When whole exome (and workup) is wholly confusing

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[1] Stanford Health Care, [2] UC Davis Health

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Case

24YO right handed male with congenital onset and minimal progression of:

- Bilateral ptosis without diplopia
- Mild dysarthria without dysphagia
- Generalized weakness
- Areflexia
- Hypotonia
- Chronic fatigue
- Intermittent vertigo and lightheadedness
- Chronic headaches

Not present: dysmorphism, structural brain or eye disease, skin changes, contractures, autonomic symptoms

Birth history: full term, uncomplicated pregnancy, elective C-section
Development: delayed motor milestones, essentially normal cognitive function
Past medical and surgical history: OSA, migraine with aura, occipital neuralgia, Zenker's diverticulum
Family history: older sister with multiple sclerosis, maternal uncle with ALS

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Examination (age 24)

Normal:
- General examination
- Mental status
- Sensation
- Coordination
- Gait

Abnormal:
- Cranial nerves: mild dysarthria, bilateral ptosis mildly worse with sustained upgaze, facial diparesis
- Motor: see table
- Bulk: low throughout
- Tone: mildly reduced in arms and legs
- Reflexes: trace patellar only

Action	Right	Left	Action	Right	Left
Shoulder abduction	**4+**	**4+**	Hip flexion	5	5
Elbow flexion	5	5	Hip extension	5	5
Elbow extension	5	5	Hip abduction	5	5
Wrist extension	5	5	Hip adduction	5	5
Wrist flexion	5	5	Knee extension	5	5
Finger extension	5	5	Knee flexion	5	5
DIP flexion at digit 2	**4**	**4**	Ankle dorsiflexion	**5-**	**5-**
DIP flexion at digit 5	**4**	**4**	Ankle plantarflexion	5	5
Digit 2 abduction	5	5	Ankle eversion	**4**	**4**
Digit 5 abduction	**4**	**4**	Ankle inversion	5	5
Thumb abduction	5	5	Great toe dorsiflexion	5	5

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Our patient, age 24

Explicit consent from the patient was given to show this video at the Carrell-Krusen conference

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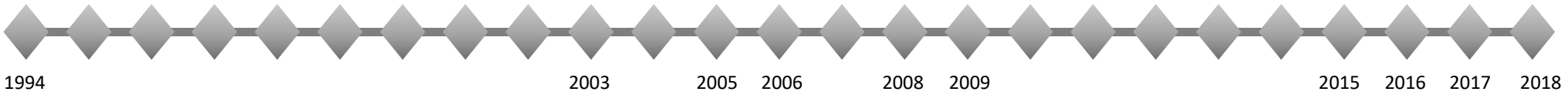
Previous workup



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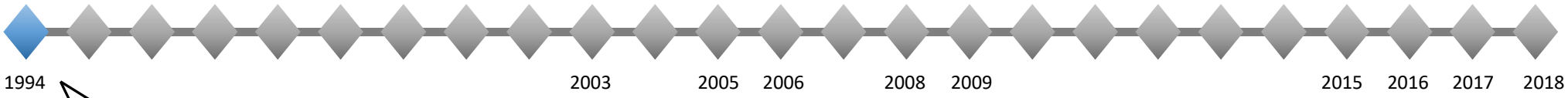
Previous workup



1994:

- Karyotype: 46XY
- Genetics screening evaluation: normal
- Skeletal survey: no evidence metabolic bone disease
- MRI brain WO: normal
- Ophthalmology and audiology exams: normal

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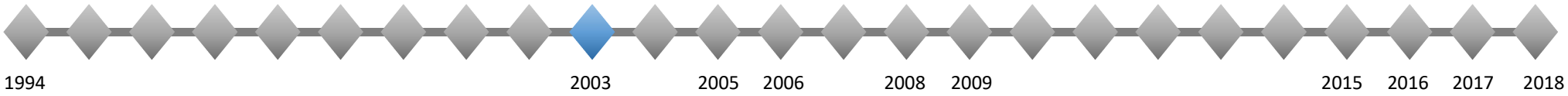
Previous workup



2003:
- EMG/NCS: "generalized myopathy"
- Lysosomal enzyme analysis: normal

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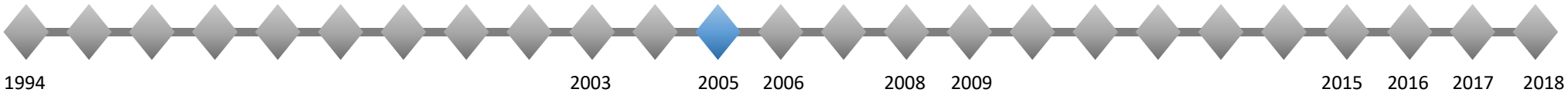
Previous workup



2005:

- EMG/NCS: early recruitment of short-duration motor unit action potentials in proximal and distal muscles, no evidence of polyneuropathy
- Left biceps biopsy: “scattered sub-sarcolemmal deposits of mitochondria but no ragged red fibers” otherwise normal

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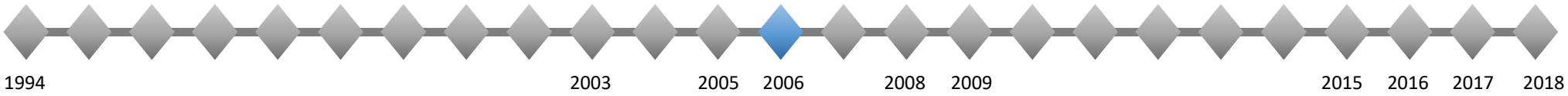
Previous workup



2006:
- Genetic testing for MELAS, MERRF, NARP
- Elevated myoglobinuria
- Decreased phosphofructokinase activity

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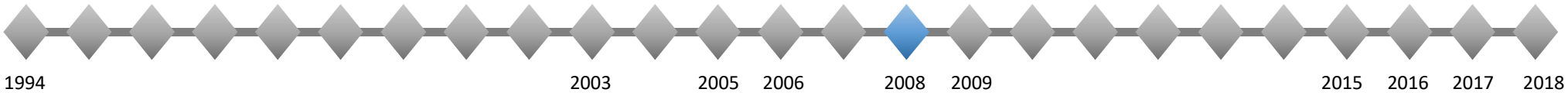
Previous workup



2008:

- Glycolytic enzyme panel: normal
- Ischemic forearm testing: normal increase in lactate and ammonia
- CK “normal”
- Plasma amino acids and urine organic acids normal
- Right vastus lateralis biopsy: normal
- CT head WO questionable clival lesion (limited by artifact from braces)

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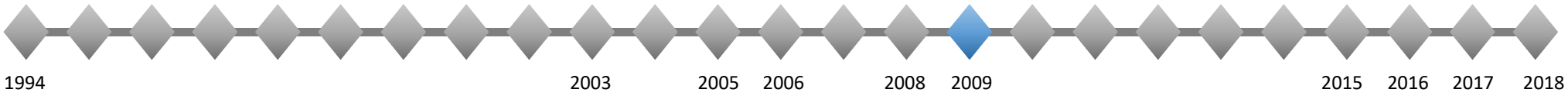
Previous workup



2009:

- MRI brain W/: No clival lesion, small foci of T2 prolongation in parietal white matter bilaterally
- MRI whole spine WO: small syrinx from T5-T10
- Sleep study: AHI 2.2, delayed REM onset, reduced REM stage duration
- ENT evaluation with tongue/pharynx hypotonia
- FEES: normal
- Vestibulonystagmogram: saccades of slow velocity and long latency with abnormal air calorics

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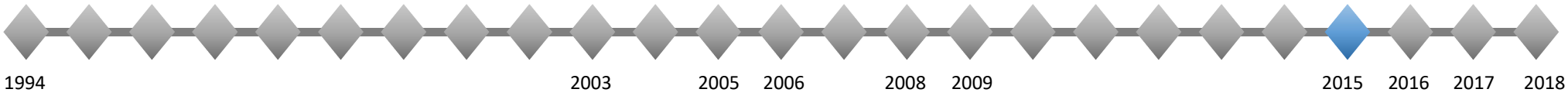
Previous workup



2015:

- Left quadriceps biopsy: FINAL PATHOLOGIC DIAGNOSIS: Skeletal muscle with mitochondrial abnormalities

Comment: The findings in this muscle biopsy are suggestive of a mitochondrial abnormality, but the features are not sufficiently developed to be diagnostic for a mitochondrial myopathy. The ultrastructural studies show abnormal mitochondrial paracrystalline inclusions and focal subsarcolemmal mitochondrial accumulation. No ragged red fibers are seen on H&E or trichrome stains, although some fibers show mildly increased subsarcolemmal staining for oxidative enzymes. The ATPase and fast and slow myosin stains show a predominance of type 2 muscle fibers. The oxidative enzyme stains including NADH and SDH show somewhat darker staining than expected for type 2 muscle fibers, likely reflecting a diffuse rather than focal increase in mitochondrial content that would be difficult to appreciate on ultrastructural studies.

