ultimately, deficient ATP synthesis.

Figures 2–3.

- The mitochondria in the zygote are derived from the ovum because mitochondria in the sperm are located in the tail region and do not enter the ovum at fertilization. There is evidence that any sperm-derived mitochondria that happen to enter the ovum are actively degraded. Thus, mtDNA is maternally inherited and does not segregate in a mendelian fashion.
- There are many copies of mtDNA in each mitochondrion (as high as 10) and most mature cells have in the order of 100 000 mitochondria. During cell division, mitochondria and this mtDNA are randomly segregated to the daughter cells. Thus, when a mutagenic stimulus hits the cell, not all copies of the mtDNA are affected, meaning that some copies will carry a mutation and other copies will not. This phenomenon where not all of the mtDNA copies are the same is known as heteroplasmy. The degree of heteroplasmy will differ from person to person within a family. The degree of heteroplasmy within a person may also differ from organ system to organ system and even cell to cell, leading to a vast array of possible phenotypes.
- There are no known DNA repair mechanisms for mtDNA resembling the repair mechanisms in nDNA. Thus, once a mutation occurs in the mtDNA it is permanent.
- Because not all of the mtDNA copies will carry a pathogenic mutation, the degree to which mitochondrial function in a cell or an organ is impaired by the mutation will vary with the degree of heteroplasmy. This leads to a phenomenon known as the threshold effect, whereby symptoms may only arise in those organs where the degree of heteroplasmy is sufficient to significantly impair mitochondrial function. The threshold is in itself variable in that some tissues and organs (such as muscle and brain) require more energy than others. For example, a patient may have a pathogenic mutation affecting 90% of his mtDNA in his skeletal muscle and present with profound muscle weakness. That same patient may have only 60% of his mtDNA affected by the mutation in the liver and have no clinically apparent liver involvement. However, that patient may have a sibling in whom 95% of the hepatic mtDNA is affected by the mutation and that sibling may present with liver failure and have no apparent muscle disease despite having the same mutation.

• Not all of the mitochondrial proteins involved in oxidative phosphorylation are encoded by the mtDNA. The mtDNA codes for only 37 genes, of which 13 represent protein subunits, and yet it is estimated that close to 1000 proteins are required for normal mitochondrial function.[1] Although the mtDNA is an important site of pathology in patients with mitochondrial defects, nDNA defects represent an even larger subgroup and are transmitted in mendelian fashion.[4]

Genetics thus indicate there are two genomes involved in making mitochondria work, mtDNA and nDNA. Inheritance patterns can range from autosomal recessive to autosomal dominant to maternal inheritance. Due to the phenomena of heteroplasmy and the threshold effect, the same mutation can present with completely different manifestations (or no manifestations at all) in different people.

Epidemiology of mitochondrial diseases

Primary diseases of the mitochondria are caused by both mtDNA mutations and nDNA mutations. (Secondary mitochondrial dysfunction is even more common than primary mitochondrial disease and is addressed more fully in the three articles that follow.) Primary disorders of the respiratory chain affect up to 1 in 5000 people and are much more common than previously realized.[4-6] Given the large number of nuclear genes involved in mitochondrial function, it is estimated that up to 1 in 5 individuals carry nuclear gene mutations, most of which are recessive so that heterozygosity for the mutation does not translate into clinical symptoms.[4] Thus, as a group, primary mitochondrial diseases are much more common than other genetic diseases such as cystic fibrosis (1 in 31 000), even though these less common diseases are more familiar to primary care providers.

Genetic counseling

Providing genetic counseling for families when mitochondrial disease has been identified in some members is complex and requires knowledge of the site of the mutation (nuclear DNA or mitochondrial DNA), the pathogenic significance of the mutation (if known), the degree of heteroplasmy in symptomatic individuals, and other variables.[7] In many cases, information about these variables may not be known. Also, in the case of mutations in mitochondrial DNA, some important variables such as the degree of heteroplasmy will influence the clinical presentation of the disease. The degree of heteroplasmy may not be known if the family is presenting for preconception counseling. In most families, the mutation causing the mitochondrial disease is not identified, making counseling about recurrence risks in future pregnancies and the potential of prenatal diagnosis even more difficult. In cases where it is highly likely that the DNA defect is nuclear in origin and where a biochemical deficiency of a complex can be identified in fibroblast culture from an affected family member, prenatal diagnosis may be possible through measuring the activity of the mitochondrial enzyme complexes in specimins derived from chorionic villus sampling or amniocentesis.[7] False negative results can occur, however, as some mitochondrial defects that cause organ dysfunction in other tissues may still have high residual activity in tissues that are amenable to prenatal sampling.[7] Given the complexity of the genetic counseling issues, it is recommended that all families diagnosed with mitochondrial disease be referred to an expert for appropriate counseling.

