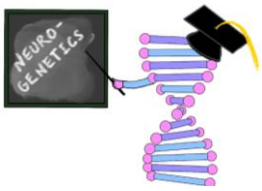


Teaching Guide

Module 4: Methylation/Imprinting/Uniparental Disomy

Slide 1: Title slide – introduce disorders of epigenetic modifications and imprinting as contributors to genetic disorders

Slide 2: Objectives, from text on slide.

Slide 3: Disclose the chief concern. Discuss what features of presentation to focus on when generating a differential diagnosis. In this case, hypotonia encompasses feeding and growth.

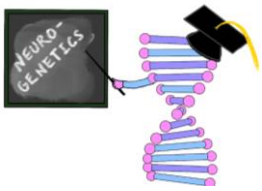
Slide 4: Spend a few minutes having participants share causes of hypotonia based on their previous knowledge. *Ask them to identify both genetic and non-genetic causes.* When a differential diagnosis is suggested, ask what other features they might expect and underlying etiology (genetic or not). **Note: that hypotonia can be peripheral or central.**

Slide 5: Acknowledge that hypotonia has a broad differential and encompasses both genetic and non- genetic etiologies, and both peripheral (e.g., myopathies, congenital myotonic dystrophy) and central nervous system causes. **For this discussion, we will focus on central causes of hypotonia.** Discuss exclusion of secondary etiologies (e.g., infection, systemic illnesses). Given the very large number of individual genetic disorders that result in hypotonia, focus on the most common disorders and classes of disorders. If these were not already discussed on the previous slide, ask about associated and distinguishing features, red flags, reasons accurate diagnosis is important to care and outcome. Discuss the underlying types of genetic changes resulting in these disorders.

Slide 6: Point out the utility of different testing modalities to detect the genetic changes commonly implicated in neonatal hypotonia. **When discussing methylation changes, point out direct (in green) and indirect (in yellow) detection.**

Slide 7: Walk through the recommended diagnostic algorithm. Discuss that early targeted testing for the most common causes is appropriate if phenotype suggestive and clinically appropriate. DM1=Myotonic Dystrophy Type 1, PWS = Prader-Willi Syndrome, and SMA = Spinal Muscular Atrophy. Note: that testing for DM1 and PWS may not be picked up by exome, multi-gene panel, or chromosomal microarray, so if it is suspected, more targeted testing should be ordered.

Slide 8: Text from slide. *Ask the learners what they would look for on exam* (dysmorphic facial features, head size, cryptorchidism, hands/feet, neurological examination.



Individuals with PWS have: **a narrow forehead, almond-shaped eyes, and a triangular mouth; short stature; and small hands and feet.** Some people with PWS have unusually fair skin and light-colored hair. Both affected males and affected females have underdeveloped genitals.

Slide 9-10: Emphasize some abnormal findings and discuss whether generally associated with hypotonia or may be specifically associated with a diagnosis. Microcephaly can be seen in several disorders including PWS, Angelman Syndrome (AS), Rett Syndrome (but this patient is male), Smith–Lemli–Opitz (SLO) syndrome. Almond shaped eyes and epicanthal folds can be seen in Down Syndrome and PWS. A high arched palate can be seen in PWS, myopathies, and other diseases. Cryptorchidism and abnormal genitalia can be seen in PWS, and pathogenic variants in the ARX gene (X-linked lissencephaly with abnormal genitalia). Short, tapered fingers can be seen in PWS and Coffin-Lowry Syndrome.

Slide 11: *Ask the learners what they might look for on family history.* For example – history of multiple spontaneous abortions to suggest chromosomal abnormality.

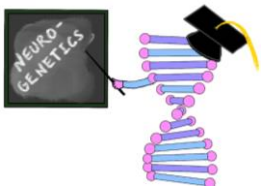
Slide 12: Read from slide. **Discuss what an unremarkable family history suggests about genetic mechanisms.** For example, it is less likely to be an inherited autosomal dominant disorder if there are no other family members identified. Other options include *de novo* monogenic change, CNV, chromosomal changes, uniparental disomy (when both members of a chromosome pair are inherited from one parent, and the other parent's chromosome for that pair is missing) or sex-linked inheritance. If there is incomplete penetrance, some individuals who carry the pathogenic variant express the associated trait while others do not.

Slide 13: Non-genetic investigations can provide clues to genetic etiology. Here, genital hypoplasia implicated on Prader Willi Syndrome among other disorders. Microcephaly confirmed by MRI.

Slide 14: Genomic investigations. Slide 7 indicated exome as first line. However, CMA can be considered at the same time given rapid turnaround and low cost**. Here, CMA was diagnostic.** Discussion for this slide should include what a region of homozygosity is and what the possible reasons are that an array may detect regions of homozygosity. For shorter regions interspersed throughout the genome we may consider parental consanguinity. For discrete regions, uniparental disomy should be considered.

Slide 15: Read slide and discuss principles of general epigenetic modification as well as imprinting specifically.

Epigenetics refers to the stable changes that modulate genomic structure (mostly DNA methylation, and histone methylation and acetylation) and gene expression in response to cell-extrinsic, cell-cell, and cell-intrinsic signals, but without modifying the DNA sequence.



Epigenetics does not follow Mendelian genetics (e.g., Punnett Square), because the imprinting from the maternal vs. paternal allele affects the inheritance pattern and phenotypic expression.

Histones are the proteins that chromosomes wind around to create nucleosomes. Close packing of histones occurs in condensed chromatin (heterochromatin), and this is transcriptionally silent. Loose packing of histones occurs in open chromatin (euchromatin), and this is transcriptionally active. In an oversimplified view, DNA methylation and histone methylation lead to transcriptional silencing, and histone acetylation leads to transcriptional activation.