- Gene Dx Mitochondrial DNA testing normal

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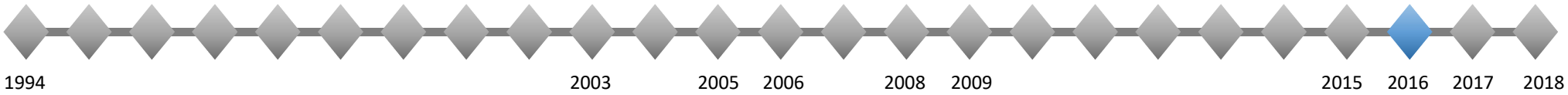
Previous workup



2016:

- Whole exome sequencing with two VUS:
  - Fukutin-related protein (FKRP)
    - c. 421 C>A heterozygous from mother
  - Sarcoglycan alpha subunit (SCGA)
    - c.469G>C heterozygous from father

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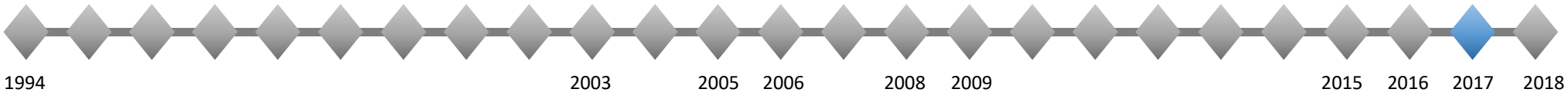
Previous workup



2017:

- EMG/NCS “These abnormal electrodiagnostic studies provide evidence for a prejunctional disorder of the neuromuscular junction.”

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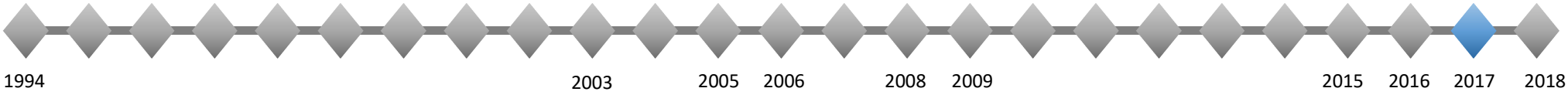
2017 EMG/NCS



Nerve / Sites	Rec. Site	Segments	Dist mm	Lat ms	Amp mV	Dur ms	Relative amplitude	CV m/s	Latency Difference	Temp °C
L Facial										
L postauricular	Nasalis	Postauricular – Naslis	100	2.8	**1.7**	6.4	-	-		-
L Accessory (spinal)										
Neck	Trapezius	Neck - Trapezius	100	2.6	1.5	10.9	-	-		-
L Median										
Wrist	APB	Wrist - APB	80	3.2	8.4	5.1	100			33.1
Elbow	APB	Elbow - Wrist	215	7.1	8.3	5.4	98.1	55	3.9	33.1
L Ulnar										
Wrist	ADM	Wrist - ADM	80	3.1	**4.5**	5.3	100			32.6
Bel Elb	ADM	Bel Elb - Wrist	180	6.4	**3.7**	5.4	82.5	55	3.3	32.5
Abv Elb	ADM	Abv Elb - Bel Elb	100	8.5	**3.6**	5.6	98.4	46	2.2	32.5
L Peroneal - EDB										
Ankle	EDB	Ankle - EDB	80	4.5	2.1	5.2	100			32.6
Fib hd	EDB	Fib hd - Ankle	280	10.2	2.0	5.3	97.5	50	5.6	32.6
Popl Fossa	EDB	Popl Fossa - Fib hd	100	12.4	1.8	5.2	88.6	45	2.2	32.6
L Tibial - AH										
Ankle	AH	Ankle - AH	80	4.0	6.3	5.9	100			32.2
Popl Fossa	AH	Popl Fossa - Ankle	360	10.7	6.2	6.4	99.6	54	6.7	32.2

F-Wave

Nerve	Minimum latency (ms)
L Median	25.6
L Ulnar	26.3
L Peroneal	40.4
L Tibial	41.7

Sensory Nerve Conduction Studies

Nerve / Sites	Rec. Site	Segments	Onset Lat ms	Peak Lat ms	Amp µV	Dist mm	CV m/s	Comment	Temp °C
L Median									
Wrist	D 2	Wrist - D 2	2.3	3.0	39.5	140	61		32.9
L Ulnar									
Wrist	D 5	Wrist - D 5	1.9	2.5	32.2	110	59		33.3
L Sural									
Calf	Ankle	Calf – Ankle	2.8	3.5	19.7	140	50		32.9
L Superficial peroneal									
Lat leg	Ankle	Lat leg – Ankle	2.2	2.9	14.4	120	55		32.9

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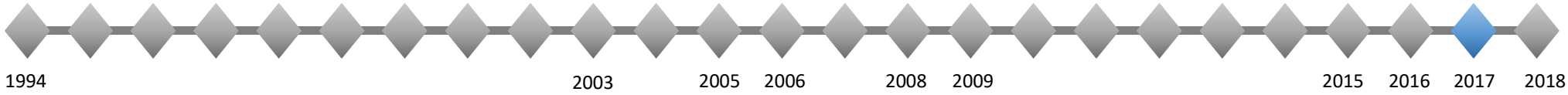
2017 EMG/NCS



EMG

Summary Table								
Muscle	**Fib/PSW**	**Fasc**	**Dur (ms)**	**Amp. (mV)**	**Poly.**	**Recruit**	**Max frequency**	**Comments**
L. Deltoid	None	None	**1-4**	**0.2-0.6**	None	Normal	40Hz	-
L. triceps brachii	None	None	7-11	0.4-1.2	None	Normal	40Hz	-
L. biceps brachii	None	None	7-11	0.4-1.2	None	Normal	40Hz	-
L. first dorsal interosseus	None	None	7-11	0.6-1.6	None	Normal	40Hz	-
L. C6 paraspinal	None	None	7-10	0.4-1.2	None	Normal	40Hz	-
L. tibialis anterior	None	None	7-10	0.2-1.2	None	Normal	40Hz	-
L. gastrocnemius (medial head)	None	None	8-12	0.4-1.2	None	Normal	40Hz	-
L. vastus lateralis	None	None	8-12	0.6-1.6	None	Normal	40Hz	-
L. iliopsoas	None	None	7-11	0.4-1.2	None	Normal	40Hz	-
L. tensor fasciae latae	None	None	8-12	0.4-1.2	None	Normal	40Hz	-

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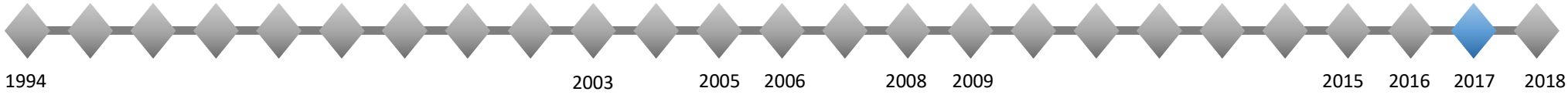
2017 EMG/NCS



