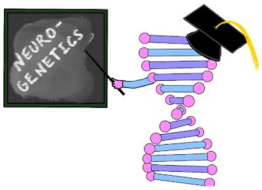


Teaching Guide

Module 10: IEM - Mitochondrial Disease

Slide 1: Introduce mitochondrial disease as subset of inborn errors of metabolism for this module.

Slide 2: Objectives, from texts on slide.

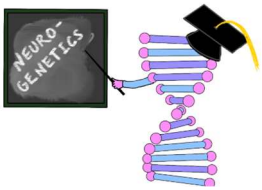
Slide 3: Disclose the chief complaint. We are identifying the **new onset of neurologic symptoms in the setting of fever.** This can be a classic presentation of mitochondrial disease.

Slide 4: Spend a few minutes having participants share genetic causes of ataxia and epilepsy based on their previous knowledge. *Try to get participants to be more specific than "mitochondrial disease" – which genes do they pick and why*. Also try to coax out differential to include other metabolic disorders and genetic ataxias that may have seizures as an additional phenotype.

Slide 5-7: A genetic differential approach to epilepsy and ataxia should have been covered in previous modules. So, participants should have some background knowledge that they would be expected to apply. Refer to these slides quickly without spending too much time.

Slide 8: This is course material where instructor will be helping participants form some concepts in acute onset neurologic symptoms, in this case seizures in the setting of ataxia. This is not about learning the specifics of the genes, but recognizing that a variety of genetic disorders, affecting many different biological processes can overlap with this presentation.

- Part of this discussion is identifying the salient issue – *is this a patient with an "ataxia disorder", "new onset epilepsy" or "both?"*
    - Clearly it is the last, then we must look for genes that affect multiple neurologic process.
- Next, *do these symptoms appear randomly or in the setting of a trigger?*
    - **The latter should be a red flag for mitochondrial disease**, although the former does not preclude mitochondrial disease. Further association with manifestations such as cognitive impairment, myoclonus, myopathy, and extra-neurologic symptoms can further push one away from a Spinocerebellar ataxia, or other monogenic disorders.
    - You do not have to spend time on the SCA nomenclature but can **point out these are not linked by gene mechanism,** but rather the symptomatic presentation of a variety of different genes (including a mitochondrial disorder, SCA28).
- Lastly, it is important to note that Neuronal Ceroid Lipofuscinoses (NCL) are on the differential here, given progression of symptoms/regression.



Slide 9: **Focus on the detailed presentation of this case**, again highlighting the red-flag symptoms that point to mitochondrial disease.

- As the participants will note, the acute presentation is non-specific and unlikely to elicit any specific elements that are attributable solely to mitochondrial disease.
- However, an additional component of this slide is to *discuss elements of the patient's medical history that further support mitochondrial disease*.
    - In particular, the notable concern with **transient regression during illness** is often attributable to mitochondrial disease.
    - It is equally important to note that she did regain her baseline after the illness, as it is often a mistake to assume a "stepwise" decline is present after every episode of regression.

Slide 10: Exam of a critically ill patient. Nothing specific to discuss here unless participants would like to focus on any symptoms.

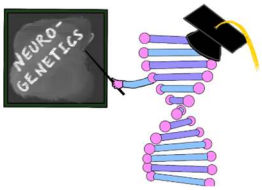
Slide 11: Discuss family history with focus on maternal family symptoms. Given that there was no history of seizures or neurologic disease, encourage discussion on things that could be clues for maternally inherited mtDNA disorders. *Ask participants their own interpretation before pointing out the red flags and whether the maternal cousins are from a maternal aunt or uncle (*aunt in this case). Spend about 3-4 minutes on this interactive exercise.

- Mother is likely affected but far less so than the proband.
- There is **high variability in disease presentation for mitochondrial patients**, and in this case, it is likely that the maternal aunt's family has symptoms as well (that are distinct from this patient).
- Migraines are common, in general, but **increased in mitochondrial disease patients** (mother and maternal grandmother).

Slide 12: Inpatient and prior outpatient workup is presented. Notable for lack of any specific diagnostic clues. Even the MRI is reported as a nonspecific, nonprogressive set of abnormalities. Summarize EEG, MRI, or EMG studies – this is not the focus of the session unless findings are pathognomonic.

Slide 13: MRI shows lack of changes over 3-4 years. The findings are nonspecific and do not predict mitochondrial disease. There is some mild left posterior peri-atrial gliosis. Cerebellar atrophy noted at 3 y/o. No new gliosis, but global atrophy and worse in cerebellum noted on follow up at 7y/o.

Slide 14: Either faculty can summarize or ask participants to comment on metabolic testing to be sent for this phenotype, if relevant.

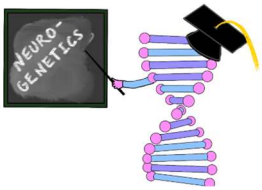


- *What are we looking for*? *How does this tie to the differential generated earlier?*
    - Please also note the lack of consistency with Lactate, and that she had many normal values – even mitochondrial disease patients can have a normal lactate.
    - Conversely, patient lactate can be elevated for many reasons in isolated situations such as GTC or sepsis – it does not have to be mitochondrial disease. It can also be elevated by improper collection such as too tight of a torniquet.

Slide 15: Genetic testing slide. Lots of negative testing and negative ES, but mitochondrial DNA testing shows a pathogenic variant at 93%. Also noted that mother has same variant at significantly lower concentration.

