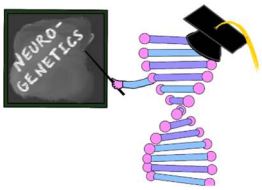


Teaching Guide

Module 3: Neuromuscular 1 – A Child with Leg Weakness

Slide 1: Title slide

Slide 2: Learning objectives for this lesson.

Slide 3: Provides a one-liner and chief complaint for the patient.

Slide 4: Differential diagnosis: Pause, and allow learners to discuss a differential diagnosis for foot drop

Slide 5: This slide discusses differential diagnosis of foot drop- both genetic and non-genetic.

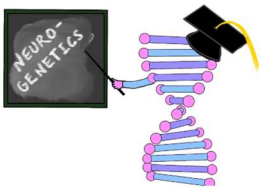
Slides 6: HPI. The instructor can either read aloud or can ask a participant to read. The greater trochanter apophysitis likely was a clinical misdiagnosis made by an orthopedist and instead represented a compression neuropathy caused by prolonged squatting. Before advancing to Slide 7 (Family History), ask the participants for their thoughts about this history. *Is this history suggestive of a particular genetic diagnosis?*

Slide 7: Have a participant read the family history. *Discuss what this suggests in terms of inheritance pattern. "Dominant disorder?"* at the bottom of the slide is hidden ahead of this discussion.

Slide 8: Ask participants *what non-genetic tests they would order*. The test results are hidden ahead of this discussion. The syringohydromyelia was not felt to be explanatory/contributory, but you could ask participants whether this could explain any of the symptoms. The EMG/NCS studies were favored to represent a chronic hereditary rather than acute acquired process. While conduction block was not seen as is often the case in hereditary neuropathy with liability to pressure palsies (HNPP), **the severe focal slowing was suspicious.**

Slides 9-10: 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). Since most genetic conditions are extremely heterogeneous (100s of genes responsible for CMT/HMSN, 1000s for neurodevelopmental disorders), single gene testing can be costly and inefficient
- 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 (e.g., *PMP22* deletion in this case), **check the fine print to make sure that gene is covered**. Believe it or not, *PMP22* is not on the Invitae Comprehensive Neuromuscular Panel!
- 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. At present I think of CMAs as a useful adjunct to exome sequencing as it may or may not be able to detect large to moderate 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 genome sequencing (GS) becomes more available.
- Exome sequencing – **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 – 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 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 is used throughout the series and provides an overview of how to resolve variants of uncertain significance (VUS). Review with participants.

Slide 13: Panel based testing was performed (Comprehensive neuropathy panel) on the proband which identified two variants, **a PATHOGENIC deletion of *PMP22* and a VUS in *SCN10A*.**

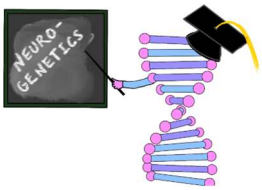
Slide 14: Here we review the two gene variants and the OMIM associated phenotypes with the group. The *SCN10A* variant is a nonsense variant which could in theory be damaging for gene function.

Slide 15: This slide hopefully gets a laugh about the challenges of dealing with VUS.

Slide 16: We begin our VUS resolution by reviewing the OMIM (Online Mendelian Inheritance in Man) entry for SCN10A. **We see that SCN10A encodes a voltage-gated sodium channel which is specific to sensory neurons.** Additionally, we see it is associated with an autosomal dominant condition called "Episodic pain syndrome, familial, 2." Closer review of the entry shows it is **characterized by adult-onset of paroxysmal pain mainly affecting the distal lower extremities associated with small fiber neuropathy.** This already should make us question the relevance of the variant to our patient's condition given the limited phenotypic overlap.

Slide 17: The Molecular Genetics section in the OMIM entry highlights what human genetic studies have been performed on SCN10A to date. It is by no means exhaustive but often is a good starting point. We see that in two family with episodic pain syndrome, heterozygous missense variants in SCN10A were identified. Both variants **caused enhanced SCN10A electrical activity and hyperexcitability of dorsal root ganglia neurons**. Thus, *SCN10A* variants which cause episodic pain syndrome are **gain-of-function**.

Slides 18-20: The next few slides provide "Variant interpretation 101."



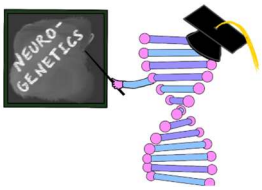
- Slide 18 – The major point here is that **gain-of-function variants are generally (not always!) missense variants** which increase gene function. The figure in the bottom left comes from the initial *SCN10A* episodic pain syndrome paper and clearly shows the missense variant results in more action potentials per input current than wild-type SCN10A. Another example of a gain-of-function variant would be one that increases the binding of a transcription factor to its target genes. As a rule, **gain-of-function variants** cause **dominant** and often ***de novo* disorders** (think of developmental and epileptic encephalopathies like SCN8A, etc).

