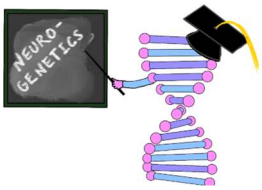


Teaching Guide

Module 17: Somatic Mosaicism

Slide 1: Title

Slide 2: Learning objectives.

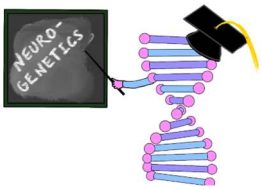
Slide 3: More details (if audience asks, and more history will be revealed in slide 6):

Slide 4: Ask audience to come up with differential diagnosis.

Slide 5: Review differential diagnosis. These are diseases that can cause focal epilepsy along with chronic weakness.

Slide 6: HPI and physical exam. Additional information in case someone asks:

- More information about the case:
    - R-handed
    - Epilepsy onset age 3
    - Semiology: when sleeping, stiffening, pulling of left side, then roll to L side and have eye deviation.
    - Developmental: Walked at 14 months and tended to drag left leg slightly. Normal language development.
    - On valproic acid, clobazam, levetiracetam.
    - No history of seizure risk factors (e.g. meningitis, traumatic brain injury, neonatal seizures)
- Heterochromia iridum is when the two irises are different colors. This can be caused by somatic mosaicism.
- In infants, it can be caused by the following conditions, with bolded ones associated with epilepsy.
    - Benign heterochromia
    - Horner's syndrome
    - **Sturge-Weber syndrome**
    - Waardenburg syndrome
    - Piebaldism
    - Hirschsprung disease
    - **Incontinentia pigmenti or Bloch-Sulzberger syndrome**
    - **von Recklinghausen disease (Neurofibromatosis type 1)**
    - **Tuberous sclerosis**



- **Parry-Romberg syndrome**

Slide 7: Brain MRI also shows **thickening/enlargement of cortex on R lateral frontal cortex** and blurring of gray/white border.

Slide 8: PET scan shows less intense red on R side (reflective of hypometabolism in R frontal lobe). Can compared to left side…

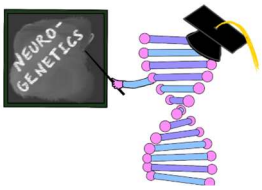
Slide 9: Note that negative gene panel does not mean that the patient's condition does not have a genetic component. "**Negative" can be thought of as non-diagnostic**, rather than ruling out a genetic cause. This holds true for other more comprehensive tests, such as exome or genome sequencing. Also note that the testing was done on saliva, which probably has as good if not **better yield for somatic variants than blood.** A study of saliva vs blood samples for SNP-microarray testing was done on individuals with syndromic ID, and there was a higher yield in saliva than blood samples (PMID: 36446895). This may be because DNA in saliva comes (in part) from the mucous membranes of the mouth (ectodermal), same germ layer as brain, while blood is mesodermal.

Slide 10: Reactive astrocytes and perivascular inflammation is non-specific and very mild. Caveat is that this biopsy was performed on the right temporal lobe not the right frontal lobe where the most significant MRI/PET changes were seen. The temporal lobe instead of frontal lobe was biopsied because of an initial concern for Rasmussen encephalitis and relative ease of access to the temporal lobe. The biopsy resulted in a brief reduction of seizure activity.

Full path report below, for facilitator to be aware of [for discussion, etc] and not necessary to be discussed in the module to adhere to timing and format:
GFAP highlights mild white matter and subpial astrogliosis. NeuN shows appropriately oriented neurons within the cortex. There is no discernible neuronal loss. No balloon neurons or dysplastic forms are seen. The white matter is mildly hypercellular with oligodendroglial hyperplasia and mild hypertensive-like vascular changes often seen in the context of seizure disorder. There are scant perivascular lymphocytes and macrophages as highlighted by CD68 and CD3 stains. There is minimal microgliosis in the white matter and no microglial nodules or perineuronal T-cells are seen. Nestin highlights the microvasculature.

