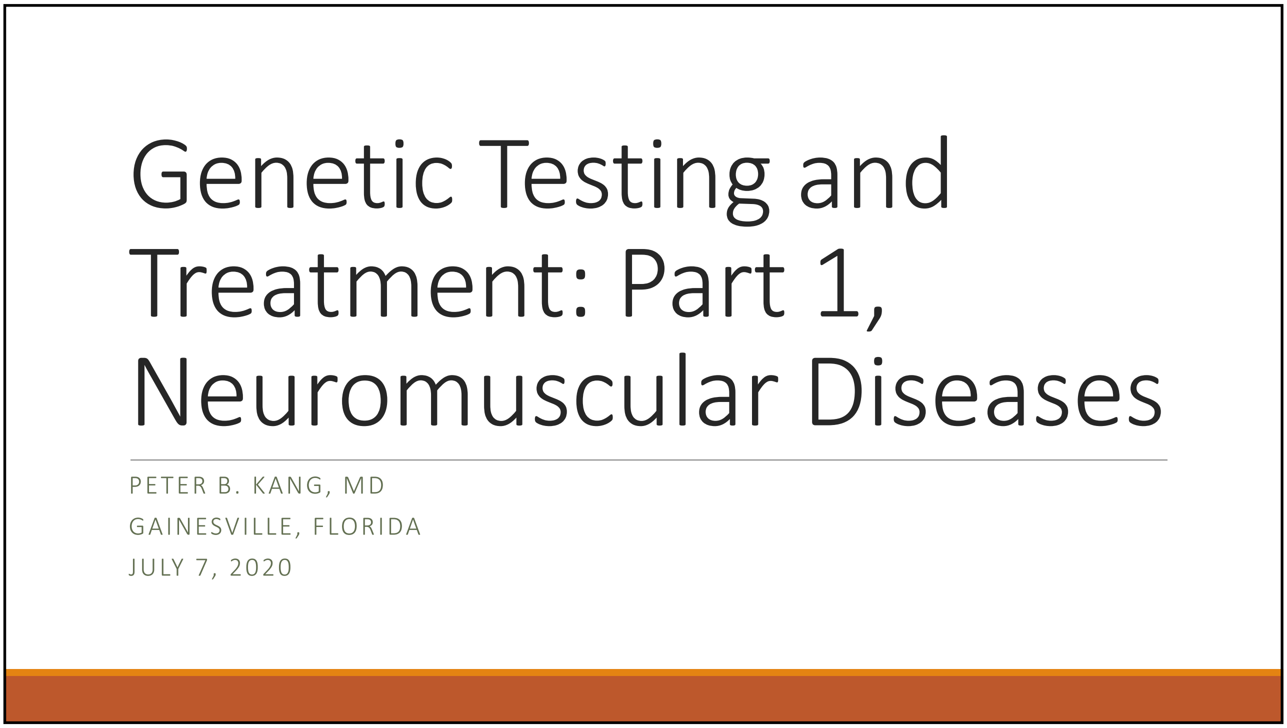

Genetic Testing and Treatment: Part 1, Neuromuscular Diseases

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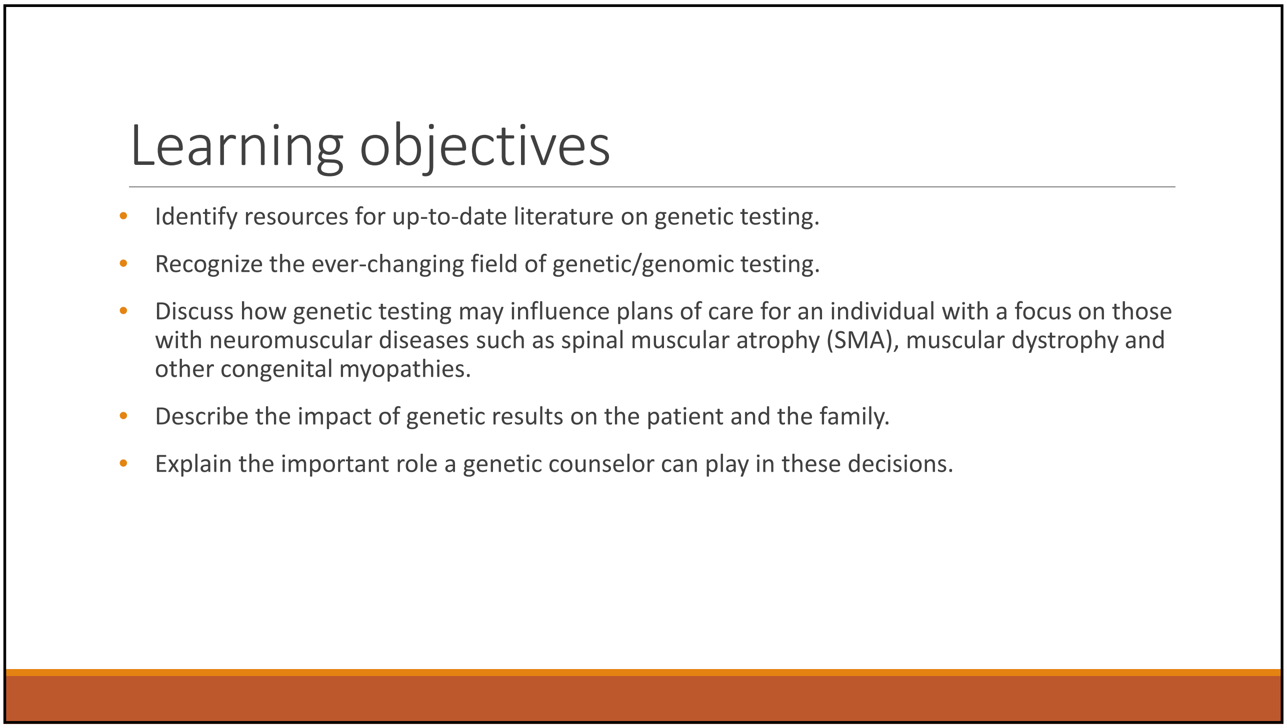
JULY 7, 2020



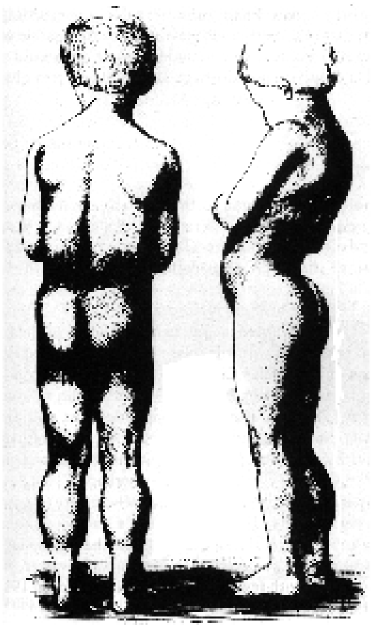
Learning objectives

- Identify resources for up-to-date literature on genetic testing.
- Recognize the ever-changing field of genetic/genomic testing.
- Discuss how genetic testing may influence plans of care for an individual with a focus on those with neuromuscular diseases such as spinal muscular atrophy (SMA), muscular dystrophy and other congenital myopathies.
- Describe the impact of genetic results on the patient and the family.
- Explain the important role a genetic counselor can play in these decisions.