Figure 4. MRI of the adult brain of patient IV-4, the Family A index patient. The arrows on this axial T2W image indicate characteristic bilateral areas of necrosis within the basal ganglia. The symmetrical necrosis of the basal ganglia is typical of Leigh syndrome.
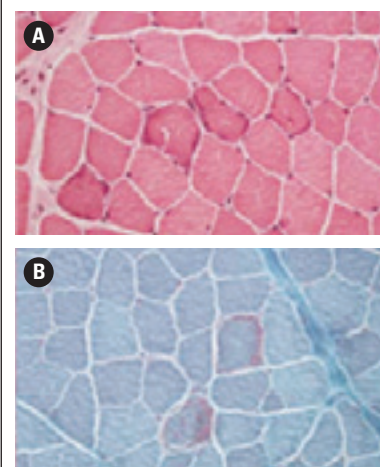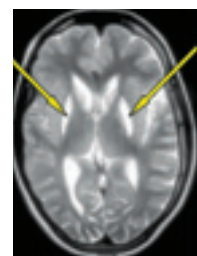

Figure 5. Histopathology results from a patient with mitochondrial disease. These laboratory images are similar to those originally obtained but no longer available for patient IV-4. The ragged-red fibres are shown using (A) basophilic granular staining of four fibres with hematoxylin and eosin stain and (B) red staining with modified Gomori trichrome stain. Images courtesy of Dr Glenda Hendson.

Figures 4–5.

Family A

Family A is a large family whose story can help doctors trying to learn about the very diverse presentations of mitochondrial disease. The index case was patient IV-4, who was diagnosed with progressive neurological disease after an initial few years of normal development. MRI showed evidence of subacute necrotizing encephalopathy (Leigh syndrome) (**Figure 4**). A muscle biopsy showed typical ragged-red fibres (**Figure 5**),
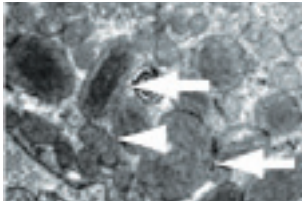
Figure 6. Electron microcopy image for a patient with mitochondrial disease. This image is also similar to one originally obtained for patient IV-4. Abnormally large mitochondria with abnormal cristae (arrows) can be compared with a normal mitochondrion (arrow head). Image courtesy of Dr Glenda Hendson.

abnormal mitochondrial structure on electron microscopy (Figure 6), and reduced levels of enzyme activity for ATP production, confirming a diagnosis of mitochondrial disease. (The biochemical deficiencies identified are shown schematically in the bottom (B) portion of Figure 2.)

Initially, mitochondrial DNA from patient IV-4 was tested for common point mutations and deletions. No such changes were found and no other family members had symptoms at that time. It was thus assumed that the patient's presentation was caused by a nuclear gene defect, most of which are autosomal recessive. Family members in generation IV were counseled that their risk of having a child affected with this presumably recessive condition was very low.

The family remained well for more than a decade until patient V-2 presented at 6 months of age with progressive developmental delay and abnormal eye movements. Findings from investigations suggested mitochondrial disease, although a muscle biopsy was not performed. Given that patient IV-4 and patient V-2 were relatives in the maternal line, concern was raised that the genetic problem in this family was not autosomal recessive (and therefore at low risk of recurrence) but might actually be present in the mitochondrial DNA (which would be at very high risk of recurrence). The family history was reviewed again and showed that patient IV-1 (mother of V-2) had an episode of transient painless monocular loss of vision. Subse-

quently a muscle biopsy was ordered and confirmed that IV-1 had the same enzyme defect as IV-4. Sequencing mitochondrial DNA was carried out and a unique pathogenic mutation of the NDI subunit gene was found and then identified in DNA from patient V-2. Other family members were screened for the mtDNA mutation and 20 were found to have this mutation, including patients III-1, III-2, IV-1, IV-2, IV-3, and IV-5. Some family members with the mutation (IV-5) are completely well, and some (IV-2) have minor nonspecific findings only, including exercise intolerance and fleeting myalgia, while others (IV-4, V-2) are very severely affected.

Conclusions

Mitochondria are intracellular organelles that make ATP. The genes required for mitochondria to function are derived from both the nucleus and from separate, maternally inherited DNA inside the mitochondrion itself. Mitochondrial disease is actually common and affects hundreds of people in British Columbia, including some members of Family A, who have a pathogenic mutation in mitochondrial DNA.

The story of Family A illustrates many features of mitochondrial disease that are considered in the articles that follow:
- Multiple different patterns of inheritance are possible, even when the clinical phenotype (such as Leigh syndrome) is suggestive of nuclear gene defects.
- Disease manifestations in people

with the same mutation can be highly variable, ranging from asymptomatic to catastrophic.
- Many typical findings of mitochondrial disease are not present in an individual case or pedigree and, in fact, symptoms may be quite nonspecific.

Competing interests
None declared.

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