2Hz Repetitive Nerve Stimulation

Anatomy/train	Rate (Hz)	Amplitude (mV)	Amplitude 4-1 (%)	Facillitation Amplitude (%)	Area (mVms)	Area 4-1 (%)	Facillitation Area (%)
L Ulnar – 4 Stim							
Baseline @ 2Hz	2	4.0	**9.2**	100	11.3	9	100
Baseline @ 2Hz	2	4.1	**12**	105	11.7	11.3	103
Baseline @ 2Hz	2	4.0	**12.7**	102	11.4	10.8	100
Immed post exer	2	**10.1**	-1	**256**	34.9	4.9	**308**
0:30 post	2	7.6	**21.3**	**192**	25.2	17.9	**222**
1:00 post	2	5.7	**22.5**	**145**	18.4	19.7	**163**
2:00 post	2	4.4	**22.8**	112	14.5	22.5	128
3:00 post	2	4.4	**22.6**	111	12.6	21.6	111

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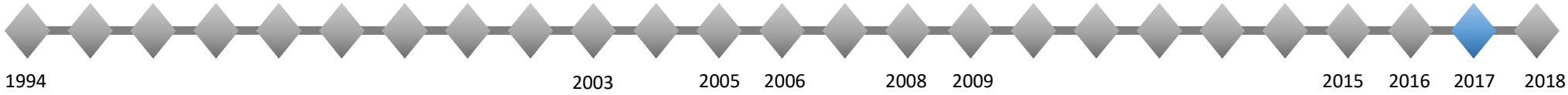
2017 EMG/NCS



2Hz Repetitive Nerve Stimulation

Anatomy/train	Rate (Hz)	Amplitude (mV)	Amplitude 4-1 (%)	Facillitation Amplitude (%)	Area (mVms)	Area 4-1 (%)	Facillitation Area (%)
L Accessory (spinal) – 4 stim							
Baseline @ 2Hz	2	1.2	**17.7**	100	8.3	23.4	100
Baseline @ 2Hz	2	1.2	**17.3**	97.4	7.6	16.9	91
Baseline @ 2Hz	2	1.0	5.8	84.8	6.7	-0.5	81
Immed post exer	2	**2.4**	2.2	**206**	23.0	7.4	**277**
0:30 post	2	**3.3**	**38.6**	**281**	21.1	37.8	**254**
1:00 post	2	2.1	**34.9**	**175**	13.1	35.5	**158**
2:00 post	2	1.6	**32**	135	9.6	30.5	115
3:00 post	2	1.4	**28.5**	121	8.2	29.7	99

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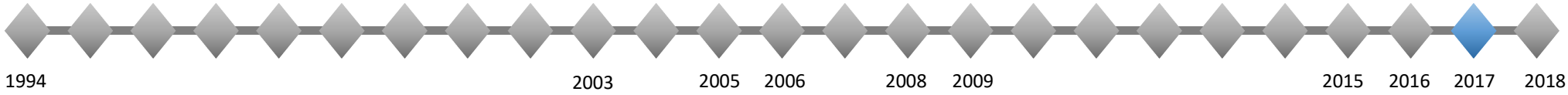
2017 EMG/NCS



2Hz Repetitive Nerve Stimulation

Anatomy/train	Rate (Hz)	Amplitude (mV)	Amplitude 4-1 (%)	Facillitation Amplitude (%)	Area (mVms)	Area 4-1 (%)	Facillitation Area (%)
L facial – 4 Stim							
Baseline @ 2Hz	2	1.5	**13**	100	3.0	18.2	100
Baseline @ 2Hz	2	1.5	**12.6**	97.6	3.2	23.9	105
Baseline @ 2Hz	2	1.5	9.2	97.1	2.8	12.5	94
Immed post exer	2	2.0	**17.6**	135	3.7	21.3	124
0:30 post	2	1.6	**20.2**	109	3.1	23.1	102
1:00 post	2	1.5	**19.6**	95.9	2.8	23.6	91.8
2:00 post	2	1.3	**20.1**	84.6	2.6	23.3	86.7
3:00 post	2	1.1	**14.4**	74.9	2.3	14.6	77.9

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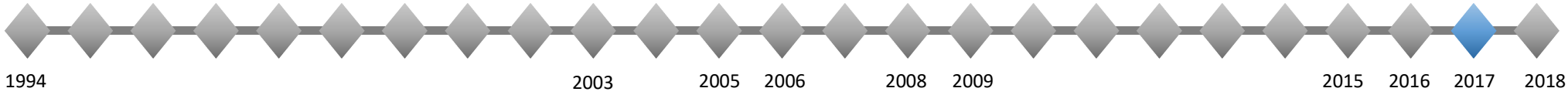
2017 EMG/NCS



2Hz Repetitive Nerve Stimulation

Anatomy/train	Rate (Hz)	Amplitude (mV)	Amplitude 4-1 (%)	Facillitation Amplitude (%)	Area (mVms)	Area 4-1 (%)	Facillitation Area (%)
Left Ulnar (repeat) – 4 Stim							
Baseline @ 2Hz	2	3.8	**24.6**	100	10.7	25.1	100
Immed post exer	2	**11.9**	**16.5**	**315**	32.4	15.9	**304**
0:30 post	2	5.8	**31.3**	**153**	16.2	30	**152**
1:00 post	2	4.1	**29.8**	110	11.7	26.3	109

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Additional workup?
Management recommendations?

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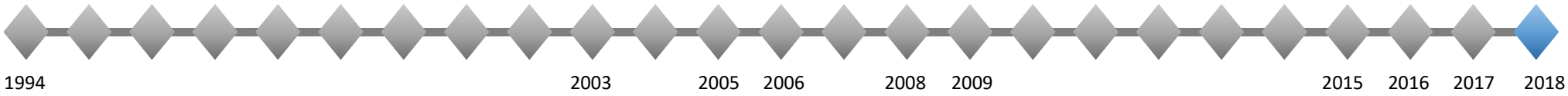
Previous workup



2018:

- Anti-VGCC antibodies: negative
- Myasthenia gravis antibody panel: negative
  - AChR binding, AChR modulating, Striational Ab
- GeneDX congenital myasthenic syndrome panel: negative
- Single fiber EMG: increased jitter and blocking

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GeneDX congenital myasthenic syndrome panel

Gene	Protein	Inheritance pattern	Diagnostic yield*
AGRN	Agrin	AR	Rare
ALG2	Alpha 1,3 mannosyltransferase	AR	Rare
CHAT	Choline O-acetyltransferase	AR	5%
CHRNA1	AChR alpha subunit	AD/AR	<1%
CHRNB1	AChR beta subunit	AD/AR	<1%
CHRND	AChR delta subunit	AD/AR	<1%
CHRNE	AChR epsilon subunit	AD/AR	[1]49%
COLQ	Acetylcholinesterase collagenic tail peptide	AR	13%
DOK7	Protein Dok7	AR	[2]10-23%

Gene	Protein	Inheritance pattern	Diagnostic yield*
DPAGT1	Dolichyl-phosphate N-acetylglucosaminephos-phorotransferase 1	AR	<1%
GFTP1	Glucosamine-fructose-6-phosphate aminotransferase isomerizing 1	AR	4%
MUSK	Muscle skeletal receptor tyrosine protein kinase	AR	Rare
RAPSN	43 kDa receptor associated protein of the synapse	AR	[3]15-20%
SCN4A	Sodium channel protein type 4 alpha subunit	AR	Rare