Outline

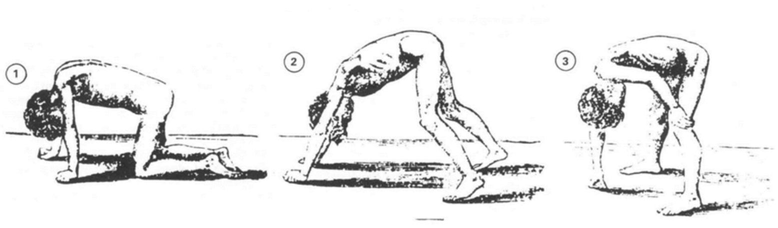
- Basics of inherited neuromuscular disease
- Why bother finding the mutation(s)?
- Genetic testing, old-fashioned and newfangled
- What to do when you have the diagnosis
- Modern libraries
- Sample case

Basics of inherited neuromuscular disease

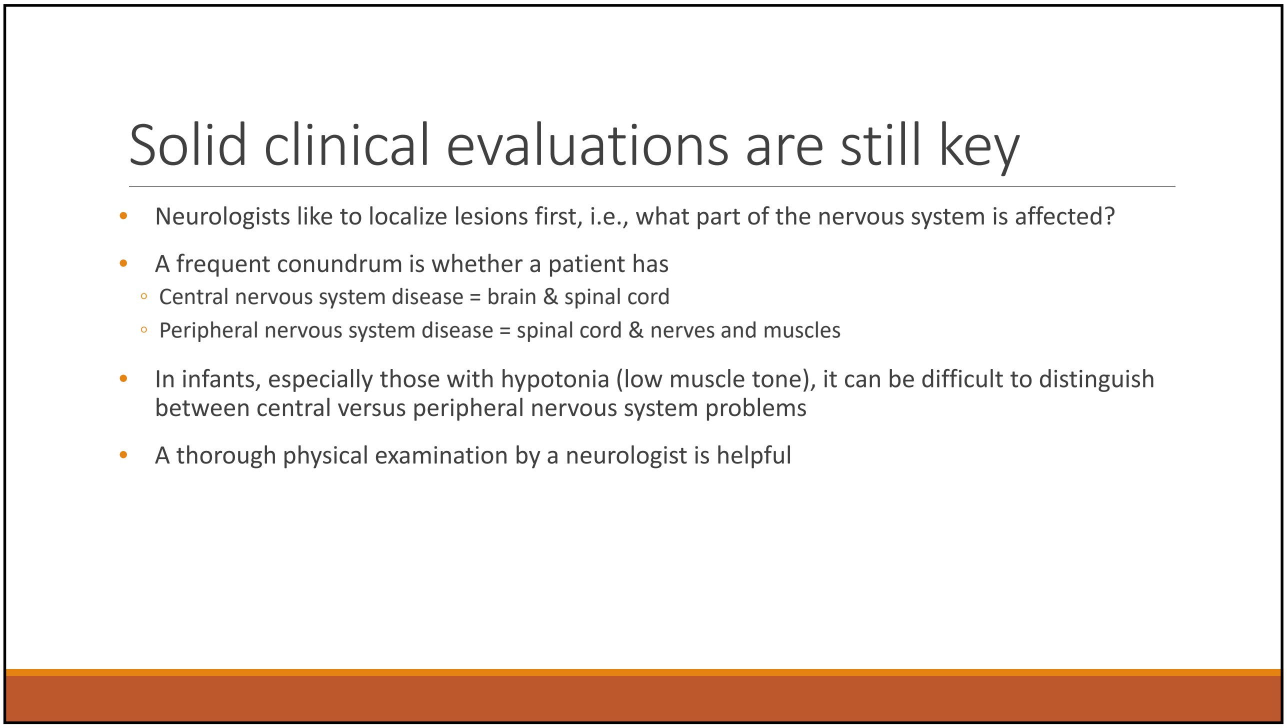


Duchenne GBA. De l’ectrisation localisee et de son application a la pathologie et a la therapeutique, 2nd ed. Paris: Bailliere;1861. p. 353-356.

Figure is a drawing based on a photograph of an early patient.

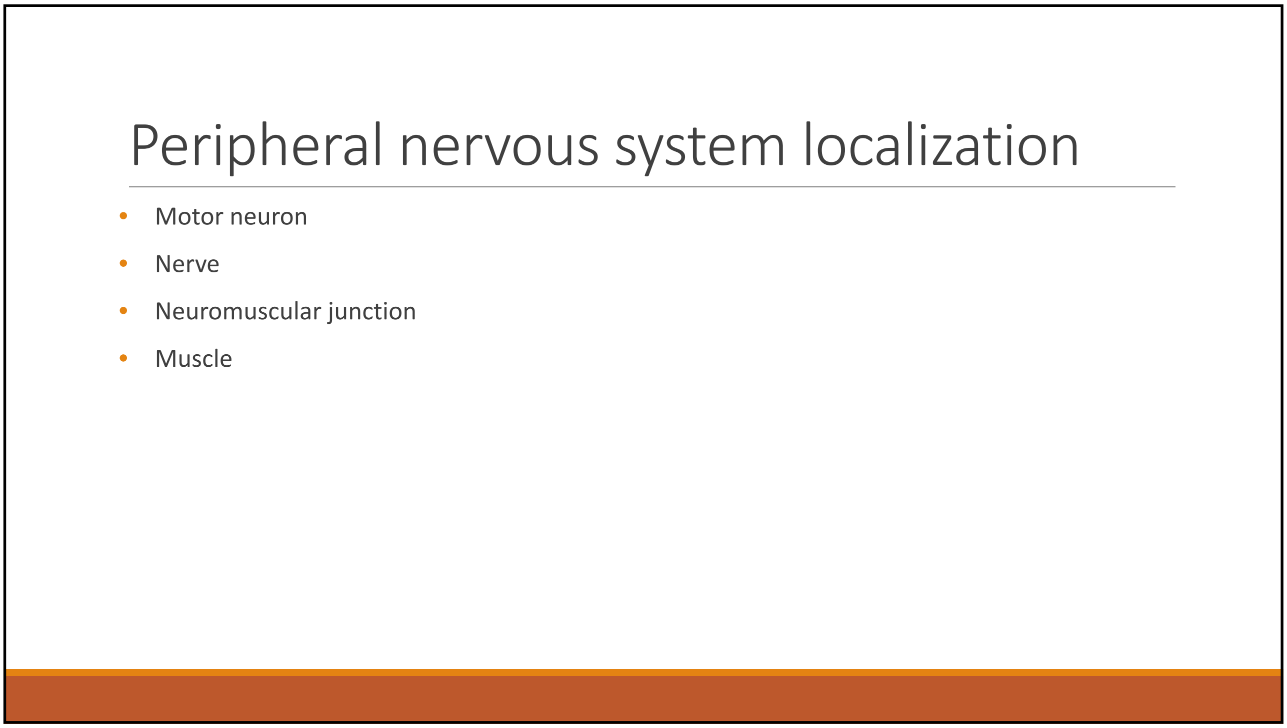


Gowers WR. Clinical lecture on pseudohypertrophic muscular paralysis. Lancet 1879;ii,73-5.
http://www.wikipedia.org



Solid clinical evaluations are still key

- Neurologists like to localize lesions first, i.e., what part of the nervous system is affected?
- A frequent conundrum is whether a patient has
  - Central nervous system disease = brain & spinal cord
  - Peripheral nervous system disease = spinal cord & nerves and muscles
- In infants, especially those with hypotonia (low muscle tone), it can be difficult to distinguish between central versus peripheral nervous system problems
- A thorough physical examination by a neurologist is helpful



Peripheral nervous system localization

- Motor neuron
- Nerve
- Neuromuscular junction
- Muscle

Peripheral nervous system localization

Motor neuron
- Spinal muscular atrophy
- Acute flaccid myelitis

Nerve
- Charcot-Marie-Tooth disease
- Guillain-Barré syndrome

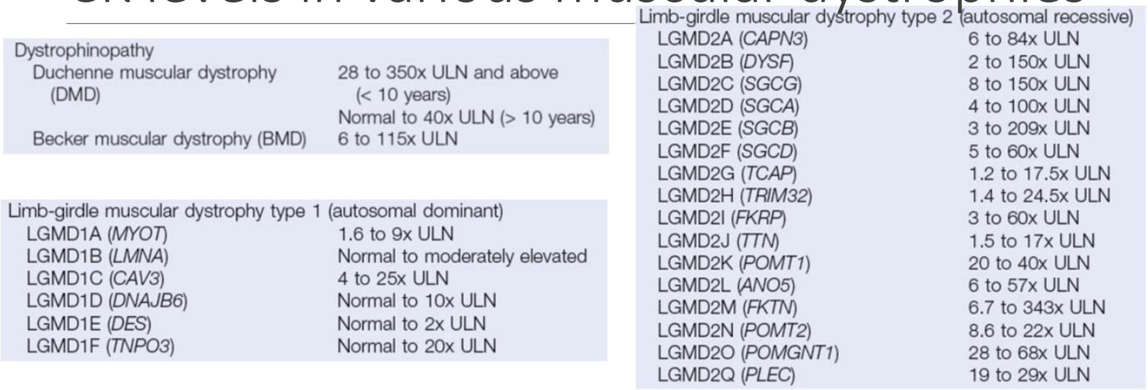
Neuromuscular junction
- Congenital myasthenic syndrome
- Myasthenia gravis

