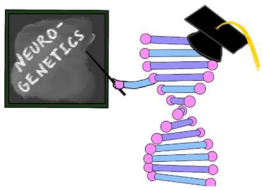


Teaching Guide

Module 1: Epilepsy 1

Slide 1: Title

Slide 2: Learning objectives

Slide 3-5: Case begins – 6-day-old term infant presents with events. **Emphasize that there is no clear history of perinatal events**, no resuscitation is required.

Slide 6: Initial work up for a neonate with seizures is standard. This infant has been home - abusive head injury is a possibility, so a **CT is warranted**. Electrolyte abnormalities, including Na, Ca, glucose are all important. Additionally, any neonate presenting with seizure **must be treated as if they could have a CNS infection and early treatment with antibiotics and acyclovir** is important while cultures are pending.

Slide 7: Neurologic examination. This exam is not unexpected in a neonate who has been given phenobarbital.

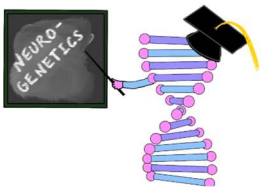
Slide 8: LTM EEG is gold standard for diagnosing neonatal seizures.

Slide 9: Seizures are quite common in the neonatal period. A detailed history is important to evaluate for the possibility of hypoxic ischemic encephalopathy, as this is the **most common cause of seizures in the neonatal period**. Ischemic stroke and intracranial hemorrhage are next on the list, respectively.

Slide 10: *Spend a few minutes having participants share genetic causes of seizures based on their previous knowledge*. If they say a particular condition, encourage them to share what additional information they know about the condition including gene, inheritance pattern, biochemical marker, treatment etc. *Lead learners into listing various genetic etiologies which could present with seizures in the neonatal period*. While the learners may group conditions into larger group like "metabolic disease," ensure that they provide some examples - such as urea cycle disorders with hyperammonemia, or Lissencephaly instead of just "brain malformations."

Slide 11: **Channelopathies are an important differential consideration** and can be associated with both more benign phenotype, such as benign familial neonatal epilepsy, or much more concerning epileptic encephalopathies. Family history could be very important when it is available to raise suspicion of such conditions.

Slide 12-15: Cortical malformations are another broad group of genetic conditions which lead to epilepsy. There are many genes related to neuronal migration and a pathogenic variant in any of these genes could lead to cortical migrational anomalies. Peroxisomal biogenesis disorders which are also sometimes known as Zellweger spectrum disorders will sometimes have



abnormalities in cortical malformation. You might get suspicious of these conditions if the infant **has some distinctive facial features like a flattened face, broad nasal bridge, high forehead, or widened, large fontanel.** Walker Warburg syndrome is a severe form of congenital muscular dystrophy that can also result in cortical malformations and eye anomalies. A high CK in the setting of a finding like cobblestone lissencephaly could raise suspicion for this.

Slide 16: Inborn errors of metabolism are an important group of disorders to consider when evaluating a neonate with seizures because the treatment for these conditions is often different than standard anticonvulsant therapies**. It's important to remember that there are MANY inborn errors of metabolism which are not detected by current newborn screening standards.** Additionally, the newborn screen varies from state to state. Additionally, sometimes these conditions present before the newborn screen has resulted. Can then go through each of the categories listed on the slide and review examples.

Slide 17: Back to the case: review work up, review that seizures were initially refractory.

Slide 18: The baby is discharged to home on an oral antiseizure regimen as well as pyridoxine.

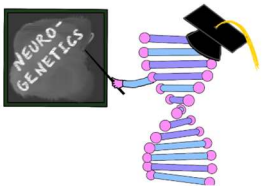
Slide 19: At follow up baby is almost 7 weeks of age, and the interval history and examination are reassuring.

Slide 20: Results from the initial hospitalization. **Metabolic studies will often have some non-specific abnormalities**, particularly if children have been on medications like antiseizure medications or have had poor nutrition due to illness. There is usually an interpretation that discusses if the pattern points towards a specific etiology. Additionally, you can also call the lab to discuss further or review with the metabolic team if available at your institution. The results in question are the results from the genetic epilepsy panel.

Slide 21-24

(Common for several modules. Depending on which module is being done, may or may not repeat all the information)

- Central Dogma developed by Francis Crick is a theory stating that genetic information flows only in one direction, from DNA, to RNA, to protein, or RNA directly to protein.
- **When there is variation or mutation or spelling error in the underlying sequence of the gene, that leads to genetic disorders.** These mutations can be inherited or de novo. In some cases, spelling errors are needed in both alleles to produce a defect in the protein, these are known as recessive disorder. Whereas in some cases, one variant in one of the alleles is sufficient to cause a genetic disorder referred to as dominant conditions.
- When analysis of a patient's genes shows a variant, but it is unclear whether the variant is related to the patient's medical condition, it is classified as a variant of uncertain significance (abbreviated as VUS). In many cases, these variants are so rare in the



population that little information is available about them. Typically, more information is required to determine if the variant is disease related.

