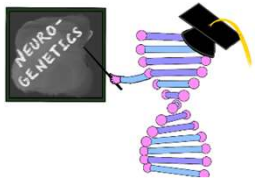
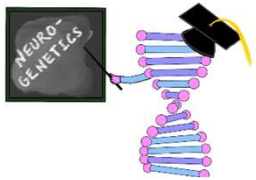
Neuromuscular 1

MODULE 3



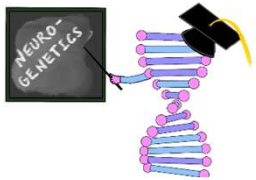
Learning Objectives

1. Develop a genetic differential diagnosis for leg weakness in children
2. Select the optimal genetic testing strategy for patients with neuromuscular diseases
3. Interpret the clinical significance of genetic variants in genetic test reports

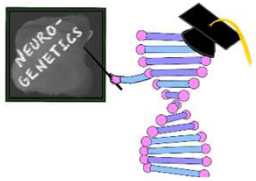


Chief Complaint

- 12-year-old male with bilateral foot drop

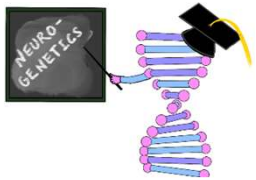


Differential Diagnosis: Foot Drop



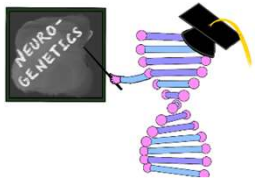
Differential Diagnosis: Foot Drop

- Hereditary neuropathies
  - Demyelinating (*PMP22*, *MPZ*, *GJB1*, *PRX*)
  - Axonal (*MFN2*, *GDAP1*)
- Dystrophic and non-dystrophic myopathies
  - Distal myopathies (*MYH7*, *KLHL9*, *DES*, *GNE*, *DYSF*, *ANO5*)
  - Duchenne & Becker muscular dystrophy (*DMD)*
  - Limb girdle muscular dystrophies (*CAPN3*)
  - Fascioscapulohumeral muscular dystrophy (D4Z4, *SMCHD1*)
- Trauma/Compartment syndrome - peroneal nerve injury
- Toxins - poorly controlled diabetes, chemotherapies, nitrous oxide, heavy metals industrial agents
- Inflammatory polyneuropathy (Guillain-Barre, etc.)



HPI

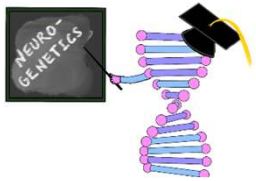
- Healthy, developmentally appropriate 12-year-old male who plays catcher for competitive baseball team
- 1 year ago – diagnosed with left greater trochanter apophysitis (?), developed unexplained bilateral arm weakness while using crutches
  - Took over a month for strength to return
- Has had bilateral foot drop for 2 months.
  - Woke up with foot weakness after a baseball game the night before
- Currently has weakness of foot dorsiflexion and eversion, great toe extension (4/5)



Family History

- Mother's legs and arms often fall asleep after a night's sleep or naps
  - Had EMG/NCS as a child and was told she had the "nerves of a 50-year-old"
- Older brother is suspected to have a muscle disease and also reports his legs and arms fall asleep easily

- *Dominant disorder?*



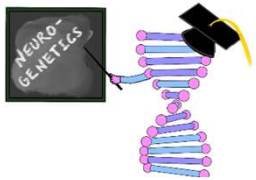
What Tests Would You Order?

Creatine kinase
- normal

MRI brain/spine - syringohydromyelia extending from T3-T12 with maximum diameter of 4 mm. No mass, cord compression, Chiari malformation, or tethered cord

Electromyography/nerve conduction study

- Focal severe slowing of bilateral ulnar nerves across elbow, distal L peroneal across ankle, distal L median nerve
- No conduction block, normal minimum F-wave latencies for L ulnar and median nerves



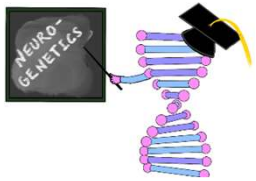
What Genetic Test Would You Order?

Single gene testing

- May be appropriate if high clinical suspicion for single gene disorder
- Genetic heterogeneity for most conditions (e.g., hereditary motor and sensory neuropathies) severely limits diagnostic utility
- May or may not include copy number analysis*

Hereditary neuropathy or neuromuscular panels

- May cover most (but not all) hereditary neuropathy associated genes
- May or may not include copy number analysis*



* Read the fine print!

What Genetic Tests Would You Order?