* In select populations with suspected CMS

[1] Founder mutation in European, Brazilian, and African populations

[2] Founder mutation in European, Canadian, and Brazilian populations

[3] Founder mutation in European and Indian populations

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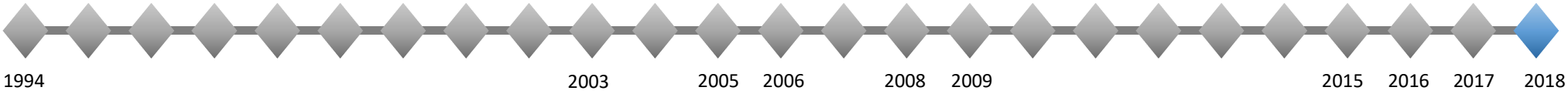
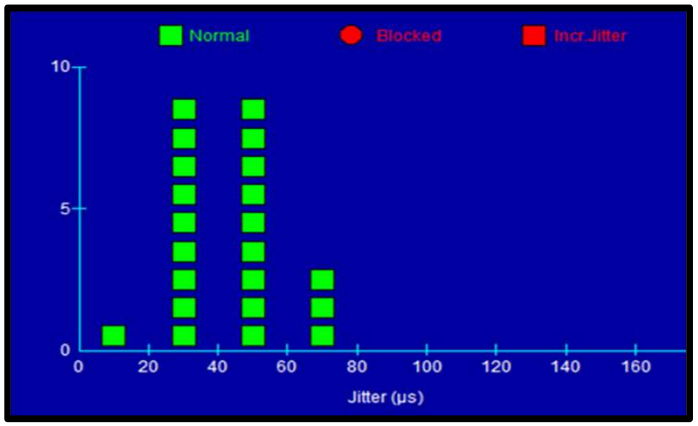
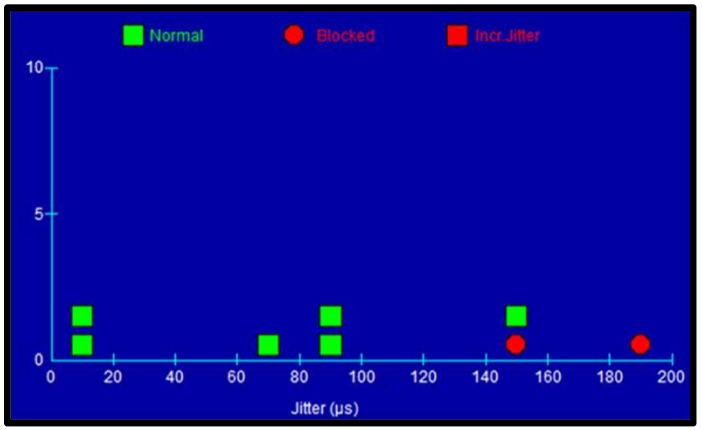
Single fiber electromyography (2018)



L. Deltoid		
	# pairs	9
	% blocked	22%
	Mean jitter	89 µs (normal <32.9µs)

L. Sternocleidomastoid		
	# pairs	20
	% blocked	0%
	Mean jitter	47 µs (normal <29.3µs)





Additional workup?
Management recommendations?

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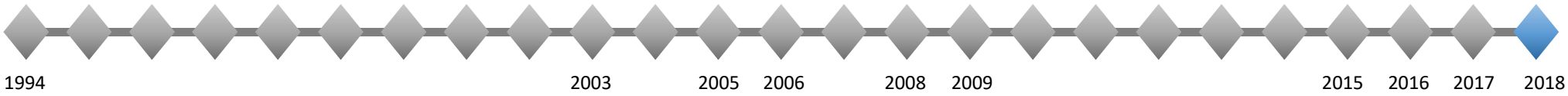
Further workup



2018:

- Repeat evaluation of whole exome sequencing identifies novel mutation in synaptotagmin 2 (gene SYT2)
  - p.L365P, c.1094T>C heterozygous de novo

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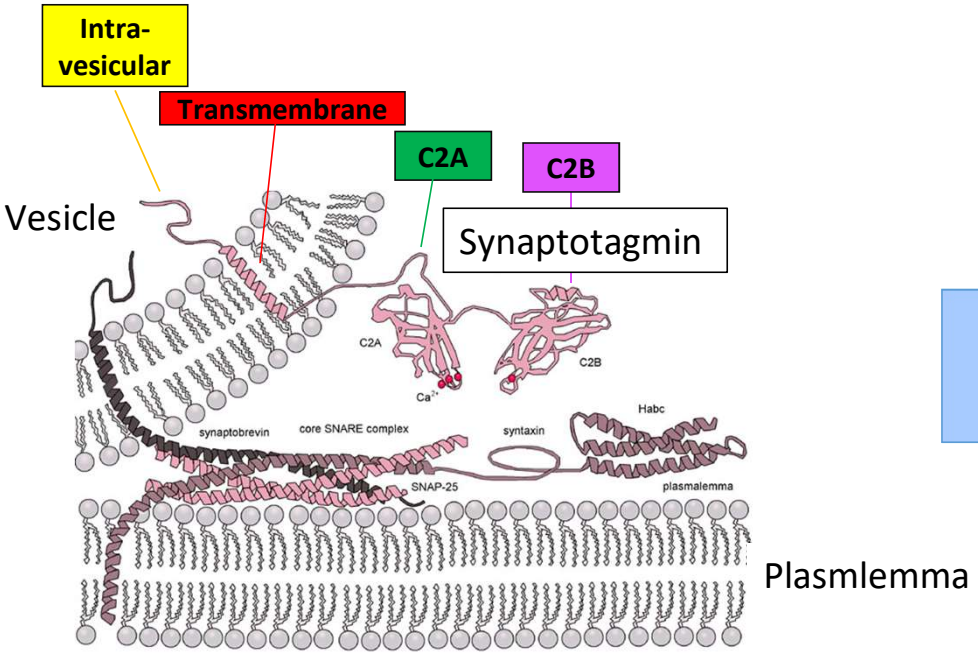
Synaptotagmin 2

Functions:

- Calcium detector for fast, synchronized acetylcholine release
- Reducing asynchronous, slow release

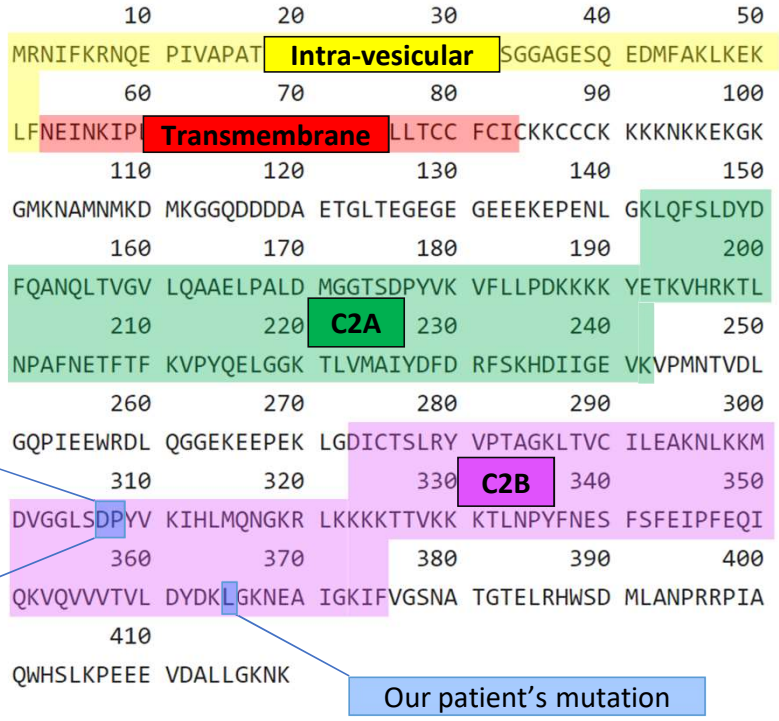
Structure:



Plasmlemma

DD Georgiev et. al. Nano and Molecular Electronics Handbook. 2007

Sequence in humans:



Previously reported pathogenic mutations

Our patient's mutation

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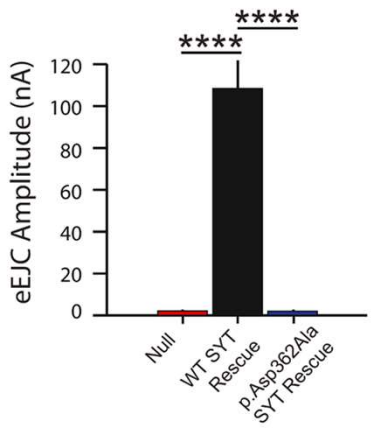
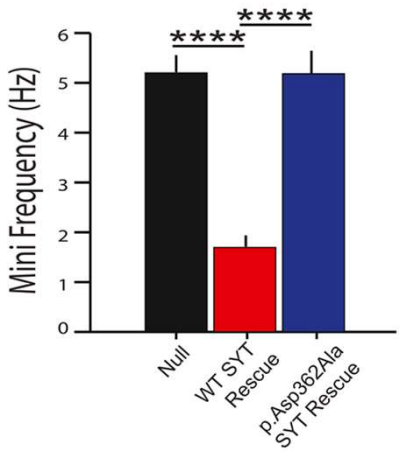
Previously reported SYT2 mutations