Muscle
- Muscular dystrophy
  - Duchenne muscular dystrophy
  - Becker muscular dystrophy
  - Limb-girdle muscular dystrophy
  - Congenital muscular dystrophy
- Congenital myopathy
- Myotonic disorders
- Inflammatory myopathy

Resources for neuromuscular evaluations

- Neurologist, adult or pediatric depending on age
- Basic clinical laboratory tests:
  - Creatine kinase (CK), aldolase, ALT, AST, LDH
  - Anti-Jo antibodies
- Electromyography (EMG) laboratory
- Muscle and nerve biopsies
  - Some neurologists do their own biopsies, especially for adults
  - In children, surgeons often do the biopsies under general anesthesia
  - Pathology laboratory and pathologist who can process and interpret
- Genetic testing, done by various facilities around the country
- Genetic counselor

CK levels in various muscular dystrophies



Dystrophinopathy	
Duchenne muscular dystrophy (DMD)	28 to 350x ULN and above (< 10 years) Normal to 40x ULN (> 10 years)
Becker muscular dystrophy (BMD)	6 to 115x ULN

Limb-girdle muscular dystrophy type 1 (autosomal dominant)	
LGMD1A (*MYOT*)	1.6 to 9x ULN
LGMD1B (*LMNA*)	Normal to moderately elevated
LGMD1C (*CAV3*)	4 to 25x ULN
LGMD1D (*DNAJB6*)	Normal to 10x ULN
LGMD1E (*DES*)	Normal to 2x ULN
LGMD1F (*TNPO3*)	Normal to 20x ULN

Limb-girdle muscular dystrophy type 2 (autosomal recessive)	
LGMD2A (*CAPN3*)	6 to 84x ULN
LGMD2B (*DYSF*)	2 to 150x ULN
LGMD2C (*SGCG*)	8 to 150x ULN
LGMD2D (*SGCA*)	4 to 100x ULN
LGMD2E (*SGCB*)	3 to 209x ULN
LGMD2F (*SGCD*)	5 to 60x ULN
LGMD2G (*TCAP*)	1.2 to 17.5x ULN
LGMD2H (*TRIM32*)	1.4 to 24.5x ULN
LGMD2I (*FKRP*)	3 to 60x ULN
LGMD2J (*TTN*)	1.5 to 17x ULN
LGMD2K (*POMT1*)	20 to 40x ULN
LGMD2L (*ANO5*)	6 to 57x ULN
LGMD2M (*FKTN*)	6.7 to 343x ULN
LGMD2N (*POMT2*)	8.6 to 22x ULN
LGMD2O (*POMGNT1*)	28 to 68x ULN
LGMD2Q (*PLEC*)	19 to 29x ULN

Kang PB, Mercurio E. Laboratory assessment of the child with suspected neuromuscular disorders. In: *Swaiman's Pediatric Neurology: Principles and Practice*, Swaiman KF, Ashwal S, Ferriero DM, Schor NF, Finkel RS, Gropman AL, Pearl PL, Shevell M, editors. 6th editon. London: Elsevier 2017. Chapter 136, p.1038-1043.

Why bother finding the mutation?

The natural outcome may be favorable

- There may be a perception that genetic diseases worsen relentlessly, or at best stay static
- This may be true of some, but not all inherited neuromuscular disorders
- The natural history of these diseases can be complex
- Example: infants with congenital myotonic dystrophy often have striking respiratory and feeding difficulties early on, but these often spontaneous improve [Campbell C et al, *Pediatrics* 2004;113:811-816]

Even supportive therapies make a difference

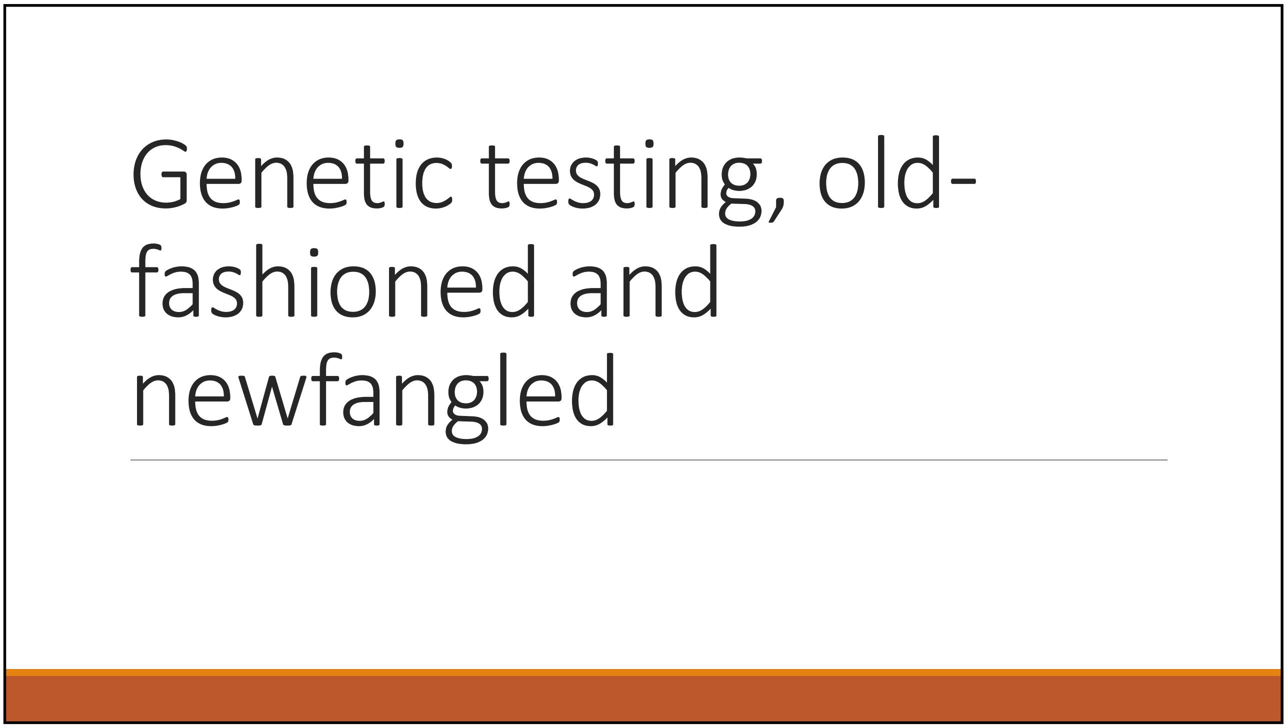
- Spinal muscular atrophy [Finkel RS et al, *Neurology* 2014;83:810-817]
  - Ventilatory support, non-invasive (via mask) or invasive
  - Nutritional support via gastrostomy (G-tube)
  - Physical therapy
  - Orthoses (braces)
- Duchenne muscular dystrophy [Birnkrant DJ et al, *Lancet Neurol* 2018;17:251-267]
  - Respiratory support
  - Physical therapy
  - Orthoses (braces)