CHILD NEUROLOGY SOCIETY

Chromosomal microarray

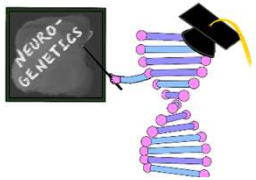
- Covers large deletions or duplications (e.g., *PMP22* deletions/duplications)
- Does not detect single nucleotide variants (SNVs) or indels (small deletions/duplications)

Exome sequencing

- Covers nearly all 20,000 protein coding genes
- May or may not detect large copy number variations (CNVs)

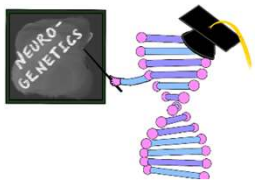
Genome sequencing

- Can detect SNVs, indels, and CNVs
- Limited availability in clinical setting
- Still evolving in the clinical setting – use with caution!



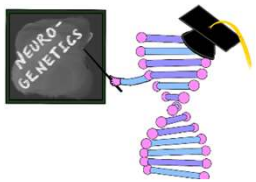
How to Interpret Genetic Test Results

- Review actual test results
- Look at disorders and modes of inheritance; discuss if they fit patient's phenotype
- Use following resources: OMIM, ClinVar, GeneReviews, PubMed



How to Interpret VUS's

- Look at inheritance pattern
- Look at phenotypic match
- Look at variant – present in population? Effect on Protein?
- Familial Segregation Studies
- Functional studies – measure enzyme or transporter activity



Our Patient's Results

CHILD NEUROLOGY SOCIETY

RESULT: POSITIVE

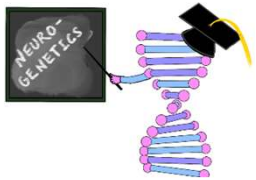
One Pathogenic variant identified in PMP22. PMP22 is associated with autosomal dominant Charcot-Marie-Tooth disease and hereditary neuropathy with liability to pressure palsies.

Additional Variant(s) of Uncertain Significance identified.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
PMP22	Deletion (Entire coding sequence)	heterozygous	PATHOGENIC
SCN10A	c.1534C>T (p.Arg512*)	heterozygous	Uncertain Significance

About this test

This diagnostic test evaluates 111 gene(s) for variants (genetic changes) that are associated with genetic disorders. Diagnostic genetic testing, when combined with family history and other medical results, may provide information to clarify individual risk, support a clinical diagnosis, and assist with the development of a personalized treatment and management strategy.



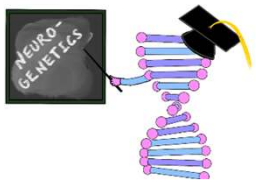
OMIM Phenotypes

PMP22 deletion

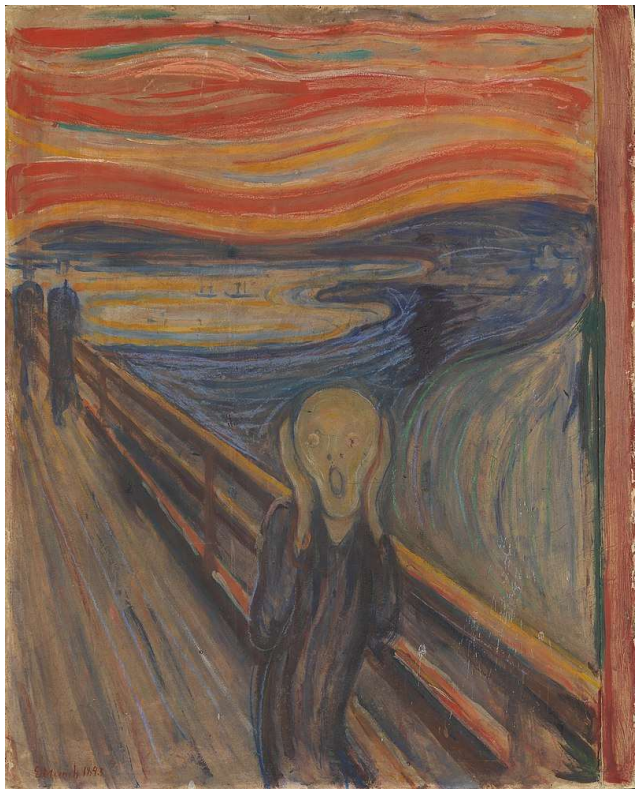
- Pathogenic
- Autosomal dominant hereditary neuropathy with liability for pressure palsies (HNPP)

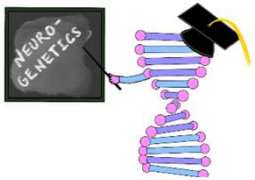
SCN10A:c.1534C>T; p.Arg512Ter

- VUS
- Autosomal dominant familial episodic pain syndrome 2



Variant of Uncertain Significance? Oh no!