-Such information may include more extensive population data, functional studies, and tracing the variant in other family members who have or do not have the same health condition. Parental testing can be useful to further delineate the variant classification.

1. Heterozygous: If the variant is found to be inherited from a parent, it would be supportive of benign status. If it is found to be de novo, it could be supportive of pathogenicity in the context of overlapping clinical features.
2. Compound heterozygous: If the variants are found to be in cis/on same allele, that would be supportive of benign status. If they are found to be in trans/ on different alleles, that could be supportive of pathogenicity in the context of overlapping clinical features. In some cases, we can measure an enzyme or transporter activity to evaluate the impact of the genetic variant on the protein, which is called a functional study.

Slide 25-26: The important next step which may be obvious when thinking about it like this, but is often overlooked, is to **review the actual genes and the conditions that they are associated with.** Are they autosomal dominant conditions? Recessive conditions? X-linked?

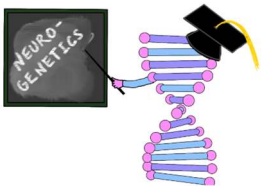
Slide 27: Interactive exercise.

Slide 28-32: Report back from small groups:

ALDH7A1 is associated with autosomal recessive pyridoxine dependent epilepsy. DOCK7 is also causative of an autosomal recessive condition, one in which there are dysmorphic features, intractable epilepsy, and cortical blindness.

In our case –

- ALDH7A1 gene- there are 2 variants.
    1. One variant is deemed a likely pathogenic variant.
    2. Another is currently classified as a variant of uncertain significance.
    3. **The most important point here is that it needs to be determined whether these variants are on opposite alleles.** If they are both on the same allele, the proband would be at most a carrier for the condition in question. If they are on opposite alleles, we would need to investigate the VUS further. Typically, we test the parents in this situation. If one parent carries one of the variants, and the other carries the other variant, then they are on opposite alleles. If one parent carries both, they are on the same allele. Of course, this does leave a dilemma. As sequencing techniques advance and more long read sequencing is available - we may be able to answer these questions without parental DNA.
- For the DOCK7 gene - there is one pathogenic variant in a recessive condition.



1. **This means that the proband is a carrier for the condition.** One caveat is that current sequencing techniques are not perfect, and there are areas in which the coverage is not as good and a variant on the other allele could be missed. Additionally, depending on the sequencing technique, deletions and duplications may be missed. If the phenotype was very possible for the patient, one could consider deletion/duplication testing.

Slide 33: A useful question to ask oneself whenever dealing with uncertain results is to ask whether there is an alternative biomarker for the condition in question.

Returning to our case- the patient is not currently seizing - is this because of the antiseizure medications? Or because she is on pyridoxine? We could consider holding pyridoxine and seeing whether seizures recur, but this might not be ideal.

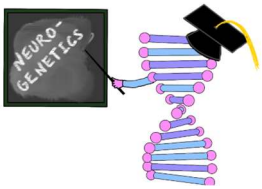
Slide 34: Let's look at where ALDH7A1 functions. It is an enzyme in the lysine metabolic pathway. When it is not working properly, there is a block in lysine metabolism and buildup of these toxic metabolites. The theory is that **these metabolites bind to pyridoxal-5-phosphate, which is the active form of pyridoxine, and render it unusable.**

Slide 35-36: The intermediaries can be tested for including alpha aminoadipic semialdehyde. The nice thing about this metabolite is that it will be elevated even if already on pyridoxine therapy. It can be tested for in the urine, plasma, or spinal fluid**. The elevations are specific to pyridoxine dependent epilepsy**, though elevations can be seen in molybdenum cofactor and sulfite oxidase deficiency. These are both much rarer the pyridoxine dependent epilepsy but are still entities to consider if the metabolic testing is all you have.

Pipecolic acid is another of the intermediary metabolites that will be elevated in pyridoxine dependent epilepsy, though in this case the levels may decrease with treatment with pyridoxine. Additionally, very high levels can be seen in peroxisomal disorders.