- Two families (US and UK) with autosomal dominant inheritance
- Variable penetrance – pattern of weakness, pes cavus, high arches common
- Repetitive nerve stimulation with baseline decrement and prolonged facilitation

Decreased synchronous release

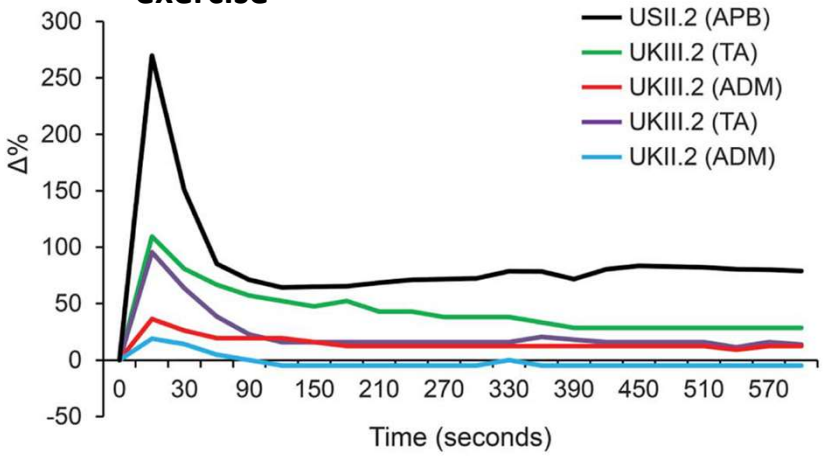


Increased asynchronous release



Hermann, et. al. Am. J. Hum. Gen. 2014

Prolonged facilitation after exercise



Whittaker, et. al. Neurology 2015

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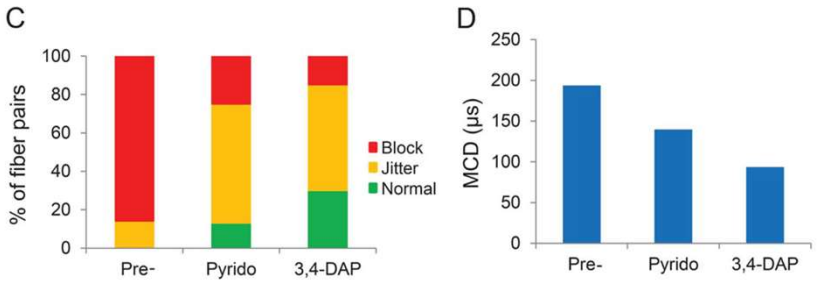
Case resolution

1994 2003 2005 2006 2008 2009 2015 2016 2017 2018



SFEMG (n=2)

2018:

- Anconeus biopsy and *in vivo* studies
  * electron microscopy and quantal analysis pending
- **Treated with 3,4-DAP and improved**

Whittaker, et. al. Neurology 2015

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Our patient's after 3,4-DAP

Anatomy/Train	Rate (Hz)	Amplitude (mV)	Amp 4-1 (%)	Amp 6-1 (%)	Facillitation (%)	Area (mVms)	Area 4-1 (%)	Area 6-1 (%)	Facillitation area (%)
Right Ulnar - ADM									
Baseline	2	8.5	9.5	8.1	100	23.5	9.4	**10.5**	100
Baseline	2	8.6	**11.3**	8.9	101	23.7	10.1	8.2	101
Immed post ex.	2	10.8	-4.3	-3.8	**127**	27.3	-0.3	-3.2	116
0:30 post	2	9.0	**16.4**	**16.3**	106	26.7	**14.5**	**12.9**	114
1:00 post	2	8.4	**17.9**	**17.5**	99.3	24.3	**14.6**	**13.9**	103
2:00 post	2	8.0	**16.9**	**15.4**	94.3	23.3	**15.4**	**14.3**	99
3:00 post	2	8.0	**18.1**	**16.9**	94.5	22.6	**14.0**	**12.5**	96.1
4:00 post	2	7.9	**16.2**	**15.3**	93.6	22.2	**15.1**	**14.2**	94.5

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Exam 40 minutes after 20mg 3,4-DAP

Explicit consent from the patient was given to show this video at the Carrell-Krusen conference

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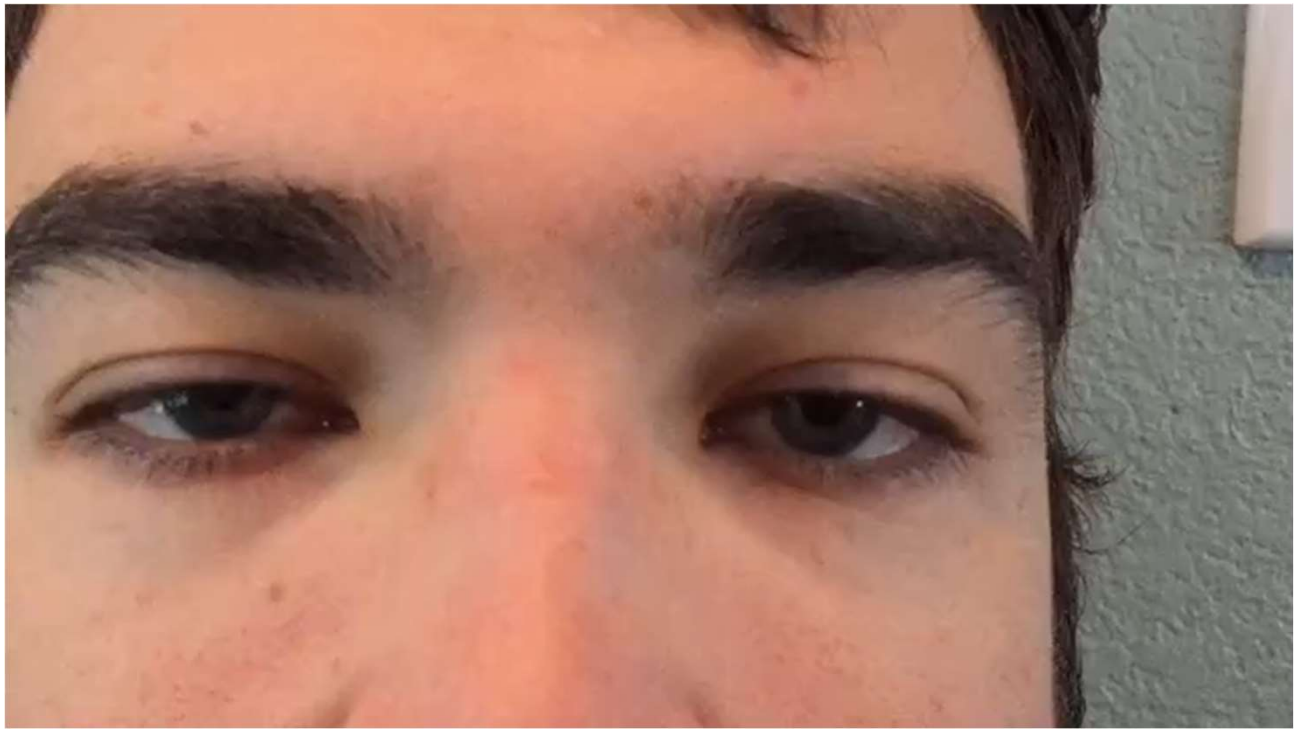
Before 3,4-DAP vs After 3,4-DAP



Explicit consent from the patient was given to show this video at the Carrell-Krusen conference