OMIM Entry for SCN10A

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50 YEARS OMIM
Human Genetics Knowledge for the World

* 604427

SODIUM VOLTAGE-GATED CHANNEL, ALPHA SUBUNIT 10; SCN10A

Alternative titles; symbols

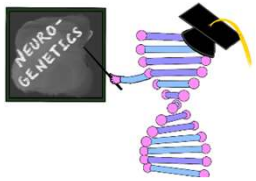
SODIUM CHANNEL, VOLTAGE-GATED, TYPE X, ALPHA SUBUNIT
NAV1.8
PN3
SENSORY NEURON-SPECIFIC SODIUM CHANNEL; SNS

HGNC Approved Gene Symbol: SCN10A

Cytogenetic location: 3p22.2 Genomic coordinates (GRCh38): 3:38,696,807-38,816,217 (from NCBI)

Gene-Phenotype Relationships

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key
3p22.2	Episodic pain syndrome, familial, 2	615551	AD	3



Adult-onset of paroxysmal pain mainly affecting the distal lower extremities associated with small fiber neuropathy

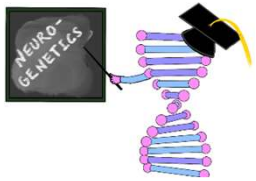
OMIM Entry for SCN10A Cont.

Molecular Genetics

In a father and son with adult-onset familial episodic pain syndrome-2 (FEPS2; 615551), Faber et al. (2012) identified a heterozygous missense mutation in the SCN10A gene (L554P; 604427.0001). An unrelated woman with a similar disorder carried a different heterozygous mutation (A1304T; 604427.0002). In vitro functional expression studies in mouse dorsal root ganglia neurons showed that both mutations caused enhanced channel electrical activities and induced hyperexcitability of DRG neurons. The findings indicated that gain-of-function mutations in SCN10A can cause an episodic pain disorder.

