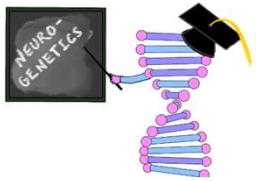
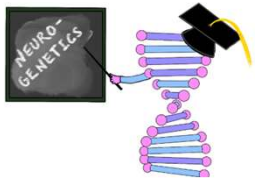
Methylation, Imprinting, & Uniparental Disomy

MODULE 4



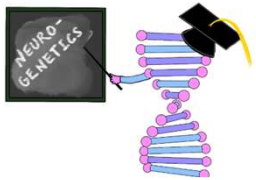
Learning Objectives

- Define uniparental disomy and its contribution to neurogenetic syndromes
- Recognize how to use methylation studies to determine parental inheritance
- Describe how maternal- vs. paternal-specific imprinting results in different diseases



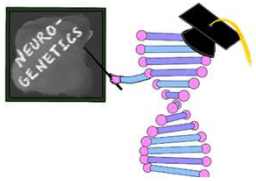
Chief Complaint

- 12-month-old male child with history of second trimester Zika Virus exposure presented with poor growth, neonatal hypotonia, and feeding difficulties.



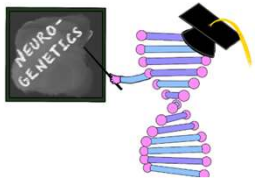
Differential Diagnosis - Interactive

Systemic/Non-Genetic	Genetic



Differential Diagnosis: Central Hypotonia

Systemic/Non-Genetic	Genetic
• Hypoxic ischemic Encephalopathy • Infection • Intracranial hemorrhage • CNS / spinal cord trauma • Cranio-cervical junction lesions • Systemic illness i.e. Congestive heart failure	• Trisomy 21 • Prader-Willi syndrome • Peroxisomal disorders • Inborn errors of metabolism • Malformations of cortical development • Monogenic disorders (numerous)



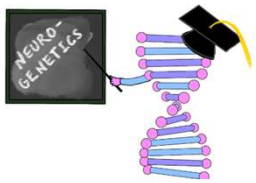
Testing Modalities

Table 1. Broad Categories of Genetic Testing Modalities for Hypotonia

Test	Aneuploidy	Large intergenic deletions/duplications	Intragenic deletions/ duplications	Monogenic SNVs and small I/Ds	Repeated element expansions[a]	Methylation changes
Karyotype or fluorescence in situ hybridization (chromosome number and identity)	Optimal test	Variable detection	Unable to detect	Unable to detect	Unable to detect	Unable to detect
Chromosomal microarray	Able to detect	Optimal test	Limited detection	Unable to detect	Unable to detect	May detect uniparental disomy or deletion leading to imprinting disorder
Next-generation sequencing–based gene panel	Unable to detect	Variable detection	Able to detect	Optimal test	Unable to detect	Unable to detect
Methylation array or bisulfite sequencing (methylation state of DNA)	Unable to detect	Unable to detect	Unable to detect	Unable to detect	Unable to detect	Optimal test
Mitochondrial sequencing (mitochondrial genome sequencing)	Unable to detect	Unable to detect	Unable to detect	Optimal test for mitochondrial genome SNV or I/Ds	Unable to detect	Unable to detect
Exome sequencing (autosomal coding sequences)	Able to detect	Able to detect	Able to detect	Optimal test	Limited detection	May detect uniparental disomy or deletion leading to imprinting disorder
Genome sequencing (coding and noncoding sequences)	Able to detect	Optimal test	Optimal test	Optimal test	Able to detect	May detect uniparental disomy or deletion leading to imprinting disorder