***The changes identified are non-specific and very mild and could conceivably be the result of seizure activity rather than related to etiology**.*

Slide 11: Subsequently, the patient's brain biopsy tissue was sent for next-gen sequencing (locally, using a brain biopsy next generation sequencing test), in 2017.



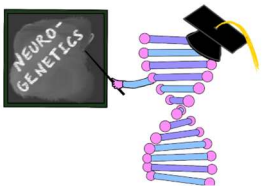
Reminders [for advanced discussion if time permits, if not move straight to the interactive exercise]:

- Nucleotide substitution means one nucleotide is changed to another one **(does not change reading frame)**
- The DNA change is notated by c.7255G>A, which means in the cDNA (if you take the mRNA sequence after introns are spliced out, and then make it into DNA instead of RNA (have thymine instead of uracil), and then go to the 7255 nucleotide position (where 1 is the "A" in the ATG start codon), in the reference sequence (notated as NM_004958.3) there is a "G" there, but in this sample, there is an "A."
    - This causes a change from amino acid Glutamate (E) to a Lysine (K) in the 2419 amino acid position. Note that 7255/3 = 2418.3333, which makes sense with 3 nucleotides making a codon.
- In this next-gen sequencing, the variant allele frequency is 10%, which means that 90% of the reads in that position had a "G" and 10% had an "A." For a germline heterozygous variant, the variant allele frequency would be 50%.

Slide 12: Have the audience break into small groups or breakout rooms and each team can be tasks for answer the following questions:

- *Team A: What does the gene code for and where is it expressed?*
    - *MTOR* encodes the protein Mechanistic Target Of Rapamycin (mTOR), which is a serine/threonine protein kinase, and it is ubiquitously expressed (genecards.org)
- *Team B: What neurological disorders do variants in MTOR cause?*
    - Focal cortical dysplasia type II (somatic variants), and Smith Kingsmore syndrome (megalencephaly, intractable epilepsy, and facial dysmorphism). Also, various tumors, and hemimegalencephaly. (https://www.omim.org/entry/601231)
- *Team C: Is the E2419K variant a gain or loss of function?*
    - Gain of function—causes constitutive activation of the MTOR protein (google, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1805553/, and other sources).
    - Found to be gain of function in functional models (e.g. yeast).
    - Still sensitive to rapamycin (everolimus is an analog of rapamycin).
- *Team D: Does the identified variant explain the clinical presentation?*
    - Yes, because MTOR is a known hemimegalencephaly gene, and even though this specific variant has not been described as much brain malformation and epilepsy, it's a known activating variant in cancers.

Slide 13: Answer to Team B, Team C questions.



Slide 14: These are testing options for somatic variants in brain. Sometimes mosaic variants can also be detected in blood or from the skin, although the level of mosaicism (variant allele frequency) in the brain may differ from that of blood or skin**. Skin is more like brain** developmentally (both are ectoderm) than blood (mesoderm). **So somatic variants affecting the brain are more likely to be found in skin** than blood if brain tissue is not available.

**Note that:

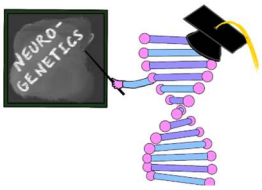
- Sequencing of brain tissue can be done from fresh tissue, a formalin-fixed, paraffin-embedded block, or on formalin-fixed, unstained slides.
- Some commercially available testing:
    - https://seattlechildrenslab.testcatalog.org/show/LAB3281-1
    - https://pathologyservices.wustl.edu/items/somatic-overgrowth-gene-set-ngs/

Slide 15: **Depth of sequencing refers to the number of reads that get mapped back to a specific point in the genome**. For example, if there are 100 next-gen sequencing reads over exon 1 of the mTOR gene, this means there's 100x coverage. But if only 10% of the reads contain a pathogenic variant, then 10 reads will have the variant and 90 will have the reference sequence. So**, if the variant allele frequency is very low (e.g. <1%), a much higher read depth is needed to detect the variant.**

- Note that (as of 6/2023) there are methods being developed to detect somatic variants during presurgical workup for epilepsy, including cell-free DNA in the CSF, and using tissue from stereo EEG electrodes to detect variants (DOI: 10.1002/ana.26080 and 10.1093/braincomms/fcad174).