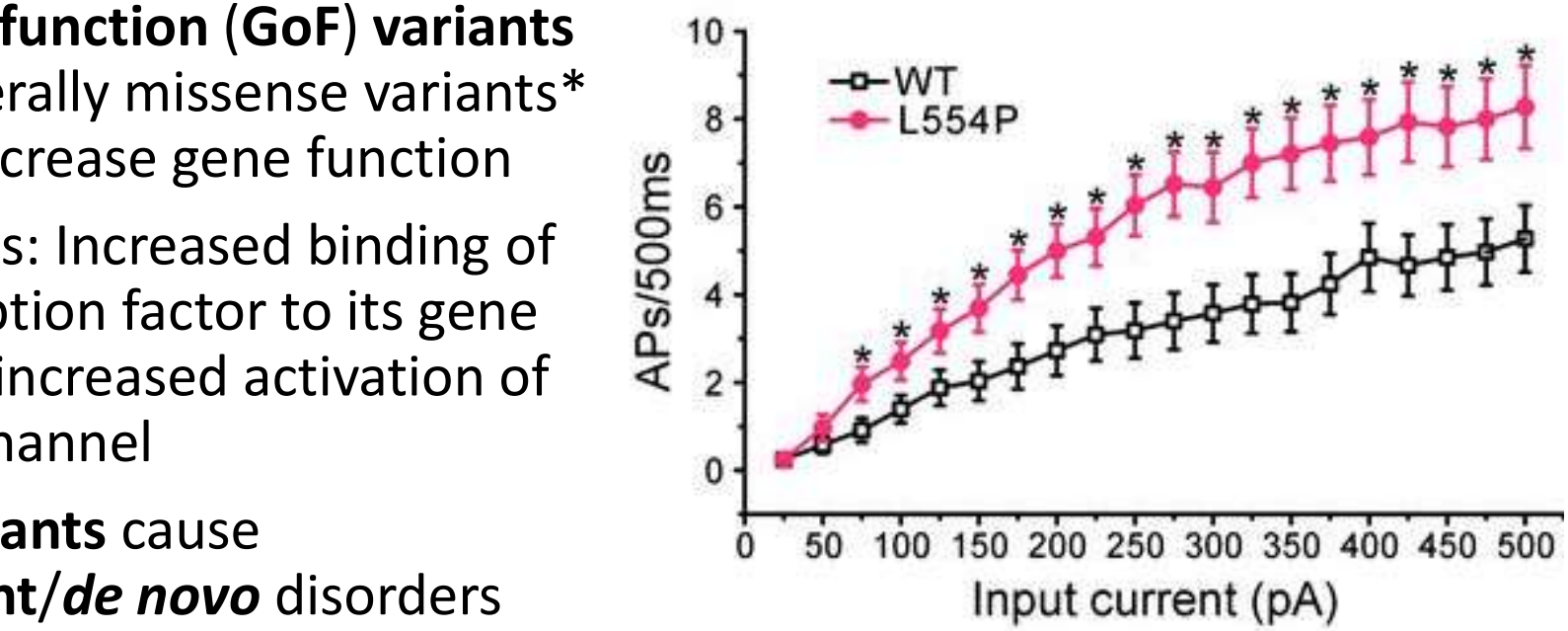
Associations Pending Confirmation

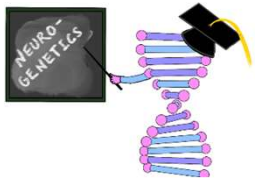
For discussion of a possible association between variants in the SCN5A (600163), SCN10A, and HEY2 (604674) genes and Brugada syndrome, see 601144.



Gain-of-Function Variants

- **Gain-of-function** (**GoF**) **variants** are generally missense variants* which increase gene function
- Examples: Increased binding of transcription factor to its gene targets, increased activation of an ion channel
- **GoF variants** cause **dominant/*de novo*** disorders

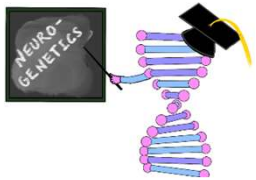




Faber CG et al. Proc Natl Acad Sci U S A. 109(47):19444-9

Loss-of-Function Variants

- **Loss-of-function** (**LoF**) **variants** disrupt gene function
- Examples:
  - Nonsense or frameshift variants causing protein truncation*
  - ±1/2 splice site variants causing abnormal mRNA splicing
  - Missense variants which disrupts gene function
    - *e.g.*, a missense variant in an enzyme catalytic domain causing loss of enzyme function
- **Some LoF variants** can be identified based on the nucleotide substitution alone:
  - **Nonsense variants** (✓)
  - **Frameshift variants** (✓)
  - **±1/2 splice site variants** (✓)
  - *Missense variants* (❌, needs functional confirmation)
  - *Other intronic variants* (❌, needs functional confirmation



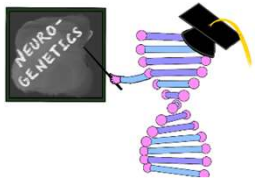
*Generally, but not always. See advanced lessons slides

Loss-of-Function Variants Cont.

LoF variants may result in *either* **dominant/de novo** disorders OR **recessive** disorders

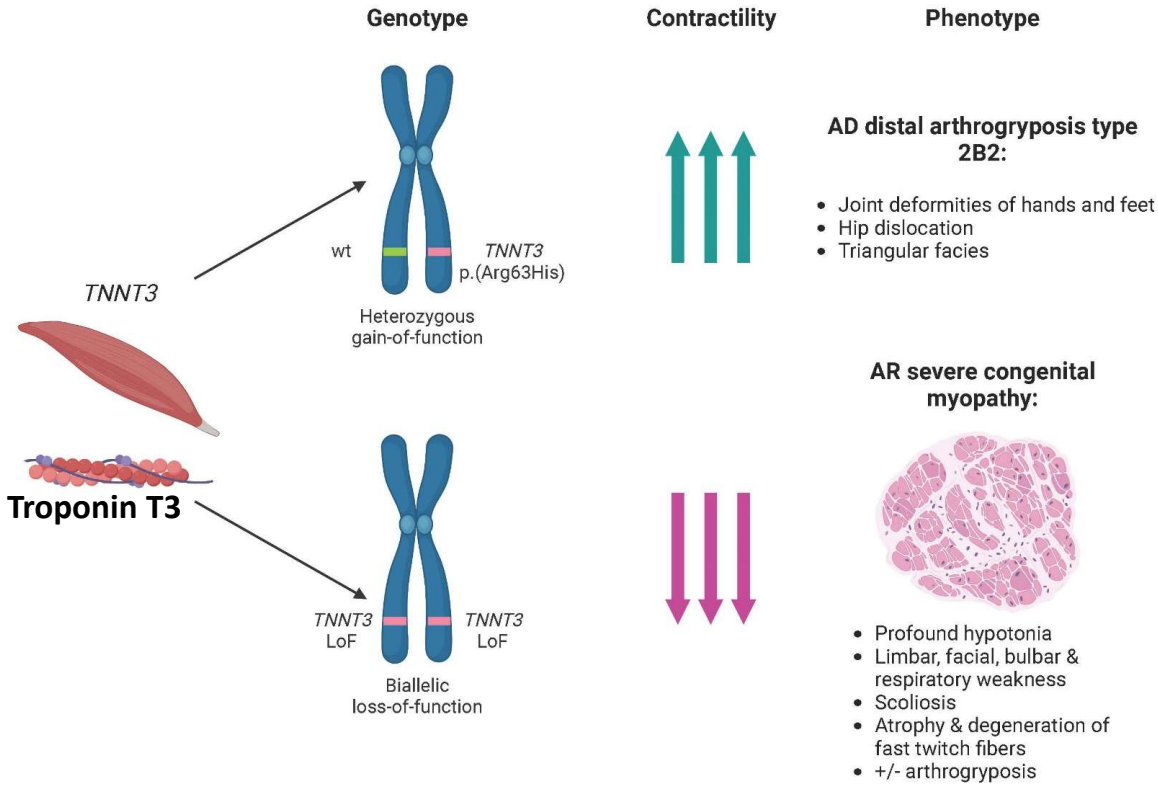
LoF variants cause **dominant/*de novo*** disorders due to **haploinsufficiency**