- Slide 19 – In contrast, **loss-of-function variants** disrupt gene function. The slide provides examples of loss-of-function variants and emphasizes some can be identified based on nucleotide substitution alone, whereas others like missense variants or deep intronic variants need functional confirmation.
    - Examples of loss-of-function variants which can be identified based on nucleotide substitution alone:
        - Nonsense variants
        - Frameshift variants
        - +/- 1 or 2 splice variants – these positions are critical for proper exon splicing, therefore variation in them almost always leads to abnormal splicing
    - Functional confirmation for missense variants might entail studies of enzymatic activity and ion channel function
    - Functional confirmation for deep intronic variants (e.g., the +5 position) would be RNA splicing studies like RNA-seq or RT-PCR

- Slide 20 – since we are talking about loss-of-function/gain-of-function, it is helpful to recognize that loss-of-function variants may cause either dominant/de novo disorders or recessive disorders.
    - For some genes, having two LoF variants in a gene = no gene function and manifestation of a recessive disease. In this case, having a LoF on one allele of the gene would be tolerated
    - In contrast, some genes are haploinsufficient. This means humans must have two working copies of the gene for health; **if you have one LoF variant, you will have a dominant disease.**

Slides 21-23: These slides build upon the concept of GoF and LoF and their relation to mode of inheritance by focusing on TNNT3, a gene associated with both dominant and recessive inheritance models.

- TNNT3 encodes troponin T3, a **critical component of skeletal muscle fibers required for contraction**.



- Gain-of-function missense variants in TNNT3 were first shown to cause autosomal dominant distal arthrogryposis type 2B2, a condition characterized by congenital joint deformities of hands and feet, hip dislocation, and triangular facies.
- Recurrent gain-of-function TNNT3 missense variants affecting the p.Arg63 amino acid increase muscle contractility and therefore result in congenital contractures.
- **In contrast, two loss-of-function function variants in TNNT3 cause a very different phenotype:** severe congenital myopathy characterized by profound neonatal hypotonia and muscle weakness, scoliosis, and muscle degeneration and atrophy.
- The distinct and more severe recessive phenotype reflects the complete loss of TNNT3 and resultant defects in muscle contractility.
- Another example of the Goldilocks principle (will reinforce with PMP22) - too much or too little contractility, end up with disease

Slide 24: Here we bring together the lessons learned to interpret the SCN10A VUS. Since **heterozygous GoF missense variants in SCN10A cause familial episodic pain syndrome 2**, a heterozygous LoF nonsense variant should not cause familial episodic pain syndrome 2. Finally, the phenotype doesn't match!

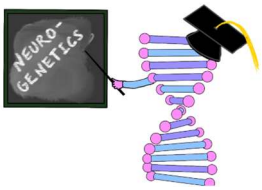
Slide 25: Here we discuss the *PMP22*-related neuropathies. *PMP22* encodes peripheral myelin protein 22, a major protein constituent of myelin sheaths.

PMP22 is highly dosage sensitive:

- Duplication causes the dominant disorder Charcot-Marie-Tooth disease 1A, the classic hereditary motor and sensory neuropathy phenotype characterized by slowly progressive distal weakness, sensory loss, and muscle atrophy.
- Deletion cause hereditary neuropathy with liability to pressure palsies (HNPP)
- Higher order copy number gains (triplication, quadruplication) may result in more severe phenotypes (early disease onset)
    - Can explain a more severe phenotype in the offspring of a less severely affected parent (triplication in child, duplication in parent)
- Thus, *PMP22* is like Goldilocks and the Three Bears: *it must be just right!*

Another relevant example of a dosage sensitive gene is *MECP2*: *MECP2* deletion causes Rett syndrome (seen primarily in girls), whereas *MECP2* duplication causes *MECP2* duplication syndrome (seen primarily in boys).

Slide 26: Many trainees may not understand why dosage sensitivity matters as a neurologist. This slide tries to bring home its importance and relevance in terms of RNA/DNA therapeutics. **Dosage sensitive genes have a very narrow therapeutic window** which complicates gene therapies and ASO development. For example, Steven Gray's lab at UTSW found that early attempts to rescue mouse models of Rett syndrome resulted in lots of dead mice.



Additionally, it is important to realize dosage matters even for genes that aren't dosage sensitive. Val Alstyne et al. recently showed that overexpression of SMN in mice results in late-onset motor neuron disease. Thus, there may be unintended consequences to our therapies which do not manifest until later in life.

Slide 27: This slide shows a figure from an article describing Ambry's experience with molecular testing for Charcot-Marie-Tooth. The main point is that *PMP22* duplications and deletions make up the lions' share of Charcot-Marie-Tooth diagnoses. Thus, **molecular testing for CMT should always include copy number analysis for *PMP22*.**

Slide 28: Here we provide a historical perspective on HNPP. It was first identified by the Dutch neurologist Prof. J.G.Y. de Jong in 1947 in a large multigenerational family with "potato grubbing palsy." Several affected individuals worked as potato grubbers which involved squatting in the fields to pull up potatoes. They would frequently suffer from a **prolonged peroneal palsy due to compression neuropathy.**

Slide 29: Thus, HNPP is both the potato grubber and baseball catcher's palsy!

Slide 30: This slide reviews the clinical features of HNPP*. I would conclude by asking the participants how they would counsel this family.* It is important to avoid or limit activities which might trigger a neuropathy including squatting, sitting cross-legged, resting elbows on a hard surface, and caution while using crutches. Cushions may be employed when such activities are unavoidable. Patients should also avoid neurotoxic medications and limit alcohol consumption.

Slide 31: Suggested reading.

Slide 32: Acknowledgements.