Some drugs work for specific genes

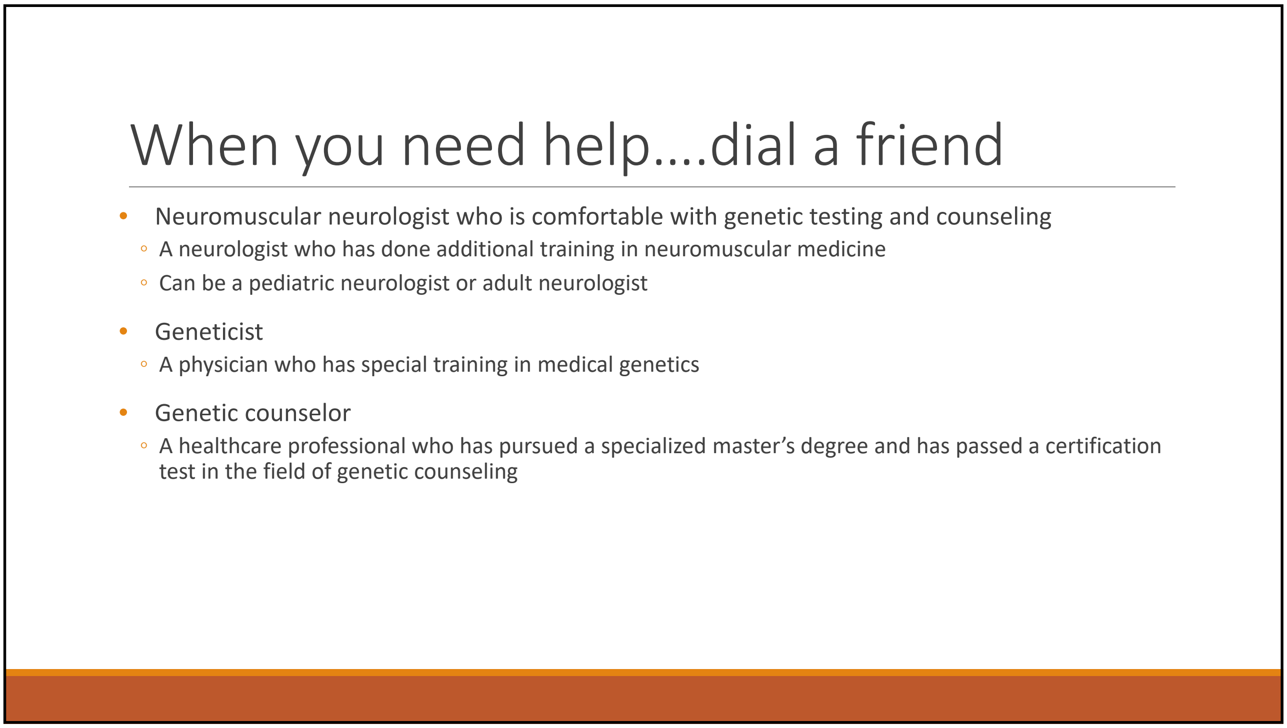
- Congenital myasthenic syndromes [Engel AG et al, *Lancet Neurol* 2015;14:420-434]
  - Choline acetyltransferase deficiency (*CHAT*)
    - Use pyridostigmine
  - Acetylcholinesterase deficiency
    - Use albuterol (salbutamol) or ephedrine
    - AVOID pyridostigmine and amifampridine
  - Dok-7 deficiency (*DOK7*)
    - albuterol (salbutamol) or ephedrine
    - AVOID pyridostigmine
  - Rapsyn deficiency (*RAPSN*):
    - Pyridostigmine, amifampridine, albuterol

The new age in neuromuscular therapy

- There have been 4 new FDA-approved therapies for inherited neuromuscular diseases that primarily affect children since 2016
- Spinal muscular atrophy
  - 2016: nusinersen (antisense oligonucleotides)
    - [Finkel RS et al, *N Engl J Med* 2017;377:1723-1732]
  - 2019: onasemnogene abeparvovec (gene therapy)
    - [Mendell JR et al, *N Engl J Med* 2017;377:1713-1722]
- Duchenne muscular dystrophy
  - 2016: eteplirsen (antisense oligonucleotides)
    - [Mendell JR et al, *Ann Neurol* 2016;79:257-271]
  - 2017: deflazacort (steroid)
    - [Griggs RC et al, *Neurology* 2016;87:2123-2131]



Genetic testing, old-fashioned and newfangled



When you need help….dial a friend

- Neuromuscular neurologist who is comfortable with genetic testing and counseling
  - A neurologist who has done additional training in neuromuscular medicine
  - Can be a pediatric neurologist or adult neurologist
- Geneticist
  - A physician who has special training in medical genetics
- Genetic counselor
  - A healthcare professional who has pursued a specialized master’s degree and has passed a certification test in the field of genetic counseling

Patterns of inheritance

- Autosomal recessive
  - You need two mutations (one on each copy of the gene) to cause disease
  - Usually clustered in a single generation/branch
- Autosomal dominant
  - You only need one mutation (on one copy of the gene) to cause disease
  - Vertical transmission (usually a patient has a parent who is affected)
- X-linked recessive
  - Mutation is on the X chromosome
  - Predominantly males, as males only need one mutation to have the disease
- X-linked dominant
  - Mutation is on the X chromosome
  - Predominantly females, mutations may be fatal in males

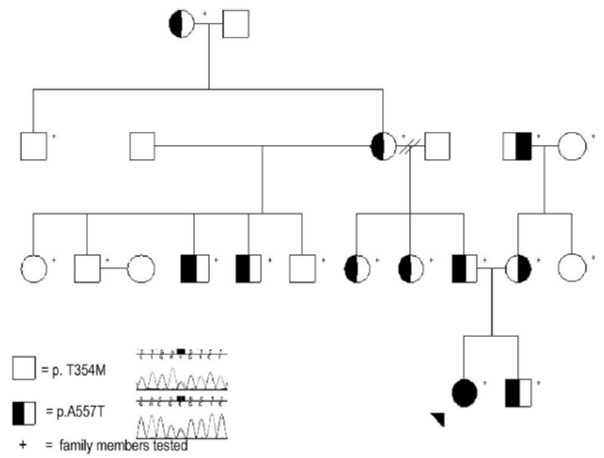


Figure 1. *Pedigree of family under study, with chromatograms. Standard pedigree symbols are used. Arrowhead indicates proband. +, individuals who were studied. The mutation inherited from the maternal line is c.1061C>T (p.T354M) in exon 7, extending back at least two generations. The mutation inherited from the paternal line is c.1669G>A (p.A557T) in exon 12, extending back at least three generations. There is no known consanguinity in the family.*

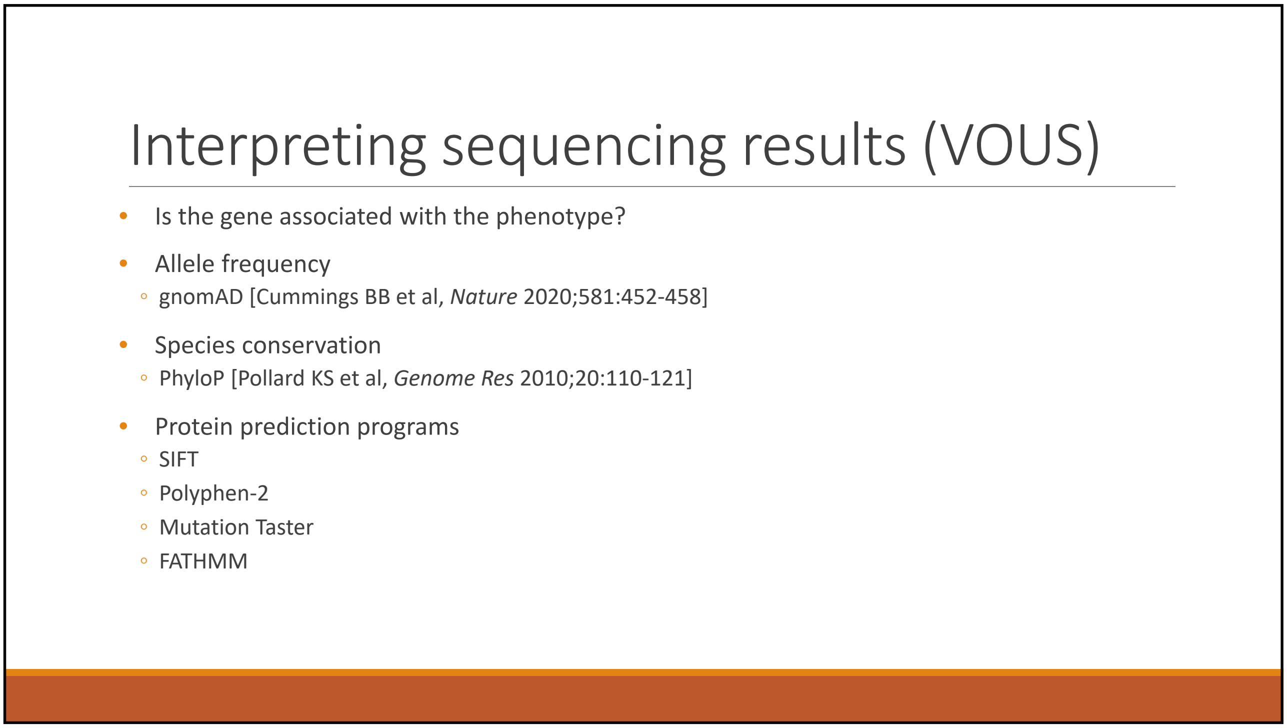
Mallory et al, *Pediatric Neurology* 2009;41:42-45