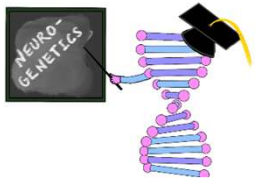
Haploinsufficiency – two working copies of a gene are required for health; a single working copy results in disease



One Gene with Multiple Diseases *and* Inheritance Models

GoF vs LoF

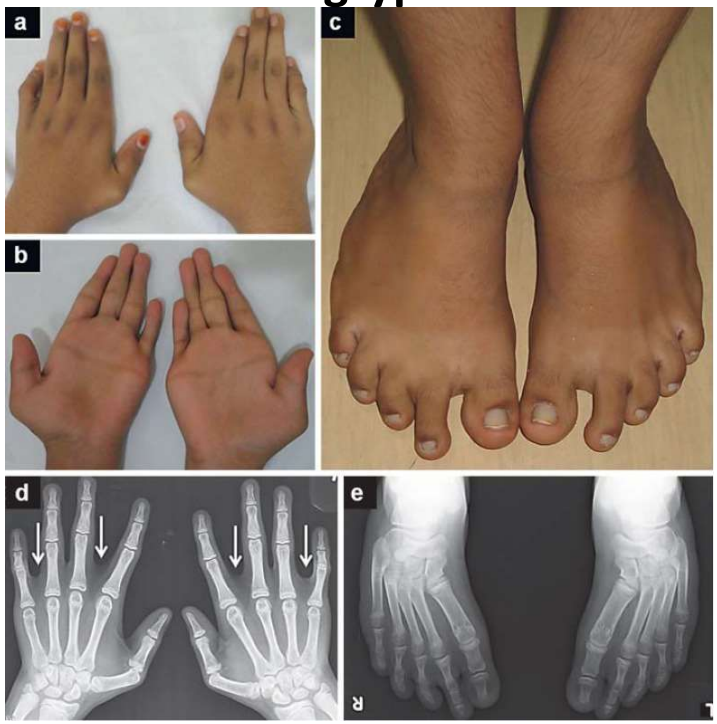


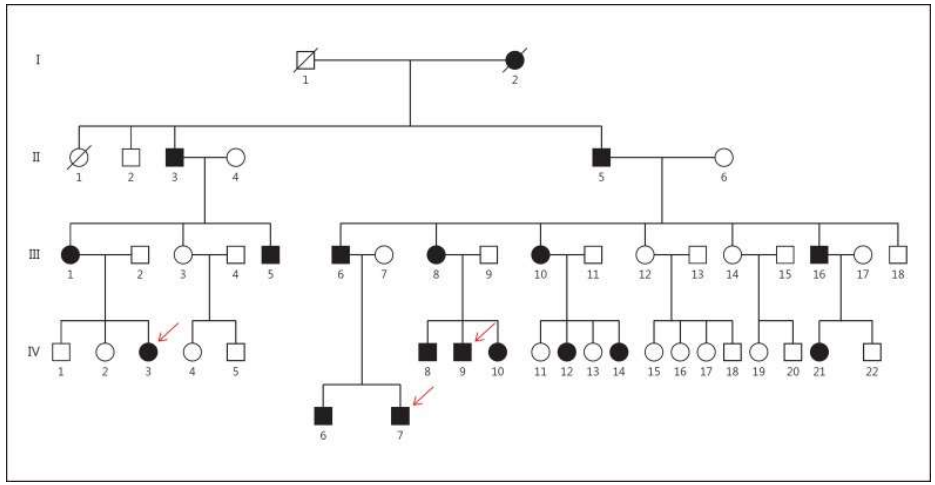


Calame, Marafi, Lupski. *Neurogenetics for the Practitioner* (in press)