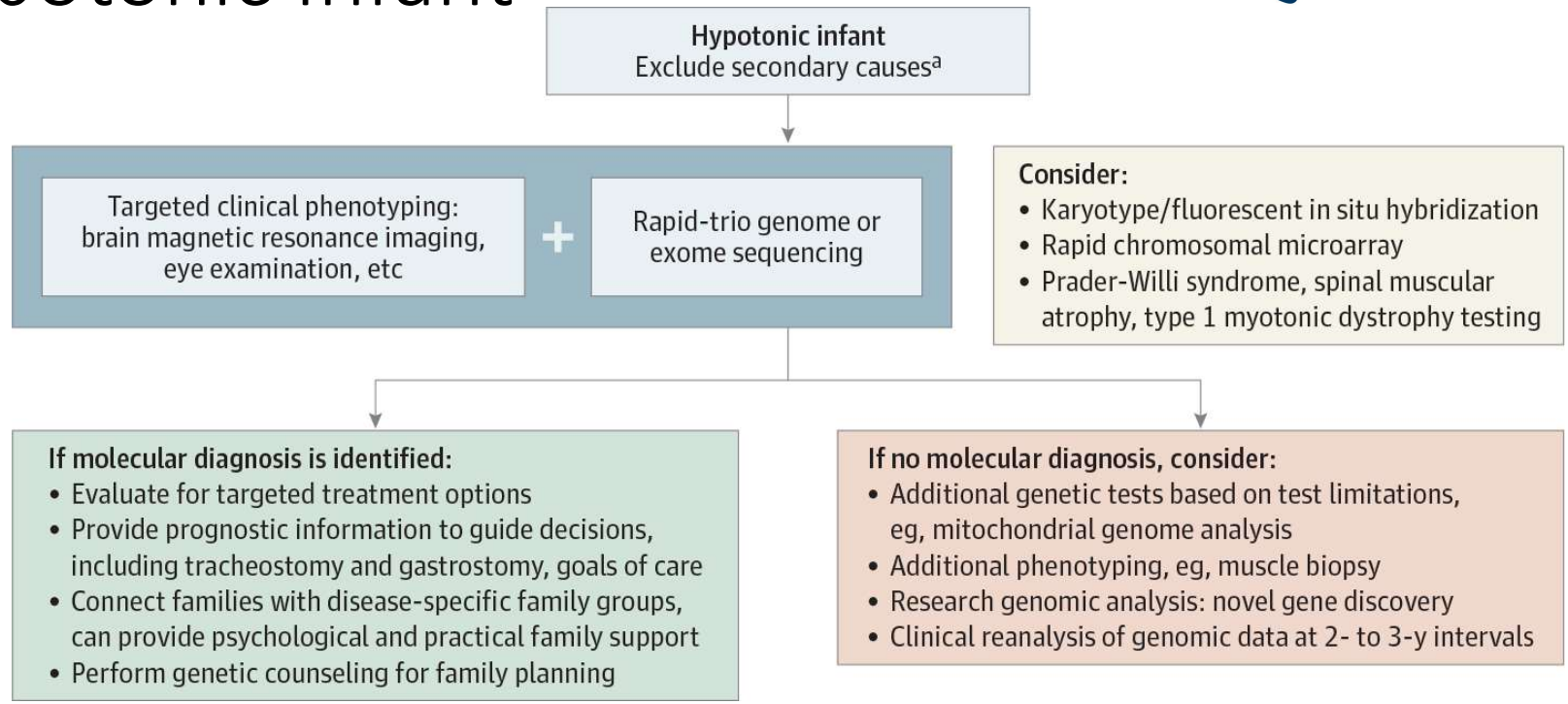
Abbreviations: I/D, insertion/deletion; SNV, single-nucleotide variant.

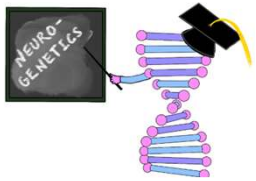
[a] Does not include targeted testing.



Morton et al., JAMA Neurol. 2022 Apr 1;79(4):405-413

Genetic Evaluation of the Hypotonic Infant



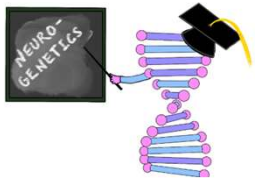


Three common causes of neonatal hypotonia that have rapid targeted testing available should be considered early in evaluation: <u>DM1, PWS, and SMA.</u>