The genetic code

- Genomic DNA
    - ↓ transcription
- Pre-messenger RNA (pre-mRNA)
    - ↓ splicing
- Messenger RNA (mRNA)
    - ↓ translation
- Protein (string of amino acids)

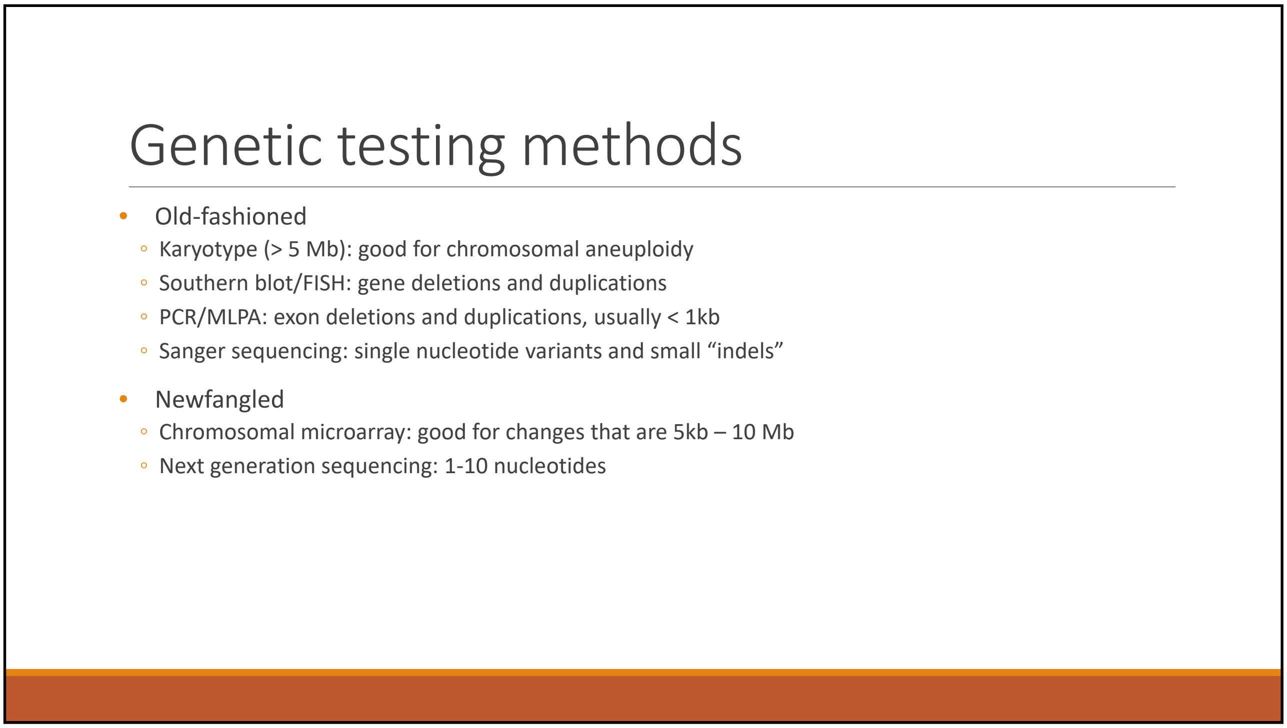
Common types of variants

- Nonsense changes: introduce stop codons, truncate proteins
    - Often disease-causing
- Frameshift: alters reading frame → alters numerous amino acids
    - Often disease-causing
- Structural variants (SVs), including copy number variants (CNVs)
    - May be disease-causing
- Missense variants (non-synonymous): alter single amino acids
    - May be disease-causing
- Synonymous variants: does not alter amino acid sequence
    - Usually not disease-causing



Interpreting sequencing results (VOUS)

- Is the gene associated with the phenotype?
- Allele frequency
  - gnomAD [Cummings BB et al, *Nature* 2020;581:452-458]
- Species conservation
  - PhyloP [Pollard KS et al, *Genome Res* 2010;20:110-121]
- Protein prediction programs
  - SIFT
  - Polyphen-2
  - Mutation Taster
  - FATHMM

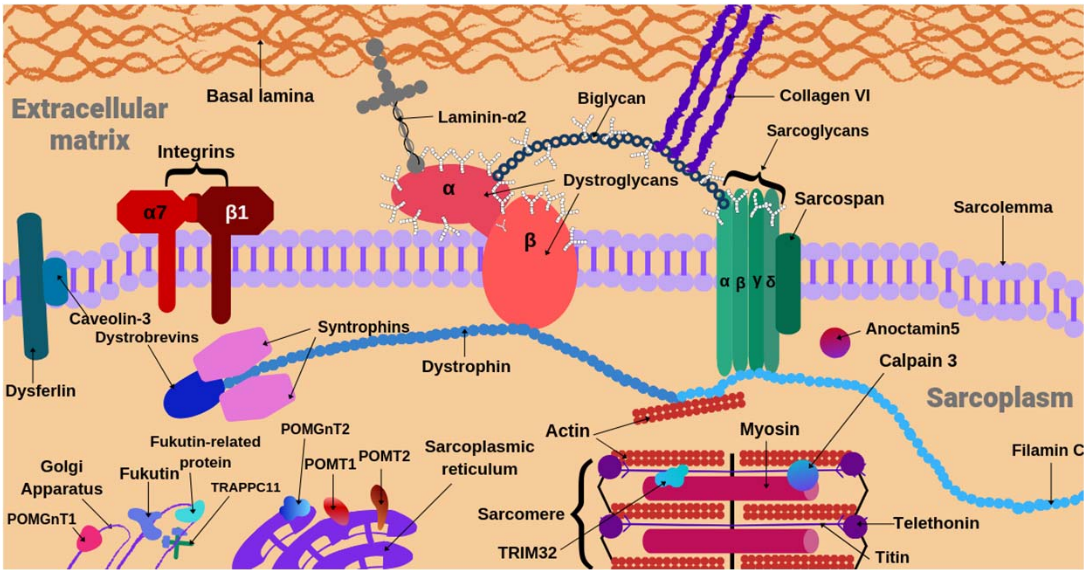


Genetic testing methods

- Old-fashioned
  - Karyotype (> 5 Mb): good for chromosomal aneuploidy
  - Southern blot/FISH: gene deletions and duplications
  - PCR/MLPA: exon deletions and duplications, usually < 1kb
  - Sanger sequencing: single nucleotide variants and small “indels”
- Newfangled
  - Chromosomal microarray: good for changes that are 5kb – 10 Mb
  - Next generation sequencing: 1-10 nucleotides

What are the limits of the old methods?