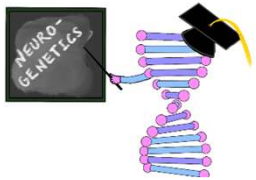
One Gene with Multiple Diseases *and* Inheritance Models Cont.

Autosomal dominant, distal arthrogryposis 2B2





Caused by **GoF** recurrent *TNNT3* **missense variant** c.188G>A (p.Arg63His) which ↑↑↑ muscle contractility



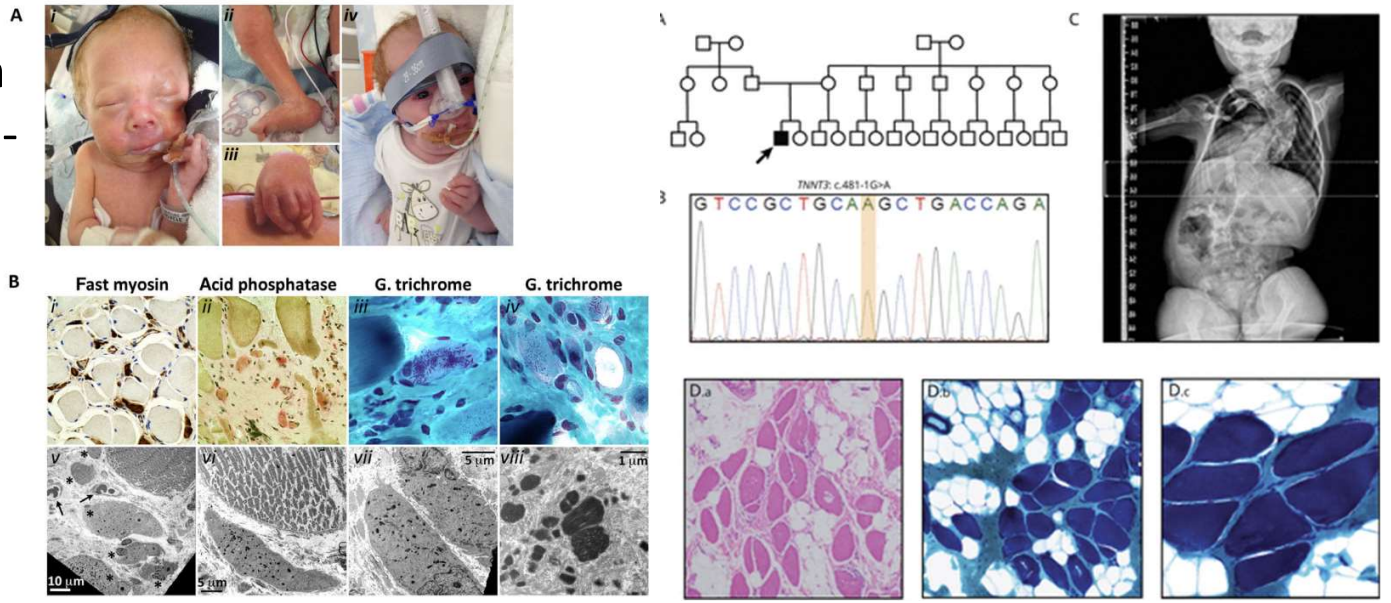
Daly SB, et al. Mol Syndromol. 2014 Aug;5(5):218-28 PMID: 25337069

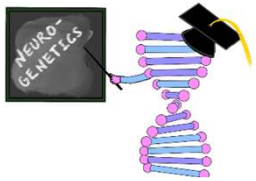
One Gene with Multiple Diseases *and* Inheritance Models Cont.

Homozygous **LoF variants** in ***TNNT3***: c.681+1G>A & c.481-1G>A

severely ↓ ↓ ↓ muscle contractile,

cause autosomal recessive congenital myopathy





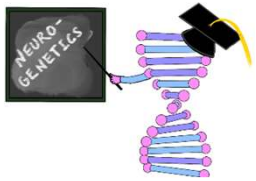
Sandaradura et al. Hum Mutat. 2018 Mar;39(3):383-388. Calame et al. Neurol Genet. 2021 Apr 26;7(3):e589.