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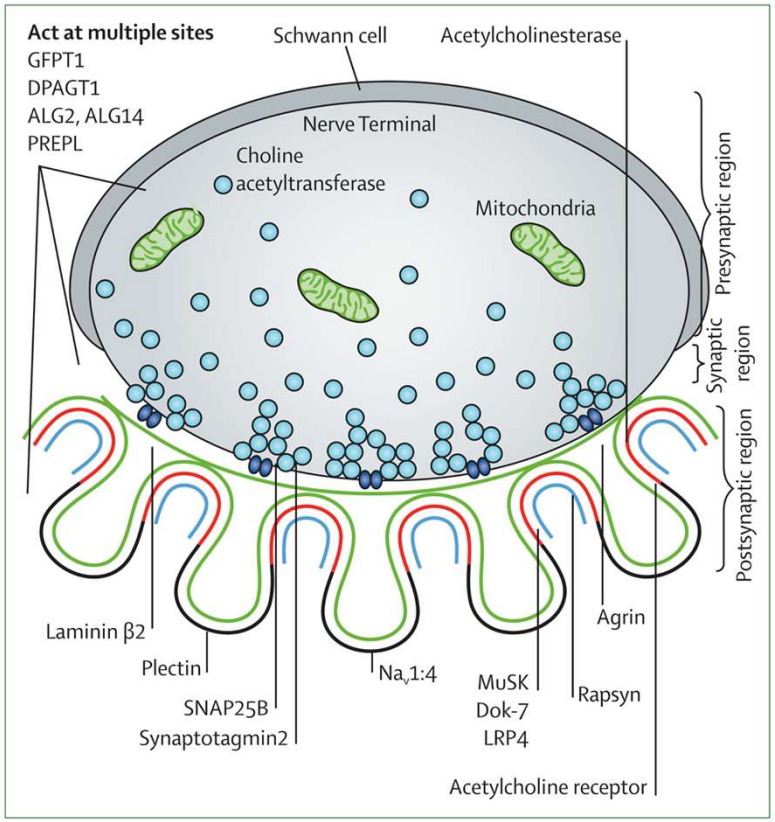
Congenital Myasthenic Syndromes



Engel, AG, et. al. Lancet Neurology (2015)

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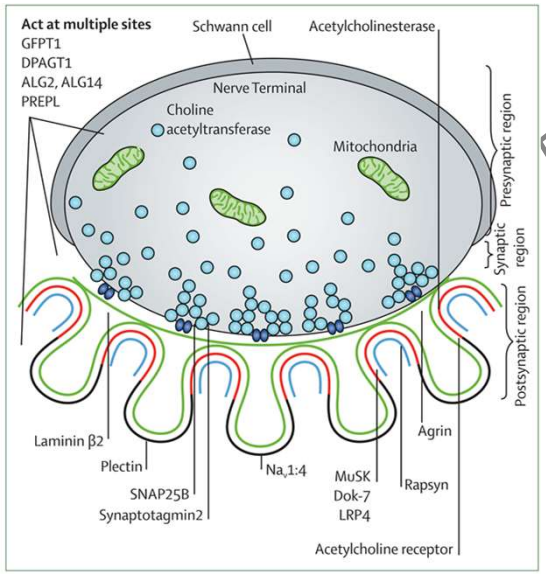
Presynaptic CMS syndromes (5.6%)



Syndrome	Proportion*
Choline acetyltransferase deficiency	5.1%
Paucity of synaptic vesicles and reduced quantal release	0.3%
SNAP25B deficiency	0.3%
Synaptotagmin 2 deficiency	?
Unconventional myosin 9	?
MUNC13-1	?
Synaptobrevin 1	?
Vesicular ACh transporter	?
PREPL	?

Engel, AG, et. al. Lancet Neurology (2015)

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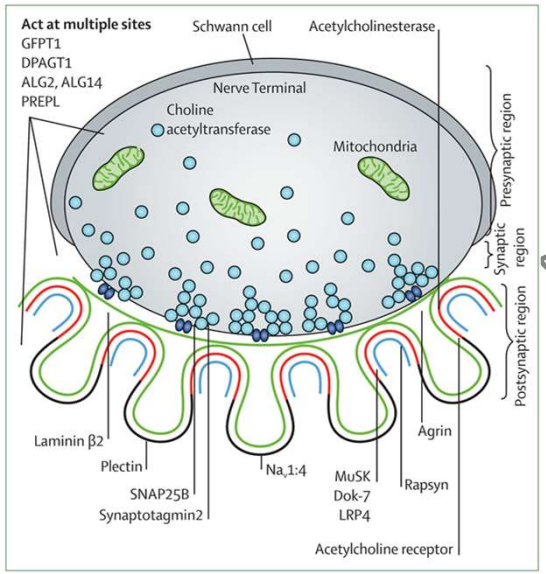
Synaptic basal lamina-associated CMS syndromes (13%)



Syndrome	Proportion*
Endplate acetylcholinesterase deficiency	12.6%
Laminin-β2 deficiency	0.3%
Laminin-α5 deficiency	?
Collagen type XIII α1 chain	?

Engel, AG, et. al. Lancet Neurology (2015)

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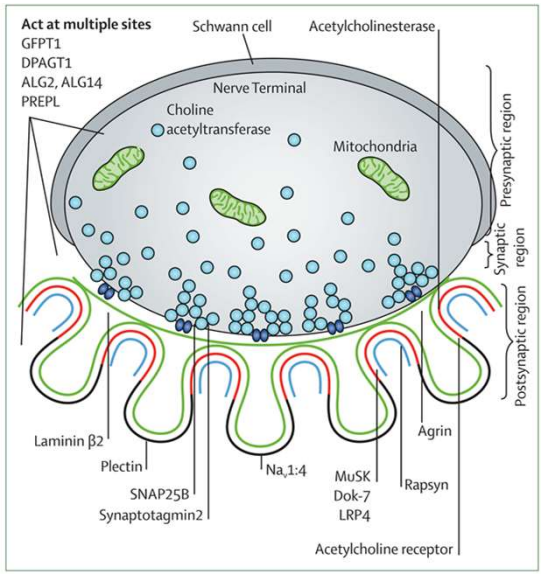
Acetylcholine receptor CMS syndromes (51%)



Syndrome	Proportion*
Primary acetylcholine receptor deficiency	33.1%
Kinetic defects in acetylcholine receptor	17.4%
Slow-channel syndrome	6.8%
Fast-channel syndrome	10.7%

Engel, AG, et. al. Lancet Neurology (2015)

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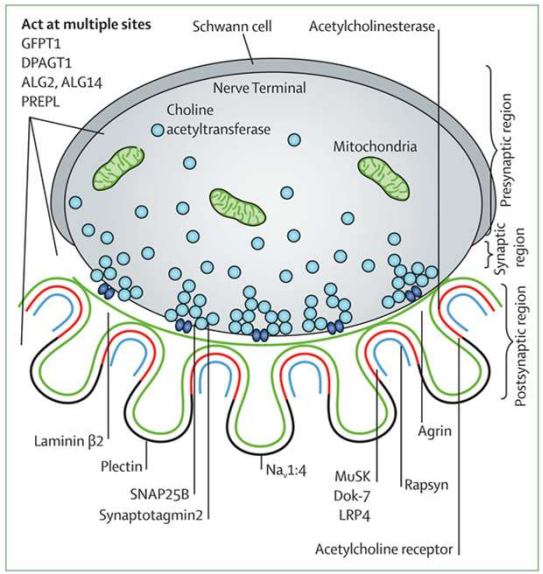
Defects in endplate development and maintenance (25.3%)



Syndrome	Proportion*
Rapsyn deficiency	14.4%
Dok-7 myasthenia	10%
LRP4 myasthenia	0.6%
MuSK deficiency	0.3%
Agrin deficiency	0.3%

Engel, AG, et. al. Lancet Neurology (2015)

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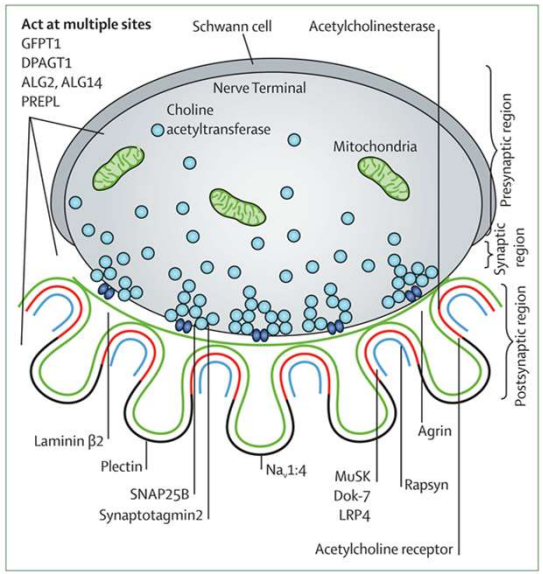
Congenital defect in glycosolation (3.7%)



Syndrome	Proportion*
GFPT1 myasthenia	3.1%
DPAGT1 myasthenia	0.6%
ALG2 and ALG 14	0%
GMPPB	?

