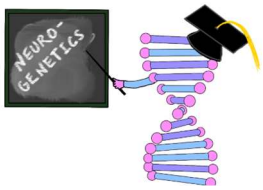


Teaching Guide

Module 13: Dual Diagnoses

Slide 1: Title slide.

Slide 2: Learning objectives for this lesson.

Slide 3: We introduce the chief complaint in the form of a one-liner. *Participants could be asked for potential genetic causes of epilepsy and delayed development.*

Slide 4: Review the patient's history with participants. *Ask if they have ever heard of monocular nystagmus and if it is associated with any genetic disorders.*

Participants could be asked about their genetic differential diagnosis at this point. While those familiar with alternating hemiplegia of childhood (AHC) will be quick to mention it, ask for the specific gene (*ATP1A3*) and also if there are any other conditions which could resemble it. Differential considerations include familial hemiplegic migraine genes (FHM1 – *CACNA1A*, FHM2 – *ATP1A2*), GLUT1 deficiency (*SLC2A1*), *SCN1A* disorders, mitochondrial disease, dopamine biosynthesis disorders, *RHOBTB2*, and *CLDN5* (Claudin-5).

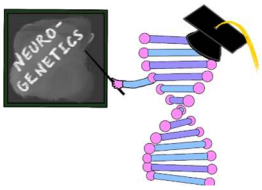
Slide 5: Continued HPI. Provides developmental history and notes **the failed hearing screen**. Ask participants *if sensorineural hearing loss is typical for AHC.*

Slide 6: We review the family history. The proband is the last child. **Note the age of the parents at time of birth.** The other two siblings have a history of ODD or speech delay, but neither are as severely affected as the proband. There is no family history of seizures, developmental delay, or hearing loss.

Slide 7: This slide details the workup. ABR confirmed moderate sensorineural hearing loss. Brain MRI showed bilateral mesial temporal lobe sclerosis [TLS] (shown in figure). Possible focal cortical dysplasia (FCD) was also observed (not shown*). Ask the participants if mesial TLS and FCD are seen in AHC.* TLS has been occasionally reported in AHC, but FCD is not a feature. The instructor can comment that FCD might contribute to the refractory focal epilepsy.

Slides 8-9: Ask participants what genetic tests they would order and why, then use the next two slides to discuss their pros and cons.

- Single gene testing – appropriate only if very high clinical suspicion for a single gene disorder (e.g., perhaps Rett syndrome, Dravet syndrome, etc). Since most genetic conditions are extremely heterogeneous (100s of genes responsible for CMT/HMSN, 1000s for neurodevelopmental disorders), the utility of single gene testing is poor.
- Panel testing – covers more genes than single gene testing but is very rarely exhaustive. Furthermore, if there are a few genes for which you have high suspicion, check the fine print to make sure that gene is covered.
- Single gene and panel testing generally covers single nucleotide variants/SNV (i.e., a substitution of one nucleotide for another) and indels (small DNA deletions and duplications). They may or may not cover copy number variants/CNV, large DNA deletions and duplications. CNV analysis



may also only be covered for a small number of genes. **Thus, it is important to read the fine print!**

- Chromosomal microarray (CMA) – detects large deletions or duplications but not SNVs or indels. Currently, I think of CMAs as a useful adjunct to exome sequencing, as WES may or may not be able to detect large to moderately sized deletions or duplications. Thus, if I want to comprehensively survey all SNVs, indels, and CNVs, I'll send ES first, then CMA if ES is not diagnostic. CMA will eventually be phased out as GS becomes more available.
- Exome sequencing (ES) – the most comprehensive clinically accessible genetic test in 2023. Superior detection of SNVs and indels in nearly all 20,000 protein coding genes. Has some ability to detect CNVs but may miss some detectable by CMA.
- Genome sequencing (GS) – will eventually replace exome sequencing in the clinical setting due to its ability to detect SNVs, indels, and CNVs. Its clinical application is still evolving, so I recommend using with caution for now.

Slide 10: Participants may have different opinions about the genetic testing strategy. Perspectives and guidelines have changed over time, and many in our field are not aware of the latest recommendations. In fact, **exome sequencing is recommended as the first-tier clinical diagnostic test for neurodevelopmental disorders** based on a meta-analysis as shown in Srivastava et al.

Slide 11: This slide is used throughout the series and provides an overview of how to think about the Interpretation of genetic testing results. Review with participants.

Slide 12: This slide shows the result of whole exome sequencing. Pathogenic variants in ATP1A3 and USH2A were identified. A variant of uncertain significance in MTOR was also identified.

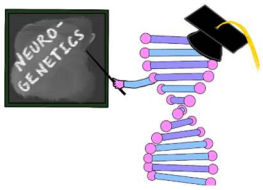
Slide 13: Review with participants the utility of OMIM and go over the entry for ATP1A3. We see that alternating hemiplegia of childhood results from pathogenic variation in ATP1A3, and this likely explains the patient's delayed development, hemiplegia, dystonia, abnormal eye movements, and seizures.