Interpretation of *SCN10A*:c.1534C>T; p.Arg512Ter

Heterozygous GoF missense variants result in familial episodic pain syndrome 2

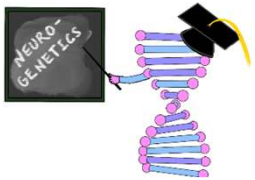
Heterozygous LoF nonsense variant is not known to cause familial episodic pain syndrome 2

Plus, the phenotype doesn't fit familial episodic pain syndrome 2!



PMP22-Related Neuropathies

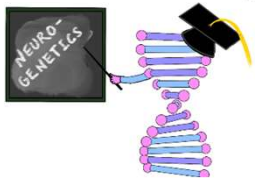
- *PMP22* encodes peripheral myelin protein 22
- *PMP22* is exquisitely dosage sensitive
  - Duplication of *PMP22* causes Charcot-Marie-Tooth disease 1A
  - Deletion of *PMP22* causes hereditary neuropathy with liability to pressure palsies (HNPP)
- *PMP22** is like Goldilocks and the Three Bears: *it must be just right*



**MECP2* is too! *MECP2* LoF = Rett syndrome, *MECP2* duplication syndrome

Why Does Dosage Sensitivity Matter?

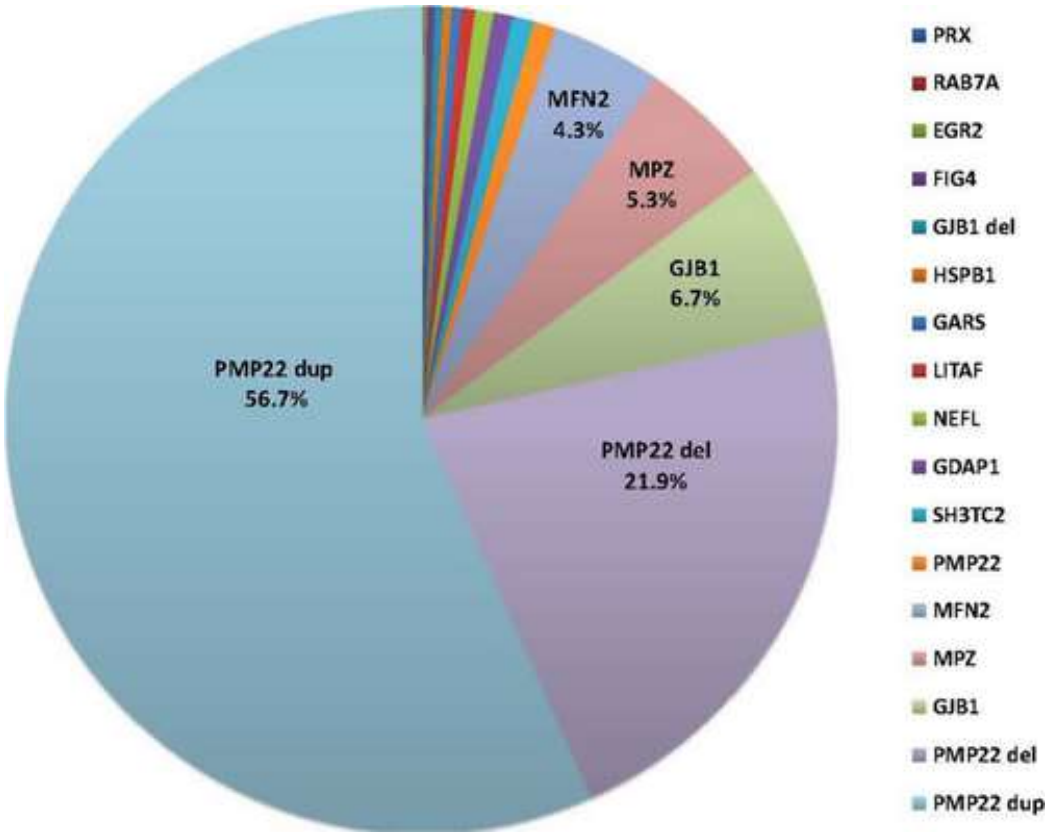
- It matters for RNA/DNA therapeutics: gene therapies (AAV), ASO, siRNA, etc.
- If too much or too little of a gene causes disease, the therapeutic window is narrow
  - e.g., early attempts to rescue Rett mice with AAV9-MECP2 → lots of dead mice
- Sometimes there can be too much of a good thing, even for genes that aren't dosage sensitive
  - e.g., AAV9-mediated SMN overexpression causes dose-dependent, late-onset motor neuron disease in mice

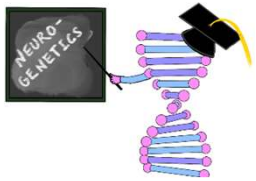


Van Alstyne M, et al. Nat Neurosci. 2021 Jul;24(7):920-940.