Slide 16-19: Explains how **imprinting disorders and regions of homozygosity result from uniparental disomy** and confer risk for neurodevelopmental disorders due to under- or over-expression of specific genes.

Slide 16: Typically, a person inherits one copy of a chromosome from their mother and one from their father.

Slide 17: Sometimes, there are regions of homozygosity in the chromosomes (in this case the entire short arm of the chromosome) that can result from inheriting both copies from 1 parent, and this is termed uniparental disomy. If there are no pathogenic variants in these regions, and there is no imprinting in those regions (which can lead to silencing of expression of genes), this may be asymptomatic.

Slide 18: Suppose the mother is carrier of a pathogenic variant on this short arm of the chromosome, in a gene that is associated with autosomal recessive disease. On the left, the person inherited that variant from their mother, so they are also a carrier. On the right, because of maternal uniparental disomy, autosomal recessive disease results.

Slide 19: Suppose the short arm of this chromosome is imprinted, designated by the green triangles, such that the maternal allele is not expressed, but the paternal allele is expressed. On the left, with typically one copy would be expressed. In the middle, with maternal uniparental disomy, disease could result from underproduction of proteins from genes in that region. On the right, disease could result from overproduction of proteins from genes in that region from paternal uniparental disomy.

FYI to presenter, but no need to review this with your audience at this point:

Specific to Prader-Willi Syndrome and Angelman Syndrome:

(from Appl Clin Genet. 2023 Apr 6;16:41–52. doi: 10.2147/TACG.S372708)

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are genetic imprinting disorders resulting from absent or reduced expression of paternal or maternal genes in chromosome

15q11q13 region, respectively. The most common etiology is deletion of the maternal or paternal 15q11q13 region.

PWS arises from the loss of function or expression of paternally derived genes. Several genes which are preferentially or exclusively expressed from the paternal chromosome have been described: *SNURF-SNRPN, MKRN3, NDN, MAGEL2, NPAP1, PWRN1, SNORD116, IPW, and SNORD115*. On the maternal chromosome, these genes have methylated CpG islands in their promoter which leads to silencing of the maternal allele. As a result, a large deletion of 15q11q13 on the paternal chromosome leads to no functional expression of these genes and results in PWS.

AS arises from the loss of function of the maternally derived ubiquitin ligase E3A, *UBE3A*, which is preferentially expressed by the maternal chromosome in the neuronal cells. *UBE3A* is not differentially regulated and is instead regulated indirectly through a paternally derived antisense transcript which prevents expression of the paternal *UBE3A*. Thus, when the maternal *UBE3A* is lost due to a large deletion of 15q11q13, paternal gene expression remains blocked and results in loss of *UBE3A* expression, leading to AS.

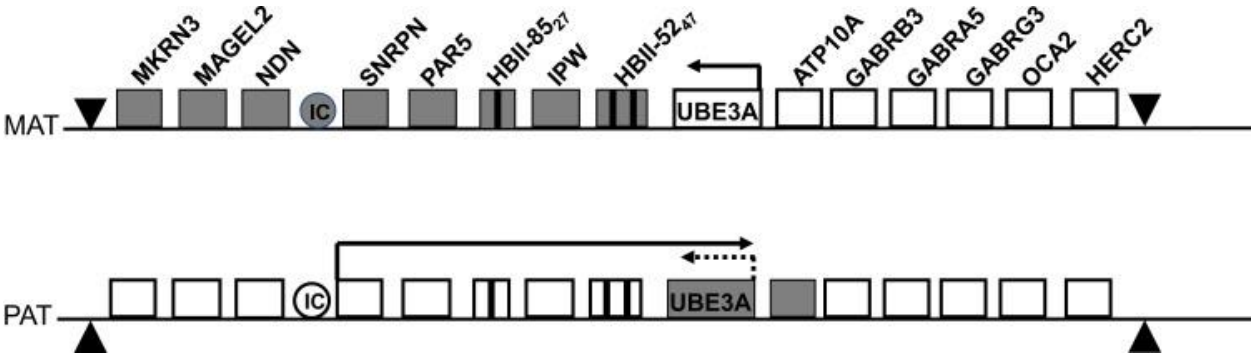
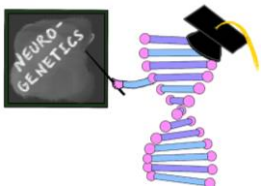


Diagram of maternal (MAT; top) and paternal (PAT; bottom) regions of human chromosome 15q11-q13. Clear boxes represent actively expressed genes; grey boxes represent those whose expression has been silenced through genomic imprinting (maternal allele) or through expression of the antisense transcript (paternal UBE3A). IC=imprinting center

Slide 20: To date, multiple chromosomes and regions have been identified that result in imprinting disorders.

Slide 21: How we test for Uniparental Disomy. Multiple tests will detect regions of homozygosity. **Methylation specific testing is gold standard** to determine parent of origin.



Slide 22-23: Return to SNP results and, in an interactive fashion, work through *what additional steps are needed to make a diagnosis.* Participants can probably Google the 15q locus and find the answer. 15q11-13 has different breakpoints, and there are certain regions associated with PWS and AS. https://rarechromo.org/ is a good resource for chromosomal microdeletion and duplication disorders.

Slide 24: Repeat of slide 6, reviewing how different test perform for methylation changes, and showing that methylation-specific PCR is optimal

Slide 25: Discuss clinical characteristics that would differentiate the two syndromes of PWS and AS while methylation studies are pending. There are some shared features but **our patient fits PWS.**

Slide 26: Text from slide

Slide 27: More information on PWS.

Slide 28: Suggested reading.

Slide 29: Acknowledgements.