Slide 37: Urine alpha amino adipic semialdehyde was sent in our case and the result was quite elevated. This provides a **biochemical confirmation of pyridoxine dependent epilepsy in this case.**

Slide 38: Pyridoxine dependent epilepsy was first recognized in this case report in 1954. A term neonate had refractory seizures. This baby had several admissions during which the baby was made NPO and given IV fluids, along with a multivitamin cocktail. During the admission, the child would be seizure free. The team began suspecting that there was something in the vitamin cocktail that was helping the seizures. Eventually, the treating team tried adding back each of the vitamins in the cocktail individually and **landed B6 as the factor that was improving the seizures.**



Slide 39: Pyridoxine is quite important as a **cofactor in neurotransmitter metabolism** including serotonin, glycine, d-serine, glutamate, and GABA. There are multiple forms of this vitamin, and it is converted to pyridoxal 5 phosphate in the brain, which is the active form.

When pyridoxine dependent epilepsy was recognized as an entity, different genes related to pyridoxine were studied.

Slide 40: It was a somewhat surprising result that most pyridoxine dependent epilepsy cases were due to pathogenic variants in a gene related to lysine metabolism. ALDH7A1, or antiquitin, is in the lysine degradation pathway. **Due to the ALDH7A1 enzyme not working properly, there is buildup of metabolites which are normally not seen**. These metabolites are thought to bind to P5P and render it unusable.

Slide 41: This condition should be considered in babies with early onset, refractory seizures. There are often multiple types of seizures, and the seizures can last quite a long time. Sometimes there are signs of encephalopathy. It may even masquerade as an HIE picture.

Slide 42: While the typical presentation is in the neonatal period, there are cases of onset in later infancy or early childhood.

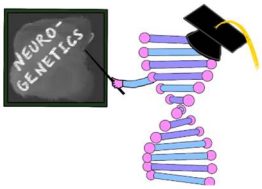
Slide 43: We have also reviewed this slide before, but these again are the biochemical markers that can be used to diagnose pyridoxine dependent epilepsy.

Slide 44: **Treatment should NOT be delayed while awaiting confirmatory testing**. One possibility is an IV challenge for pyridoxine. There are various ways to do this, but all involve giving a total of 100 mg of pyridoxine IV (sometimes split up into 50 mg followed by 50 mg) while a child is on IV monitoring. It is important that this is done in a closely monitored setting, like an ICU, as the IV challenge can result in apnea in cases of actual pyridoxine dependent epilepsy. **This is most useful when the EEG is quite abnormal at baseline** (i.e., a burst suppression pattern) and one is looking for improvement of the EEG in the minutes to hours after administration of IV pyridoxine.

There are plenty of case reports of an unclear response, or no change after an IV load, and in these cases, enteral pyridoxine should be continued until biochemical testing returns or an alternative etiology is determined. Because of issues with the IV challenge, one could simply begin with adding oral pyridoxine in a case of refractory epilepsy.

Slide 45: Treatment of pyridoxine dependent epilepsy is with lifelong pyridoxine. Typically, additional antiseizure medications can be weaned off once pyridoxine is started.

Slide 46: Typically, intellectual disability is seen in 75% of patients**, though there are 25% who have normal intellectual development**. There has been at least one case report of a child where the diagnosis was known prenatally, and the mother was started on supplementation



during the pregnancy itself. This child never had a seizure and was still known to have neurodevelopmental disabilities. It is now thought that the lack of available pyridoxine is what causes the seizures, but it may be the metabolic intermediaries that build up which are responsible for the neurodevelopmental outcome.

Slide 47: Therefore, a "triple therapy" has been proposed in the literature. This diet involves 3 arms.

1. The first is of course pyridoxine supplementation.
2. The second aspect involves a lysine restricted diet.
3. The third aspect is supplementation of arginine. This is because lysine and arginine use the same intracellular transporter system. Supplementing arginine can decrease uptake of dietary lysine by competitive inhibition.

Slide 48: **The idea is if you reduce the intake of lysine, you reduce its metabolism**, and therefore the production of these toxic intermediaries. Commercially available lysine-free formulas are those that have been developed for glutaric aciduria type 1 and are also low in tryptophan. This will need to be supplemented. If the formula is not well tolerated, then protein can be decrease to low RDA for age. There are some suggestions that if lysine reduction is not undertaken, a higher level of arginine may be used to try to decrease lysine uptake further.

It's important to note 2 things-

1. The triple therapy cannot be done without an experienced metabolic dietician.
2. These treatment recommendations are largely based on case reports and case series. We don't have head-to-head evidence for outcomes in pyridoxine alone versus triple therapy.

Slide 49: This presentation has discussed the most common genetic cause of pyridoxine dependent epilepsy, which is the ALDH7A1-related cause. However, there have been some other conditions with pyridoxine responsiveness reported including these 3 entities listed.

Slide 50: Take home points.

Slide 51: Suggested reading.

Slide 52: Acknowledgements.