PMP22-Related Neuropathies

- *PMP22* duplications and deletions are the most common causes of demyelinating hereditary motor and sensory neuropathies



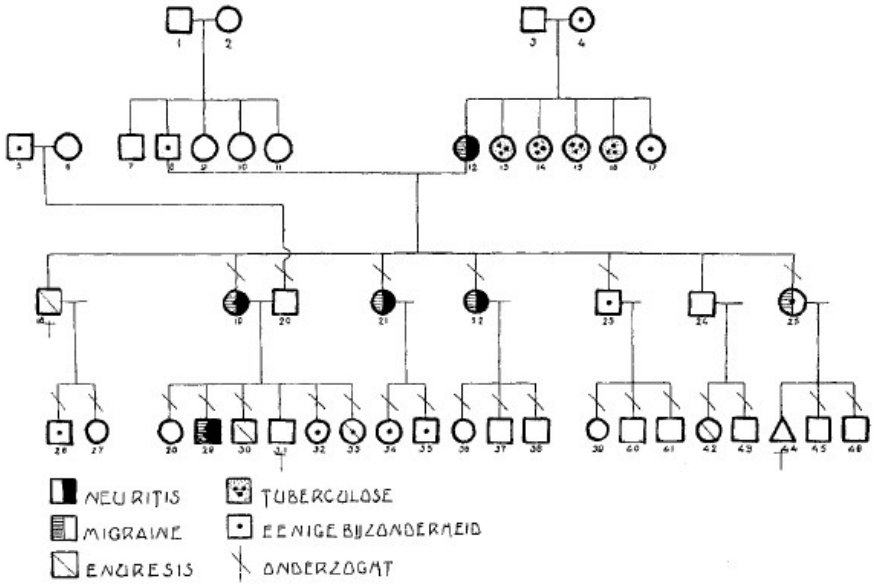


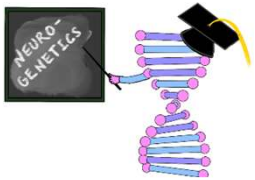
DiVincenzo C, et al. Mol Genet Genomic Med. 2014 Nov;2(6):522-9.

Hereditary Neuropathy with Liability to Pressure Palsies

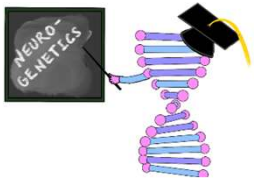
- First identified by Prof. J.G.Y. de Jong in 1947
- “Potato grubbing palsy”

Figure 1. J.G.Y. de Jong (1909–1998).





Koehler PJ. Neurology. 2003 Apr 8;60(7):1211-3. PMID: 12682341.


NEURO-
GENETICS

Hereditary Neuropathy with Liability to Pressure Palsies

Recurrent acute sensory and motor neuropathy in single or multiple nerves often triggered by transient compression

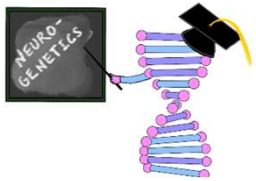
Often painless though neuropathic pain can occur

Complete recovery or mild residual disability from each compressive neuropathy

May develop distal symmetric polyneuropathy resembling CMT1A with time

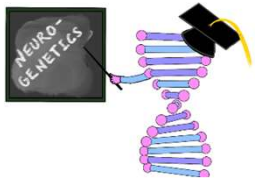
Typical NCS findings

- **Diffuse sensory NCV slowing**
- **Prolonged distal motor latencies**
- **Minor reduction in motor NCV**



Suggested Reading

- Koehler PJ. Hereditary neuropathy with liability to pressure palsies: the first publication (1947). Neurology. 2003 Apr 8;60(7):1211-3. PMID: 12682341.
- Bird TD. Charcot-Marie-Tooth Hereditary Neuropathy Overview. 1998 Sep 28 [updated 2023 Feb 23]. GeneReviews®. PMID: 20301532.
- Calame DG, Marafi D, Lupski JR. Neurogenetics for the Practitioner (in press)



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