Morton SU *et al. JAMA Neurology* 2022

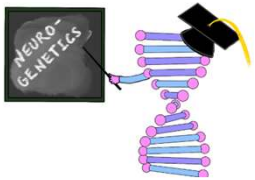
HPI

- Now 2-year-old male child shows poor growth, microcephaly, developmental delay, hypotonia, neonatal feeding difficulties and subsequent hyperphagia, severe OSA, bilateral cryptorchidism, and congenital esotropia.
- Initially hypotonia attributed to Zika exposure but no spasticity and degree of hypotonia disproportionate to cognitive changes
- Language and economic/social barriers delaying genetic evaluation



Exam

- **HEENT**: Head circumference measures < -2 std dev; <2nd %); microcephalic with normal hair distribution and pattern; bitemporal narrowing; symmetric face; pupils equal, round, and reactive to light bilaterally; almond shaped eyes; epicanthal folds; ears normally formed set and posteriorly rotated; normal nose; normal philtrum, high arched palate, and symmetric facial movements.
- **Neck**: Supple with no extra skin or webbing; no adenopathy.
- **Chest:** Clear to auscultation bilaterally; chest cage symmetric w/o pectus deformity; nipples normally formed and spaced.
- **CV:** Regular rate and rhythm with no murmurs, rubs, or gallops. Pulses full and symmetric in all extremities.
- **Abdomen:** Soft, nondistended with no HSM or masses; bowel sounds present.
- **Back:** Spine normal with no sacral tufts or dimples.
- **GU:** Left testicle palpable in the scrotal sac; right not palpable. Penile length appears within normal limits.
- **Extremities:** Normally formed digits with normal nails and creases; full range of motion of all major joints; no joint hypermobility. Tapered fingers, hypoplastic fingernails.
- **Skin:** No areas of hyper or hypopigmentation; no café-au-lait macules; no visible telangiectasias on skin or mucous membranes; no rashes. No thinned/translucent skin. No striae/abnormal scars.
- **Neuro:** Severe hypotonia; gross motor strength relatively normal compared to the degree of hypotonia, brisk reflexes, alert, social, and interactive, near age-appropriate language milestones.



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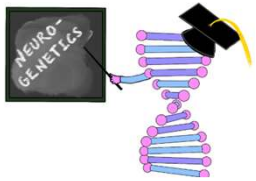
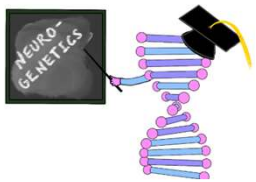
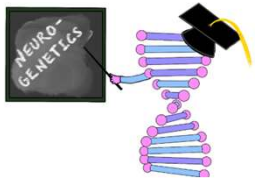


Family History - Interactive



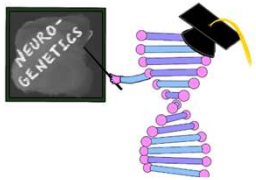
Family History - Unrevealing

- Siblings
  - 4-year-old full brother, normal milestones
  - 8-year-old paternal sister, normal milestones
  - Mother also had first trimester miscarriage (with a separate partner).
- 34-year-old mother with hypertension
- 30-year-old father – healthy



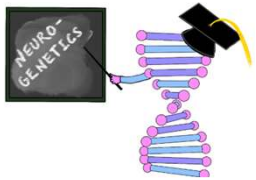
Investigations (Non-Genetic)

- Testicular ultrasound
  - Right testicle in right inguinal canal. Left testicle not identified.
- Abdominal ultrasound - left mild hydronephrosis
- MRI brain age 2 - Microcephaly. Diminished white matter volume
- Routine EEG normal
- Metabolic testing unremarkable

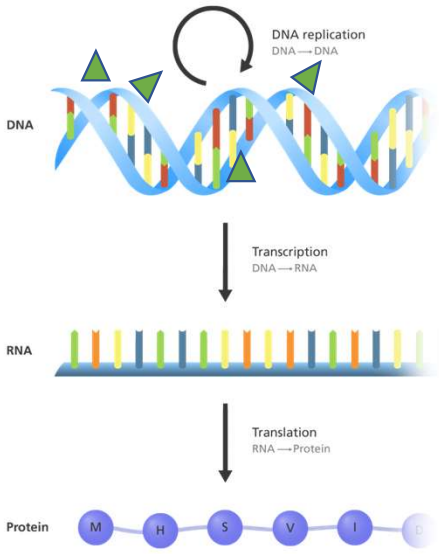


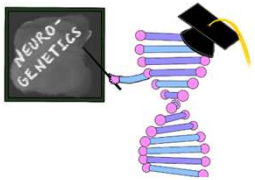
Investigations (Genomic)