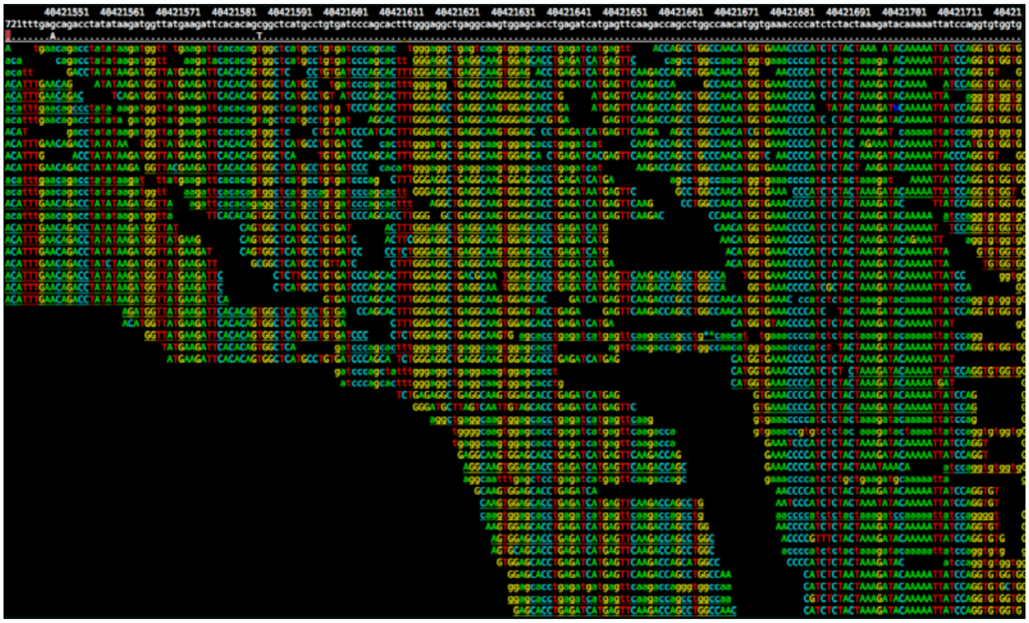
- There are just too many genes!
- Panel testing based on Sanger sequencing technology was very expensive
- In order to target genetic testing to the 1 or 2 most likely candidates, patients previously needed invasive tests such as muscle and nerve biopsies much more frequently than today



Many though not all LGMD-associated proteins localize at the sarcolemma
Raghav Kalra

Next generation sequencing

- Methods
  - Targeted sequence capture
  - Whole exome sequencing
  - Whole genome sequencing
- Common themes
  - Generation of DNA libraries
  - Massively parallel sequencing



Courtesy Tim W. Yu, MD, PhD

Yield of next generation sequencing

- 25-26% for suspected Mendelian disorders
  - [Yang et al, *JAMA* 2014;312:1870-1879]
  - [Lee et al, *JAMA* 2014;312:1880-1887]
- 46% for inherited neuromuscular disorders on a targeted sequence capture panel
  - [Ankala et al, *Ann Neurol* 2015;77:206-214]
- 40-50% for limb-girdle muscular dystrophy on exome sequencing
  - [Ghaoui et al, *JAMA Neurol* 2015;72:1424-1432]
  - [Reddy et al, *J Hum Genet* 2017;62:243-252]
  - [Saha et al, *Physiol Genomics* 2018;50:929-939]

Next generation sequencing alone may miss:

- Duchenne muscular dystrophy (DMD)
- Becker muscular dystrophy (BMD)
- Spinal muscular atrophy (SMA)
- Charcot-Marie-Tooth disease type 1A (CMT1A)
- Myotonic dystrophy (DM1 and DM2)
- Facioscapulohumeral muscular dystrophy (FSHD1)

Why?

- Next generation sequencing is good for
  - Single nucleotide (missense/nonsense) mutations
  - Insertions or deletions of 1-10 nucleotides
- Next generation sequencing is not currently reliable for larger mutations without in depth analyses that are beyond the scope of most clinical genetic diagnostic laboratories

Dystrophin mutations

DMD	BMD
50-65% deletions	65-70% deletions
5-10% duplications	10-20% duplications
20-35% sequence variants (including nonsense mutations)	10-20% sequence variants

Darras BT et al, GeneReviews 2011

How to order a neuromuscular genetic test

- Key considerations
  - What specific disease you are looking for
  - What gene(s) may be the likely culprit(s)
  - What types of mutations are found in those genes
  - These factors can help guide what test is needed
- Example
  - Duchenne muscular dystrophy
  - *DMD* (dystrophin) gene
  - Deletions, duplications, single nucleotide variants
  - A genetic test that looks for these mutation types in *DMD*

What to do when you have the diagnosis

The most important point

- Make sure you have the right diagnosis!
- Don’t be misled by the notorious variant of unknown significance (also known as VUS or VOUS)
- If you are not comfortable interpreting a genetic test report for a family, ask a neuromuscular neurologist, geneticist, or genetic counselor for help

How to counsel a family

- When discussing prognoses, be realistic but don’t dwell on the negative
  - Rigid prognoses may turn out to be wrong if a new therapy is developed
- Mention risk of recurrence with future pregnancies, parents may need testing if not tested already
- Other symptomatic family members should be tested regardless of age
- Asymptomatic siblings generally should not have carrier testing in childhood for recessive diseases unless there are medical implications, but should consider this before starting families (American Academy of Pediatrics, American College of Medical Genetics)

Modern libraries

Old fashioned literature searches

- Card catalogs
- Books
- Medical journals in print

Modern information gathering

- Online journals
- Online databases
  - NIH-affiliated
    - OMIM
    - Pubmed
  - UpToDate
  - WebMD
  - Academic medical center websites
- Social media

Sample case

History

- Adolescent male presents to clinic
- 2-year history of proximal arm and leg weakness
  - First symptoms: difficulty with stairs, rising from chair
  - Currently: cannot run
- Fatigue and bilateral hand tremors
- Early development normal
- Previously played varsity sports
- Family history non-contributory

Physical examination

- Mild facial weakness
- Diffuse weakness, worse proximally, bilateral thigh atrophy
- No myotonia
- Deep tendon reflexes intact in arms, absent at patellae, reduced at ankles
- Normal gait, could not stand on toes, + Gowers sign

Laboratory

- Brain MRI by report unremarkable
- TSH normal
- CK 1600+ & 1900+ U/L [24-204]
- ESR normal
- CRP normal
- Von Willebrand Antigen borderline low
- Anti-Jo-1 antibody negative
- Carnitine total, free, esterified/free all normal

Genetic testing

- Next generation muscular dystrophy sequencing panel showed no definite pathogenic mutations
- *ANO5* heterozygous VOUS
- *GAA* heterozygous VOUS
- *SYNE1* heterozygous VOUS

Muscle biopsy: right quadriceps

- Neurogenic features: grouped atrophy (type 2 especially), fiber type grouping, targetoid fibers, nuclear bags
- Myopathic features: myofiber degeneration, chronic regenerative changes, fibrofatty infiltration
- This combination of features suggests severe chronic neurogenic changes, myofiber degeneration, and significant fibrofatty infiltration, consistent with transition to end-stage muscle
- No evidence for specific disorders that can display both neurogenic and myopathic features: myofibrillar myopathy, mitochondrial disease, muscular dystrophy

EMG

- Left median and bilateral sural sensory responses normal
- Left median, peroneal, and tibial motor nerve conduction studies normal, including F response latencies
- Concentric needle electromyography showed abnormalities in all muscles tested: ongoing denervation and chronic reinnervation
  - Left genioglossus
  - Left biceps brachii, first dorsal interosseous
  - Left tibialis anterior, medial gastrocnemius, vastus lateralis, extensor hallucis longus

Definitive diagnostic test

- Spinal muscular atrophy genetic testing
- *SMN1*: 0 copies
- *SMN2*: at least 3 copies

- Note: patients with SMA usually have deletions on both copies of the SMN1 gene, which makes SMA an autosomal recessive disease