Engel, AG, et. al. Lancet Neurology (2015)

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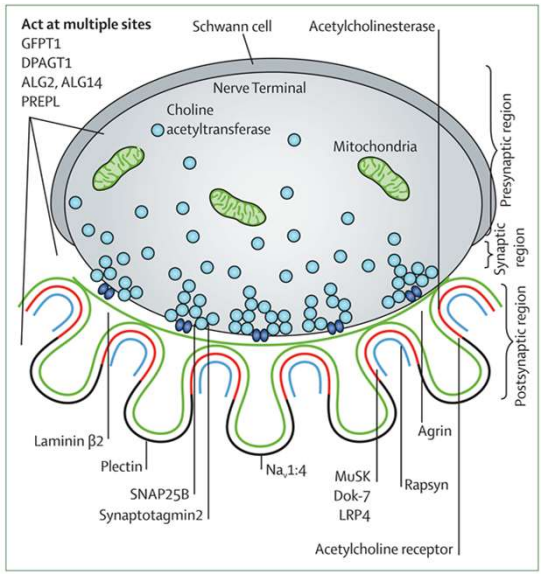
Other myasthenic syndrome (1.4%)



Syndrome	Proportion*
PREPL deletion syndrome	0.3%
Na-channel myasthenia	0.3%
Plectin deficiency	0.6%
Myasthenia associated with centronuclear myopathies	0.3%
Myasthenia aassociated with mitochondrial citrate carrier deficiency	?

Engel, AG, et. al. Lancet Neurology (2015)

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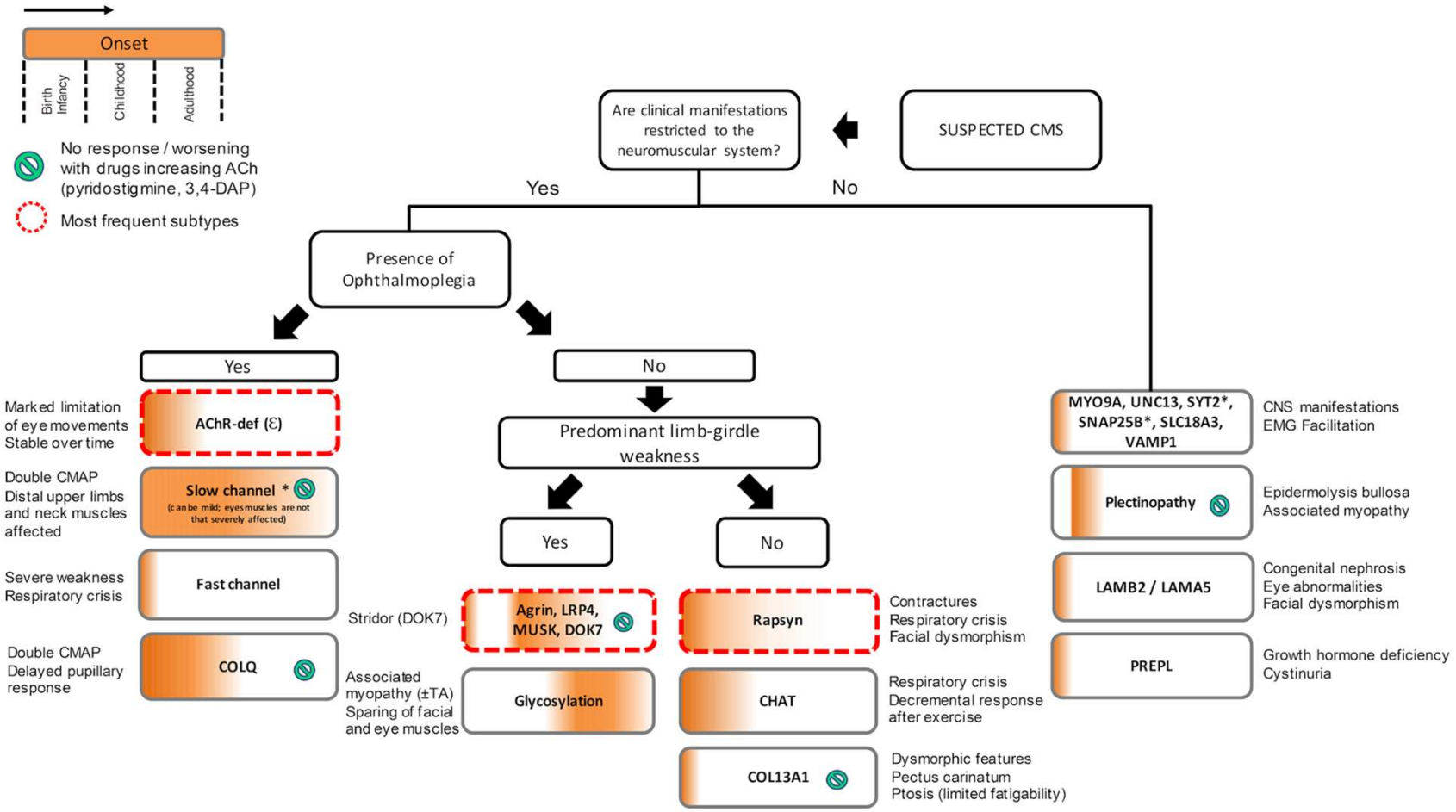

Rodriguez, PM, et. al. Int. J. Mol Sci (2008)

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Treatment of CMS

Treatment types:

- Acetylcholinesterase inhibitors
- 3,4 Diaminopyridine
- Beta-adrenergic agonists
- Channel blocking agents

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Treatment of CMS

Treatment types:

- Acetylcholinesterase inhibitors

- 3,4 Diaminopyridine
- Beta-adrenergic agonists
- Channel blocking agents

Acetylcholinesterase inhibitors:

Examples:
- pyridostigmine, neostigmine

Frequently useful for:
- Presynaptic syndromes, AChR deficiency, fast channel syndromes

Occasionally useful for:
- Congenital defect in glycosolation

Can worsen:
- Acetlycholinesterase deficiency, slow channel syndromes, most endplate maintenance syndromes

Contraindications:
lower dose in renal insufficiency

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Treatment of CMS

Treatment types:
- Acetylcholinesterase inhibitors
- **3,4 Diaminopyridine**
- Beta-adrenergic agonists
- Channel blocking agents

3,4-diaminopyridine:

Examples:
- 3,4-diaminopyridine, amifampridine (Firdapse®)

Frequently useful for:
- Presynaptic syndromes

Occasionally useful for:
- Primary acetylcholine receptor deficiency

Can worsen:
- Acetlycholinesterase deficiency, slow channel syndromes, most endplate maintenance syndromes

Contraindications:
Seizures

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Treatment of CMS

Treatment types:
- Acetylcholinesterase inhibitors
- 3,4 Diaminopyridine
- **Beta-adrenergic agonists**
- Channel blocking agents

Beta-adrenergic agonists:

Examples:
- Oral albuterol, salambutol, ephedrine

Frequently useful for:
- none

Occasionally useful for:
- endplate maintenance syndromes, Acetylcholinesterase deficiency

Can worsen:
- None

Contraindications:
Use of MAOs and SDRIs, general anesthesia

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Treatment of CMS

Treatment types:
- Acetylcholinesterase inhibitors
- 3,4 Diaminopyridine
- Beta-adrenergic agonists
- **Channel blocking agents**

Channel blocking agents:

Examples:
- Fluoxetine, quinidine

Frequently useful for:
- Slow channel syndromes

Occasionally useful for:
- none

Can worsen:
- Fast channel syndromes

Contraindications:
- Quinidine – cardiac arrythmia, cardiomyopathy

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Limitations to genetic testing