- SNP Microarray
  - arr[hg19] **15q11.2-q13.1** (23810397-28525505)x2 hmz. Region of homozygosity observed
  - This region is located on the long arm of chromosome 15 from band q11.2 to band q13.1 from nucleotide 23,810,397 to 28,525,505 and size 4.7 Mb.
    - Numerous genes are mapped within this homozygous region.
  - The presence of a large homozygous region in a single chromosome suggests uniparental disomy. Further molecular testing is recommended to evaluate this interpretation.



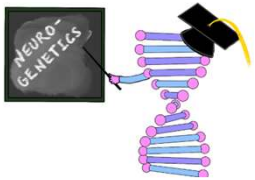
Epigenetic Modification - Definitions

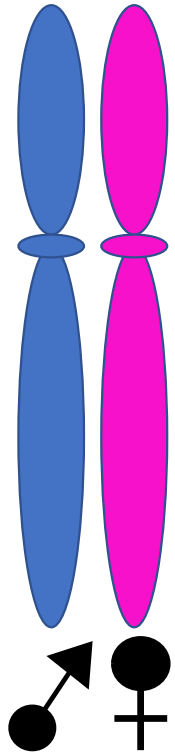




- Control systems for modulating genomic structure and activity in response to cell-extrinsic, cell-cell, and cell-intrinsic signals
  - DNA methylation and hydroxymethylation
  - Histone modifications, nucleosome modification, and chromatin remodeling
  - Noncoding RNAs
  - RNA editing
- Imprinting – differential methylation of DNA (and therefore gene expression/silencing) dependent on parent of origin

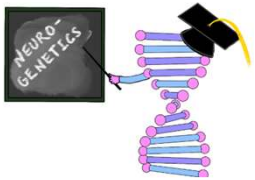
Regions of Homozygosity: Uniparental Disomy

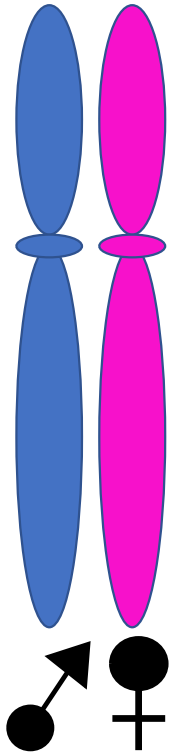




Typical

Regions of Homozygosity: Uniparental Disomy





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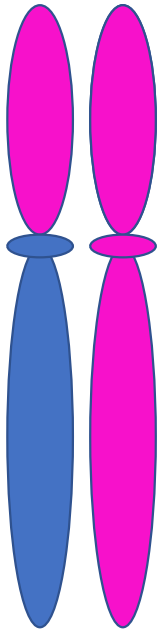
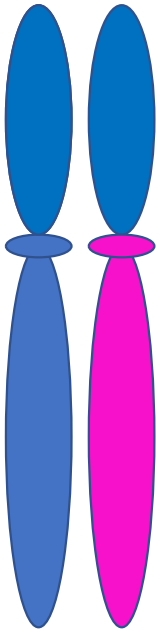