Werdnig & Hoffman

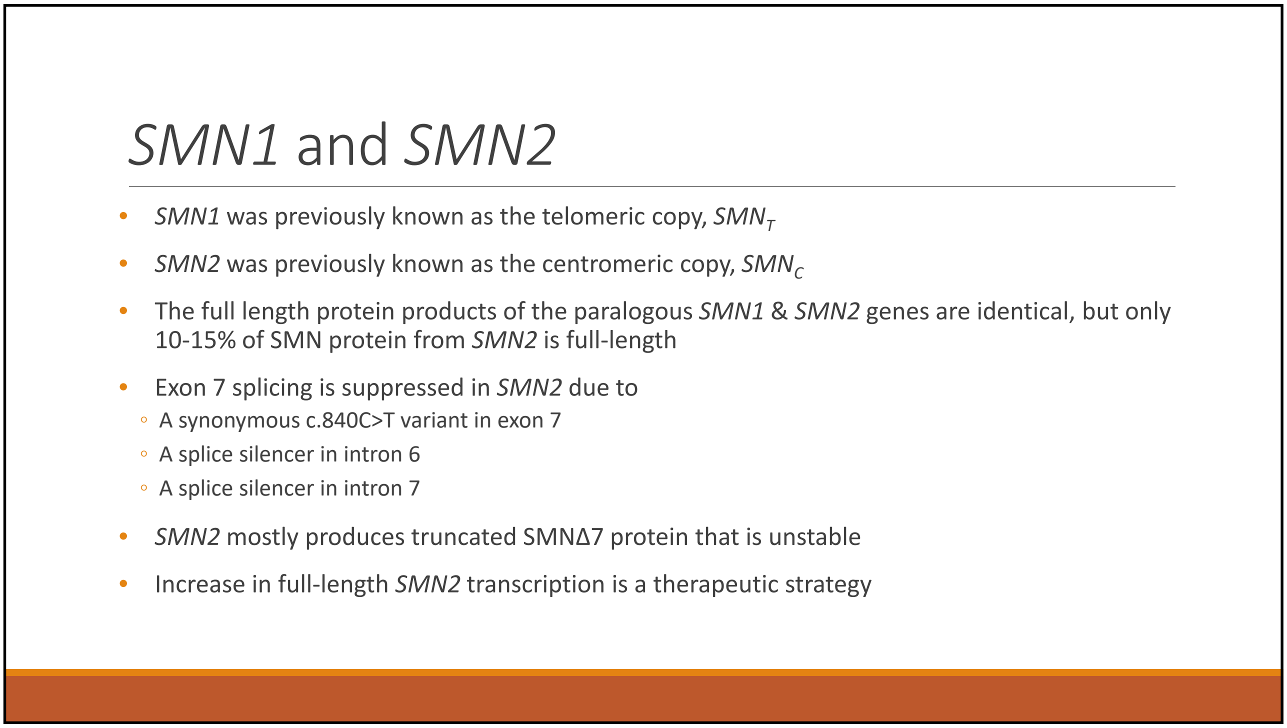
- Werdnig G. Zwei frühinfantile hereditäre Fälle von progressive Muskelatrophie unter dem Bilde de Dystrophie, aber auf neurotischer Grundlage. Arch Psychiat Nervenkr 1891;22:437-480.
- Hoffman J. Ueber chronische spinale Muskelatrophie im Kindesalter, auf familiärer Basis. Deutsch Z Nervenheilk 1893;3:427-470.

SMA basics

- Most common inherited cause of death in infancy
- Incidence 1 in 6,000 to 1 in 10,000 live births
- Diagnosis traditionally made by EMG
- Now more commonly made by genetic testing, though EMG still plays a role in certain situations
- Homozygous deletion in *SMN1* on chromosome 5q causes most cases

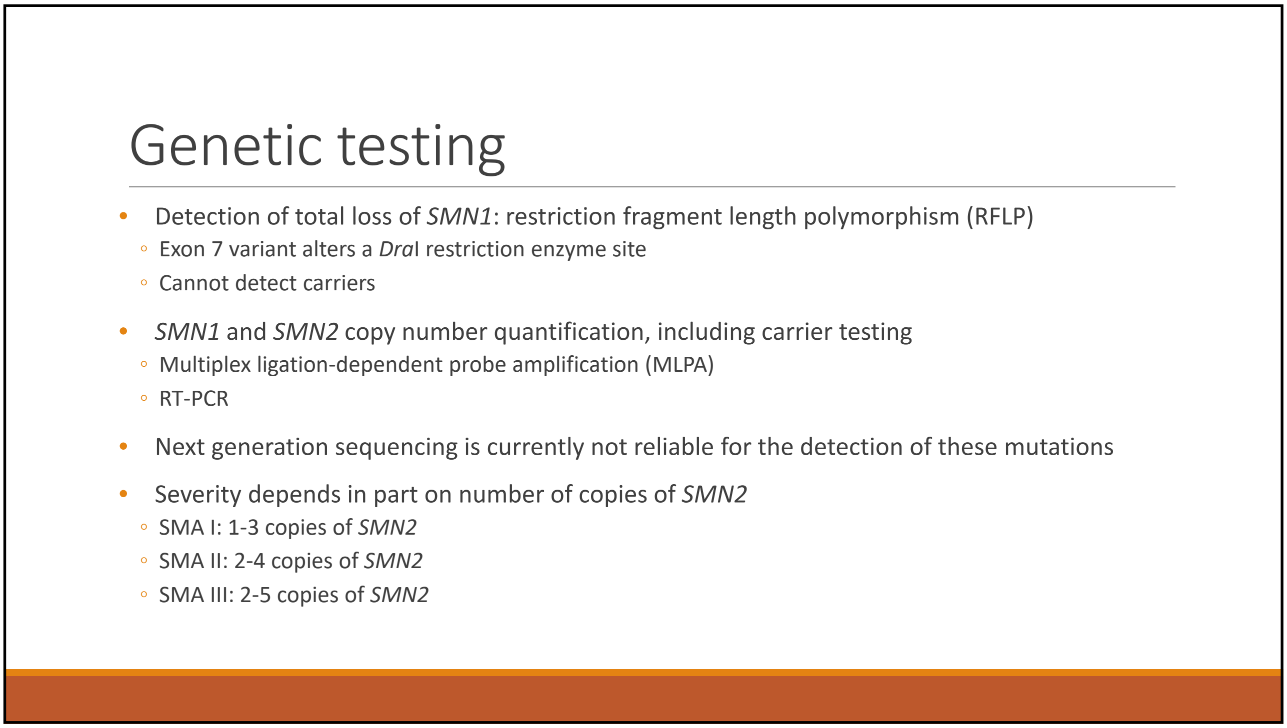
Subtypes

- I – never sit or walk, onset < 6 months (Werdnig-Hoffman disease)
  - 1A – prenatal onset with arthrogryposis (also referred to as type 0)
  - 1B – classic SMA I with poor head control
  - 1C – milder SMA I with better head control
- II – sit but never walk, onset 6-18 months (Dubowitz disease)
- III – sit and walk, onset > 18 months (Kugelberg-Welander disease)
  - IIIa – onset 18-36 months
  - IIIb – onset > 36 months, slightly better prognosis
- IV – adult onset



SMN1 and *SMN2*

- *SMN1* was previously known as the telomeric copy, SMN_T
- *SMN2* was previously known as the centromeric copy, SMN_C
- The full length protein products of the paralogous *SMN1* & *SMN2* genes are identical, but only 10-15% of SMN protein from *SMN2* is full-length
- Exon 7 splicing is suppressed in *SMN2* due to
  - A synonymous c.840C>T variant in exon 7
  - A splice silencer in intron 6
  - A splice silencer in intron 7
- *SMN2* mostly produces truncated SMNΔ7 protein that is unstable
- Increase in full-length *SMN2* transcription is a therapeutic strategy



Genetic testing

- Detection of total loss of *SMN1*: restriction fragment length polymorphism (RFLP)
  - Exon 7 variant alters a *Dra*I restriction enzyme site
  - Cannot detect carriers
- *SMN1* and *SMN2* copy number quantification, including carrier testing
  - Multiplex ligation-dependent probe amplification (MLPA)
  - RT-PCR
- Next generation sequencing is currently not reliable for the detection of these mutations
- Severity depends in part on number of copies of *SMN2*
  - SMA I: 1-3 copies of *SMN2*
  - SMA II: 2-4 copies of *SMN2*
  - SMA III: 2-5 copies of *SMN2*