Slide 16: Team group activity – break up into small groups to discuss these 5 questions.

- Team A: *What is the general understanding of how mtDNA disorders cause disease?*
    - Purpose here is to suggest that mtDNA variants suggest abnormal oxidative phosphorylation by coding an abnormal protein that is (usually) part of the electron transport chain.
- Team B: *What are the canonical diseases caused my mtDNA disorders*?
    - Purpose here is to identify MELAS, primarily.
    - Also, should discuss Myoclonic Epilepsy with Ragged Red Fiber (MERRF), Neuropathy, Ataxia, Retinitis Pigmentosa (NARP) and Leber's Hereditary Optic Neuropathy (LHON).
    - Lastly, the discussion from this group should introduce the notion of mtDNA deletion disorders (1.1-10kb large scale deletion of mtDNA, causing Kearns Sayer, Pearson, and progressive external ophthalmoplegia).
    - Bonus points for groups that end up discussing mtDNA depletion disorders (but probably won't have time).
- Team C: *What is the mode of inheritance of mtDNA disorders and who should be screened?*
    - Purpose is to open discussion about maternal inheritance (as distinct from traditional mendelian inheritance). The team should spend some time distinguishing features of maternal inheritance.



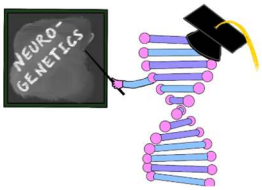
- Team D: *Do you think this gene is the cause of your patient's symptoms? How will you decide?*
    - Purpose is to raise the discussion point of how one determines a genotype-phenotype association when diseases are heterogeneous, like this one.
    - *What supports the diagnosis (clinical red flags, absence of alternate diagnosis, high level heteroplasmy)? What goes against it?*
        - Lactate, MRI – neither of which is sensitive or specific enough to rule out mitochondrial disease!
- Team E: *What biomarkers are used for mitochondrial diseases? How did they apply in this case?*
    - Purpose is like Team D, but more focused on evaluation of patient.
    - *Was there any lab test suggestive of mitochondrial disease*?
        - Isolated lactate is probably not.
    - *What do we make of the MRI?*
        - Too variable in mitochondrial unless the classic findings are present).

Slide 17: Discussion of modes of inheritance. Focusing on fact that mitochondrial disease can be either mendelian or maternal. But the key take home point that everyone should know at the end of this is that **all maternal inheritance is mtDNA**.

Slide 18: Discussion of different types of mitochondrial disease. Incorporate discussion from group A and B.

Slide 19: Caveats unique to mitochondrial disease – only about 60% of patients are diagnosed with mitochondrial genome + exome. Slightly increased with genome (maybe up to 70%, generously). Many patients will remain unofficially diagnosed at the end of the genetic testing odyssey. Also, **interpretation of both VUS and heteroplasmy levels is important for mitochondrial genetic testing**.

Slide 20- 24: Didactic on the unique features of mtDNA inheritance. Despite a clear mode of inheritance, **the expression of mitochondrial disease deriving from mtDNA is much more complex** than mendelian inheritance.



There are three unique properties we will discuss here:

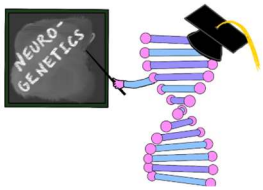
- Cellular heteroplasmy:
    - A mixture of normal and variant mtDNA in a patient, usually approximated by a blood test, but urine testing is also used.
    - As there are many copies of mtDNA within each mitochondrion, and hundreds of mitochondria in each cell, there are potentially thousands of copies of the mitochondrial chromosome in each cell. As a result of this, when a mitochondrial gene undergoes mutation, **there will be two populations of mtDNA's within the cell: those that have the mutation, and those that don't.** This is called heteroplasmy.
    - Homoplasmy occurs when 100% of mtDNA copies are identical-these copies can be normal or mutated.
- Bottleneck effect:
    - The process of an oocyte has a variable amount of heteroplasmy from its sister oocytes. The variant mtDNA is squeezed through a “bottle” into each oocyte, resulting in each cell having a different amount of mtDNA. This results in a different fertilized egg when embryogenesis begins.
- Threshold effect:
    - This is the notion that you **need a certain “level” of heteroplasmy** to have a phenotype. Dependence on ATP generated by oxidative phosphorylation varies from organ to organ. Therefore the "sensitivity" of an organ to a mtDNA mutation may vary.
        - For example, brain and muscle function, which are highly dependent on rapid energy production, are easily compromised by mtDNA mutations which impair oxidative phosphorylation. The same proportion of mutant mtDNA produces far less liver dysfunction.

Slide 25-26: Text from slides. Discussion of specific mtDNA disorders and how to counsel patients about these disorders.

Slide 27-29: Discussion of MRI findings in mitochondrial disorders – nomenclature of a metabolic stroke. Advanced imaging that may help with identify at risk brain areas for metabolic stroke. Unlike ischemic strokes, these lesions may resolve completely. There are bilateral, symmetric lesions that occur in many mitochondrial disease (i.e. Leigh Syndrome) that may be called “metabolic strokes” by some, but they are distinct from these MELAS like lesions.

Slide 30: Take home points.

Slide 31: Suggested reading.



Slide 32. Acknowledgements.