Slide 16: Schematic showing the difference between germline **mosaicism (where some but not all egg cells contain a variant)** and then all cells in that patient are affected, and somatic mosaicism (where some cells in the patient are affected).

- Note that for diseases that are thought to be caused by a *de novo* in the proband (when with trio genetic testing of blood samples, the parents don't have the variant but the proband does), the recurrence risk for future pregnancies is typically very low. But if one parent has germline mosaicism of that variant, then in this case, the risk for future pregnancies is much higher.
- Germline mosaicism: Variation is present in germ cells in a parent, but no other cells (or very few other cells) in their body; if child inherits variant, all cells expected to be affected.
- Somatic mosaicism: Variation is NOT present in parents; variant occurs during cell division, becomes present in a subset of cells.



Slide 17: Another table showing differences between inherited variants (typically referring to when the parent has the variant in all their cells) and somatic variants.

*Brain biopsies are infrequent in genetics; the goal is to try to take an affected tissue in the least disruptive way possible, and often the negative aspects of an invasive procedure outweigh the positives of completing genetic testing since a given diagnosis/treatment is not guaranteed.

Somatic mutations present in all cells of the human body:

- Consequence of multiple mutational processes
- Intrinsic slight infidelity of DNA replication machinery
- Exogenous or endogenous mutagen exposures
- Enzymatic modification of DNA
- Defective DNA repair

Slide 18: Depending on when somatic variants occur during corticogenesis, different cell types may be affected.

Cortical development—origins of pyramidal neurons and astrocytes in the cerebral cortex.

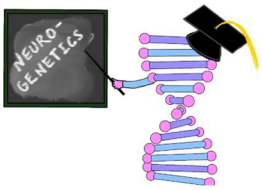
A. A neuroepithelial cell (red) at the ventricular zone serves as progenitor for both a pyramidal neuron (green-blue) as well as a radial glial cell (gold).
B. A newly differentiated neuron (blue) migrates along a radial glial process.
C. Neurons (blue) continue to migrate as intermediate progenitor cells (small yellow) form.
D. Intermediate progenitor cells begin to generate neurons (blue).
E. The progenitor cells in the ventricular zone begin to give rise to astrocytes (dark green). Interneurons (purple) generated elsewhere migrate tangentially.

CP, cortical plate; IZ, intermediate zone; VZ, ventricular zone.

The VZ early in development has a thickness of ~10 cell bodies (50 to 100 mm). The CP ranges in thickness from two to three cell bodies at the earliest stages of development, eventually forming a mature cerebral cortex that is 2 to 4 mm thick.

Slide 19: **In inherited and de novo variants, all the cells in the proband carry the variant**. The earlier a somatic variant occurs; the larger portion of the brain is affected.

- A/B- Represents autosomal dominant inherited epilepsy- in this case inherited from the proband's mother who has the variant; thus, the variant is present in half of her oocytes, and can be passed to offspring. An example would be a channelopathy like a KCNQ2 variant. MRI of the brain is typically normal in these individuals.



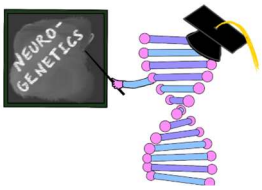
- C/D- **Represents an autosomal dominant variant that arose de novo-the parents themselves are unaffected**, but the pathogenic variant arises in the formation of the gametes, in this example the sperm, as a sporadic variant. The variant is thus present in every cell in the body. In this example, the variant is on LIS1 and associated with lissencephaly and the imaging demonstrates this simplified gyral pattern.
- E/F-This represents an **early post zygotic mutation-one that occurs after the formation of the zygote,** but early enough such that a large population of the cells are affected (including in the leukocytes, which is what tested for most clinical genetic testing). The variant will be visible in a mosaic pattern (not all cells affected). In this example, the variant is in the DCX gene - which is required for normal migration of neurons from the ventricular region to the superficial cortex. The population of cells which carry the pathogenic variant only migrate halfway creating this extra band of grey matter.
- G/H- Represents a late-post zygotic mutation - **present only in certain tissues in a mosaic pattern.** In this case of hemimegaloencephaly, the mutation is present only half of the brain.