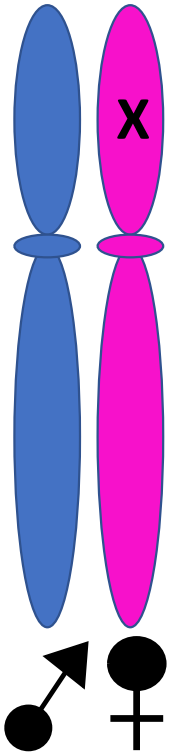
Maternal Uniparental Disomy



Paternal Uniparental Disomy

Regions of Homozygosity: Uniparental Disomy

X=pathogenic variant



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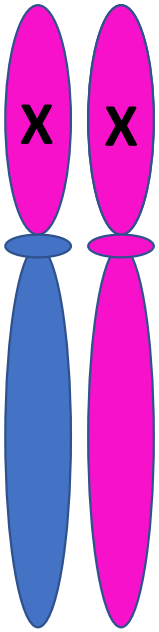
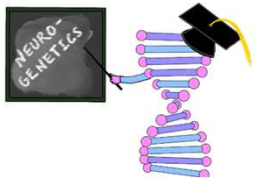
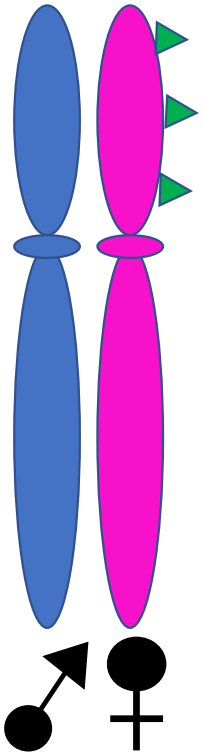


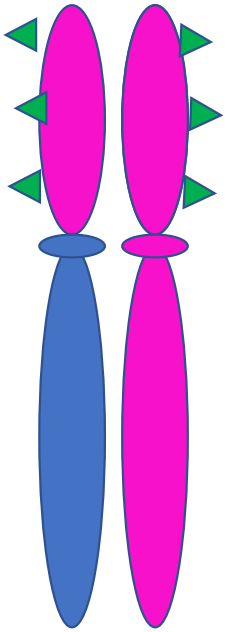
Maternal Uniparental Disomy → Autosomal recessive disease

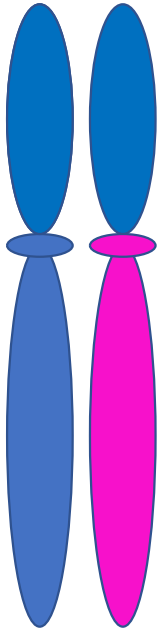


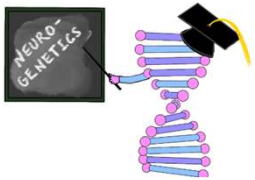
Regions of Homozygosity: Uniparental Disomy



Imprinted/ silenced allele





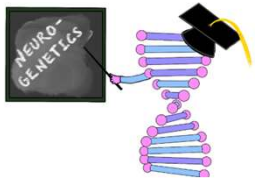


Typical

Maternal Uniparental Disomy underproduction

Paternal Uniparental Disomy overproduction

Chromosomes Implicated in Imprinting Disorders Due to Uniparental Disomy



Maternal UPD

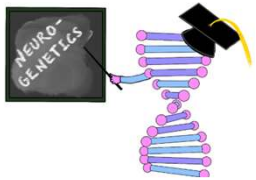
- Chromosome 7
- Chromosome 11
- Chromosome 14
- Chromosome 15
- Chromosome 20

Paternal UPD

- Chromosome 6
- Chromosome 11
- Chromosome 14
- Chromosome 15
- Chromosome 20

Testing for Uniparental Disomy

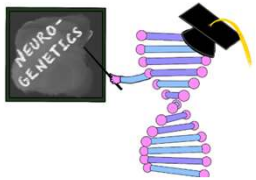
- Multiplex PCR of short tandem repeat markers from patient and parental trios
- Chromosomal Microarray – regions of homozygosity– cannot determine parent of origin
- Analysis of SNP distribution from trio genotype data in the context of exome or genome sequencing
- Methylation-specific PCR (MS-PCR) and methylation specific multiplex ligation-dependent probe amplification (MS-MLPA)
  - Based on interrogation of the methylation status of differentially methylated regions



Interactive

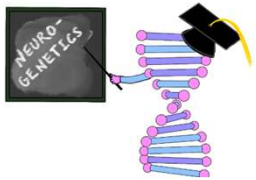
Patient Results: arr[hg19] **15q11.2-q13.1** (23810397-28525505)x2 - hmz region of homozygosity observed

- *Is this an imprinted region?*
- *Are there disorders of uniparental disomy from one or both parents?*
- *What is the next step in testing?*