Antisense oligonucleotide development

- Intronic splice silencing regions identified in intron 6 and intron 7, confirmed by mutagenesis of those sites
- A set of antisense oligonucleotides (ASOs) targeting these introns were tested
- ASOs 09-23 and 10-27 were found to target the intron 7 silencing region
- Tail vein injection of ASOs 09-23 and 10-27 into *hSMN2* transgenic mice augmented inclusion of exon 7 compared to saline and ASO 0-0 controls
- Effect was notable in liver and kidney, but not spinal cord, indicating that the ASOs do not cross the blood-brain barrier (hence intrathecal infusions)
- [Hua Y et al, *Am J Hum Genet* 2008;82:834-848]

Antisense oligonucleotide therapy

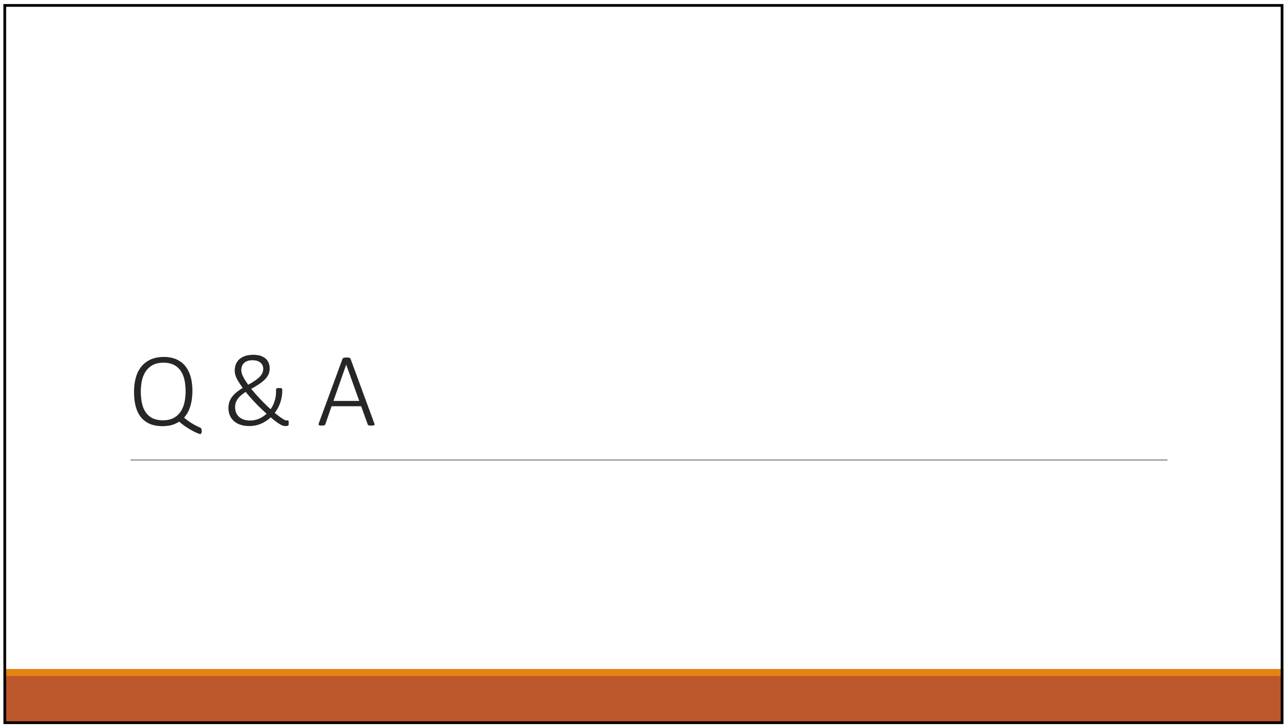
- A series of human clinical trials have recently been published
  - [Chiriboga C et al, *Neurology* 2016;86:890-897]
  - [Finkel RS et al, *Lancet* 2016;388:3017-3026 (SMA I)]
  - [Finkel RS et al, *N Engl J Med* 2017;377:1723-1732 (SMA I)]
  - [Mercuri E et al, *N Engl J Med* 2018;378:625-635 (SMA II)]
- Nusinersen approved by the US Food and Drug Administration
- Additional studies in other subpopulations of SMA in progress

Gene therapy

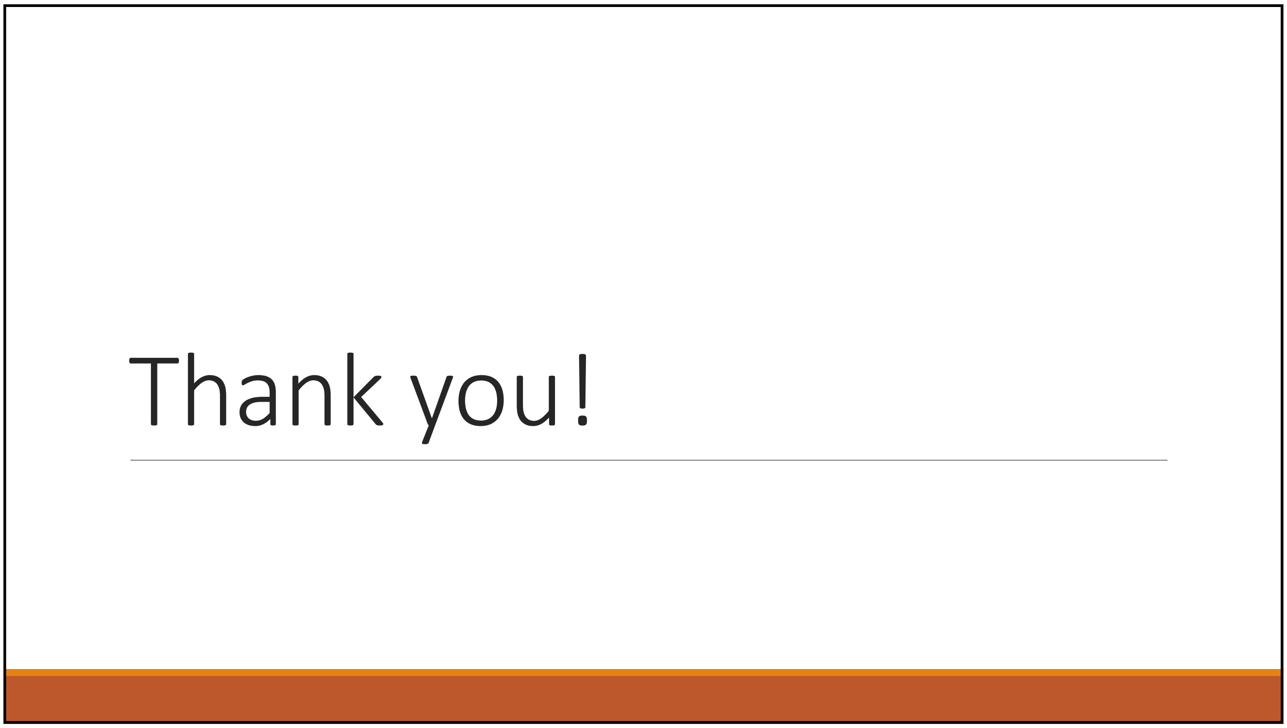
- 15 patients with SMA I
- AAV9-SMN was administered intravenously (crosses blood-brain barrier)
- Primary outcome: safety
- Secondary outcomes: event-free survival, CHOP-INTEND scores
- No safety concerns arose in this study, survival and motor function improved
- [Mendell JR et al, *N Engl J Med* 2017;377:1713-1722]
- Approved by the FDA in 2019

The history of SMA

- 1891: Werdnig’s description of SMA (Hoffman in 1893)
- 1995: cloning of *SMN1* and *SMN2*
- 1999-2000: good outcome measures developed
- 2008: identification of promising antisense oligonucleotides
- 2012-2014: natural history studies
- 2016: FDA approval of nusinersen
- 2019: FDA approval of onasemnogene abeparvovec
- Total = 125 years until the first FDA-approved therapy!
- The timeline is long, but illustrates the power of genetic knowledge



Q & A



Thank you!