Slide 14: This slide shows the Revised AHC criteria from 2021. Review the criteria and note that the patient meets all essential and major criteria and most minor criteria. Play the video showing monocular nystagmus.

Slide 15: Review the OMIM entry for USH2A with participants. Biallelic pathogenic variants in USH2A cause Usher syndrome type 2A, a condition characterized by sensorineural hearing loss at birth and later development of retinitis pigmentosa. This likely explains the patient's hearing loss.

Slide 16: This slide introduces the concept of dual molecular diagnoses. For example, a patient who inherits two pathogenic variants associated with Tay-Sachs disease and a de novo pathogenic variant associated with Kabuki syndrome ends up with a blended phenotype. In a large review of exome sequencing data from a clinical diagnostic lab, dual molecular diagnoses were found in almost 5% of patients and frequently involved combinations of autosomal dominant, autosomal recessive, and X-linked diseases.

Slide 17: In some cases, the two molecular diagnoses are completely distinct, for example KBG syndrome and ichthyosis vulgaris or in this case AHC and Usher syndrome 2A. The blended phenotype



seen in dual diagnosis patients may resemble other conditions and cause diagnostic confusion. It is worth noting that sensorineural hearing loss is seen in a different phenotype related to ATP1A3 phenotype - the CAPOS phenotype, and there are rare case reports of a more overlapping phenotype.

Slide 18: Here we return to the genetic test results and ask, *what about the MTOR variant?*

Slide 19: MTOR variants can cause two phenotypes:

- Somatic variants in the brain are often found in focal cortical dysplasia type II.
- Germline variants can be seen in an autosomal dominant condition called Smith-Kingsmore syndrome. Smith-Kingsmore syndrome is characterized by macrocephaly, seizures, umbilical hernia, and dysmorphic facial features. This does not sound much like our patient at first glance.

Slide 20: Since Smith-Kingsmore syndrome is a poorly described condition (only a few patients described in OMIM), **it can be helpful to review the primary literature in PubMed**. Here is an article published in 2016 describing germline and somatic MTOR mutations.

Slide 21: Review the content of the paper with participants. Germline de novo missense MTOR variants were seen in 6 individuals. They had focal epilepsy, mild or no brain malformations, and DD/ID (Developmental Delay/Intellectual Disability) or normal development.

Slide 22: This introduces the concept of dual diagnosis with overlapping phenotypes. The blending of **two overlapping phenotypes may result in greater phenotypic severity**. For example, if the MTOR variant is pathogenic, it could explain the refractory focal seizures and perhaps the FCD in this patient.

Slide 23: Here we discuss tools for VUS resolution. Please review with participants. We will focus on the two with arrows.

Slide 24: We review gnomAD data (population database with >100,000 exomes and genomes) and see the variant is absent. Since gnomAD largely excludes individuals with severe developmental disorders, its absence is expected. Conversely, if it was found in several individuals, that would reduce the likelihood of pathogenicity.

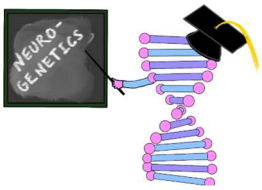
The variant is also found to be highly conserved (phyloP100 score of 9.435 – this reflects evolutionary conservation within vertebrates). This value can be obtained from Varsome and other sources.

The variant is also predicted damaging/deleterious by CADD (score 23.5). A score of 10 is in the top 10% of all predicted damaging variants, 20 in the top 1%, 30 in the top 0.1%, etc. CADD scores can be obtained at the listed website.

Finally, parental testing confirms the variant is *de novo*.

Taking all these factors into account, the variant can be reclassified as likely pathogenic per ACMG criteria.

Another approach would be reach out to MTOR experts for advice, functional evaluation of the *MTOR* variant.



Slide 25: This slide is taken from the Genetics in Medicine paper that spells out the consensus criteria that can be used to classify a variant of uncertain significance.

Slide 26: *Why should we pursue the MTOR variant?* This slide makes the case. First, MTOR-related disease is potentially treatable by everolimus or vigabatrin. Secondly, it may explain the patient's drug-resistant epilepsy. Finally, it may have prognostic implications.

Slide 27: Some patients may have even more complex phenotypes. For example, this patient from Karaca et al. had an especially severe phenotype resulting from homozygous variants in AP4B1, AMPD2, and NOTCH2. The figure shows how the three variants result in a complex blended phenotype.

This may be more common in consanguineous families due to increased absence of heterozygosity (a surrogate for runs of homozygosity, shown in the figure by gray shading) but can also be seen in non-consanguineous families like the focus of this report.

Slide 28: Another important consideration is that multiple molecular diagnoses can result in intrafamilial or interfamilial variability. For example, the milder phenotype seen in BAB6229 in comparison with his older brother BAB6228 is explained by the fact that he is only homozygous for the BCOR variant and not the MED17 and SPG7 variants found in the older brother.

Slide 29: Suggested reading.

Slide 30: Acknowledgements.