Interactive

- Patient Results: arr[hg19] **15q11.2-q13.1** (23810397-28525505)x2 – hmz: region of homozygosity observed
- *Is this an imprinted region?*
  - Yes
- *Are there disorders of uniparental disomy from one or both parents?*
  - Maternal uniparental disomy – Prader Willi Syndrome
  - Paternal uniparental disomy – Angelman Syndrome
- *What is the next step in testing?*
  - Methylation specific PCR



Limitations of Testing

CHILD NEUROLOGY SOCIETY

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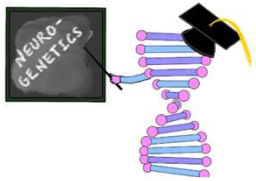
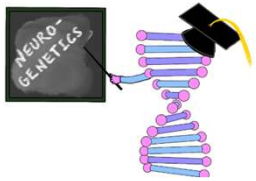


Chromosome 15 Uniparental Disomy

Maternal UPD

Prader Willi Syndrome

- Severe hypotonia and feeding difficulties in early infancy
- Excessive eating in early childhood
- Delayed motor milestones and language development
- Some degree of cognitive impairment
- Hypogonadism - genital hypoplasia, incomplete pubertal development, and, in most, infertility
- Short stature
- Temper tantrums, stubbornness, manipulative behaviors, and obsessive-compulsive characteristics
- Characteristic facial features, strabismus, and scoliosis



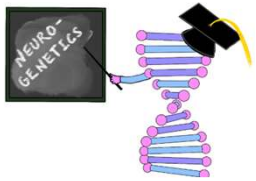
Paternal UPD

Angelman Syndrome

- Severe developmental delay or intellectual disability
- Severe speech impairment
- Gait ataxia and/or tremulousness of the limbs
- Unique behavior with an apparent happy demeanor that includes frequent laughing, smiling, and excitability
- Microcephaly
- Seizures

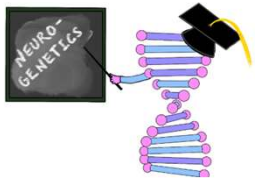
Diagnosis?

- Methylation specific PCR RESULT:
  POSITIVE FOR PRADER-WILLI SYNDROME
  - This individual has only the maternally inherited allele consistent with a diagnosis of Prader-Willi syndrome.



Prader-Willi Syndrome (PWS)

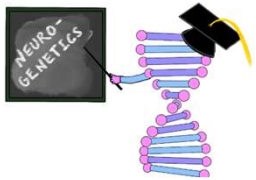
- Loss of function or expression of paternally derived genes
- On the maternal chromosome, these genes have **methylated CpG islands** in their promoter which leads to silencing of the maternal allele
- 60-70% of cases due to 15q11q13 deletion
- Second most common cause is uniparental disomy



- <u>Genes exclusively expressed on paternal chromosome:</u>
  - *SNURF-SNRPN*
  - *MKRN3*
  - *NDN*
  - *MAGEL2*
  - *NPAP1*
  - *PWRN1*
  - *SNORD116*
  - *IPW*
  - *SNORD115*

Suggested Reading

- Morton SU, Christodoulou J, Costain G, et al. Multicenter Consensus Approach to Evaluation of Neonatal Hypotonia in the Genomic Era: A Review. *JAMA Neurol*. 2022;79(4):405-413. doi:10.1001/jamaneurol.2022.0067
- del Gaudio, D., Shinawi, M., Astbury, C. *et al.* Diagnostic testing for uniparental disomy: a points to consider statement from the American College of Medical Genetics and Genomics (ACMG). *Genet Med* **22**, 1133–1141 (2020). https://doi.org/10.1038/s41436-020-0782-9
- Qureshi IA, Mehler MF. Epigenetic mechanisms underlying nervous system diseases. *Handb Clin Neurol*. 2018;147:43-58. doi:10.1016/B978-0-444-63233-3.00005-1



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Core members:
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  - Jeff Strelzik (CNMC)

Committee members:
- Daniel Calame (Baylor)
- Divakar Mithal (Northwestern)
- Christa Habela (Hopkins)
- Kristin Baranano (Hopkins)
- Lisa Emrick (Baylor)
- Margie Ream (Nationwide)
- Julie Ziobro (UM)

Additional Members:
- Alexa Taylor (CNMC)