Slide 20: Some focal diseases occur because of a second-hit somatic variant. This means that all cells are heterozygous (inherited or de novo) for a variant, and then there is **second variant that occurs in somatic cells (post-zygotic).** This can occur with cancer and can also explain focal disease in some of the mTORopathies (tuberous sclerosis, DEPDC5, NPRL2, and NPRL3).

- Note that this is different from the genetic mechanism of haploinsufficiency, where have a heterozygous variant causes disease typically because half the amount of protein produced is not enough for normal function.
- "Loss of heterozygosity" is a phrase often used to describe the process that leads to the inactivation of the second copy of a tumor suppressor gene. During this process, a heterozygous cell receives a second hit in its remaining functional copy of the tumor suppressor gene, thereby becoming homozygous for the mutated gene. Mutations that inactivate tumor suppressor genes, called loss-of-function mutations, are often point mutations or small deletions that disrupt the function of the protein that is encoded by the gene; chromosomal deletions or breaks that delete the tumor suppressor gene; or instances of somatic recombination during which the normal gene copy is replaced with a mutant copy.

Slide 21: This is a recommended diagnostic workflow for malformations of cortical development (MCD). In the lower left purple, molecular testing in alternative tissue (skin, brain) is mentioned.

- Note that this is just one diagnostic test in a much larger scheme for the approach to MCDs.
- VOUS= variant of uncertain significance
- GOUS = gene of uncertain significance



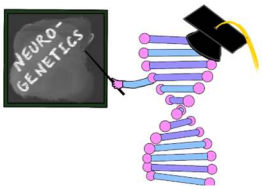
Slide 22: The mTOR pathway is an important in MCD and epilepsy, as hyperactivity of the mTOR pathway caused by mutations of mTOR pathway genes can cause focal cortical dysplasia type 2 (FCD), hemimegalencephaly (HMG), megalencephaly (MEG), tuberous sclerosis complex (TSC), and familial focal epilepsy with variable foci (FFEVF). **The mTOR pathway is a master regulator of metabolism and has important roles in cortical development.**

Slide 23: Back to our patient… Once the hyperactivating MTOR variant was found, and because of the scientific literature supporting that **rapamycin (and analogs such as everolimus) can inhibit mTOR activity in cells carrying this variant,** the patient was started on everolimus (off-label, as this medication is approved for treating TSC). Seizures significantly improved on this medication, and he has not needed epilepsy surgery. This shows the power of precision diagnosis and treatment.

ESES – electrical status epilepticus in sleep

Slide 24 [save for advanced discussion with interested participants]: Outside of the mTOR pathway, there are several other genes that can have somatic mosaicism and are associated with neurological disease in children.

- **SLC35A2 encodes an enzyme involved in glycosylation** and is on the X-chromosome. Heterozygous girls with truncating variants in this gene can have congenital disease of glycosylation.
    - As a side note, remember that X-inactivation occurs in cells with two X chromosomes, such that only one X chromosome expresses its genes. Typically, this is random, but sometimes it's skewed, meaning the wildtype allele or the mutant allele is expressed more. The skewing can be different depending on cell type (neurons might have different skewing than in the blood, but blood cells are easier to measure).
    - **Missense and truncating somatic variants in SLC35A2** can result in non-lesional focal epilepsy, focal cortical dysplasia type 1, mild malformations of cortical development (mMCD), and a more recently described entity mild malformation of cortical development with oligodendroglial hyperplasia and epilepsy (MOGHE).
- With double cortex or subcortical band heterotopia, the concept of random X-inactivation is also important in girls that have **heterozygous pathogenic variants in the DCX gene**. This is because the cells that inactivate the mutant allele can complete the migratory process to form the normal cortex, but those that inactivate the wildtype allele only express the mutant allele and then accumulate in the white matter as a heterotopic band.
- PMG = polymicrogyria



Slide 25: Suggested Reading.

Slide: Acknowledgements.