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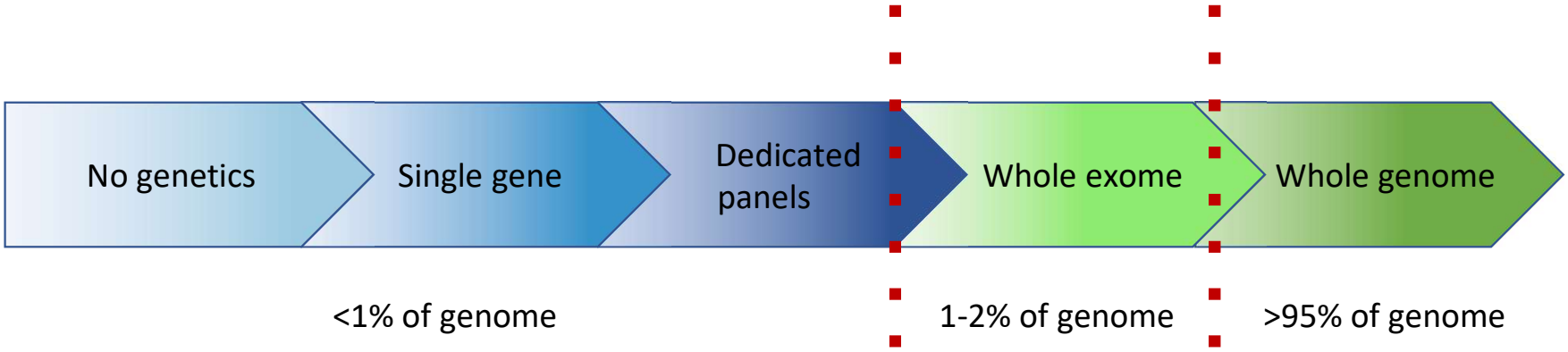
Limitations to genetic testing



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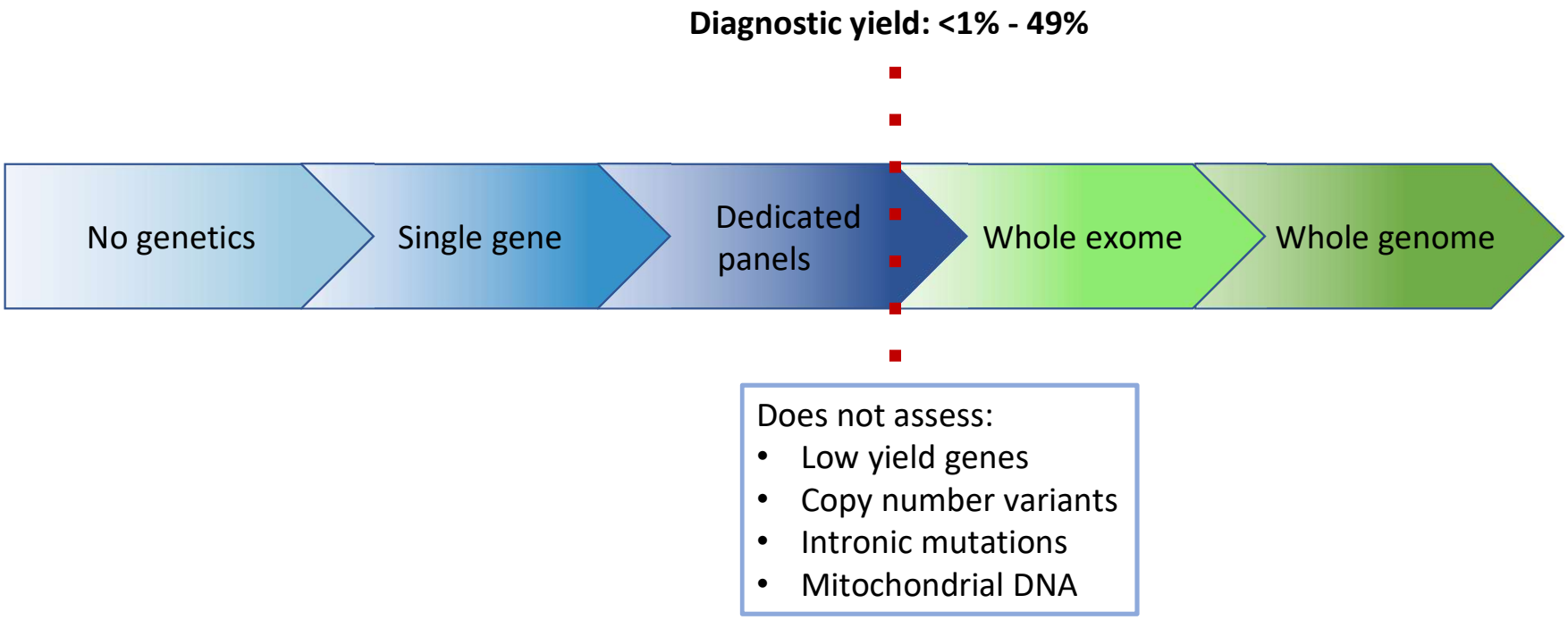
Limitations to genetic testing



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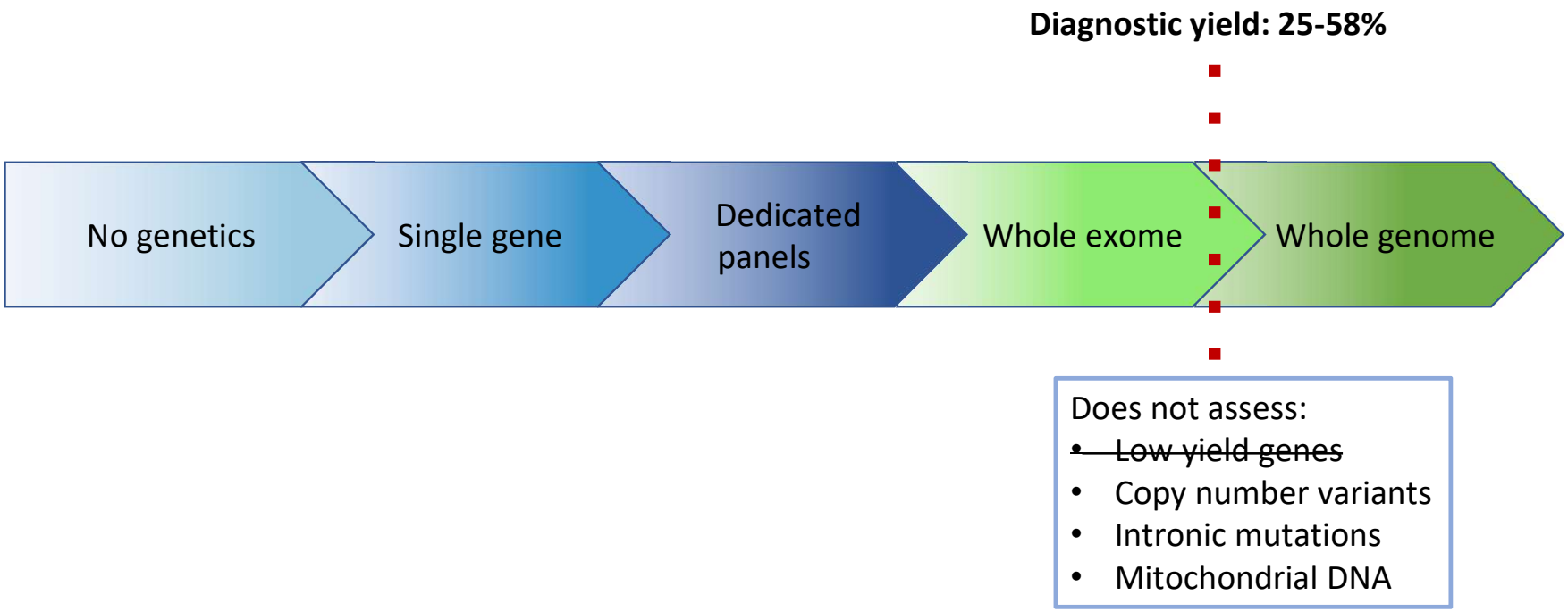
Limitations to genetic testing



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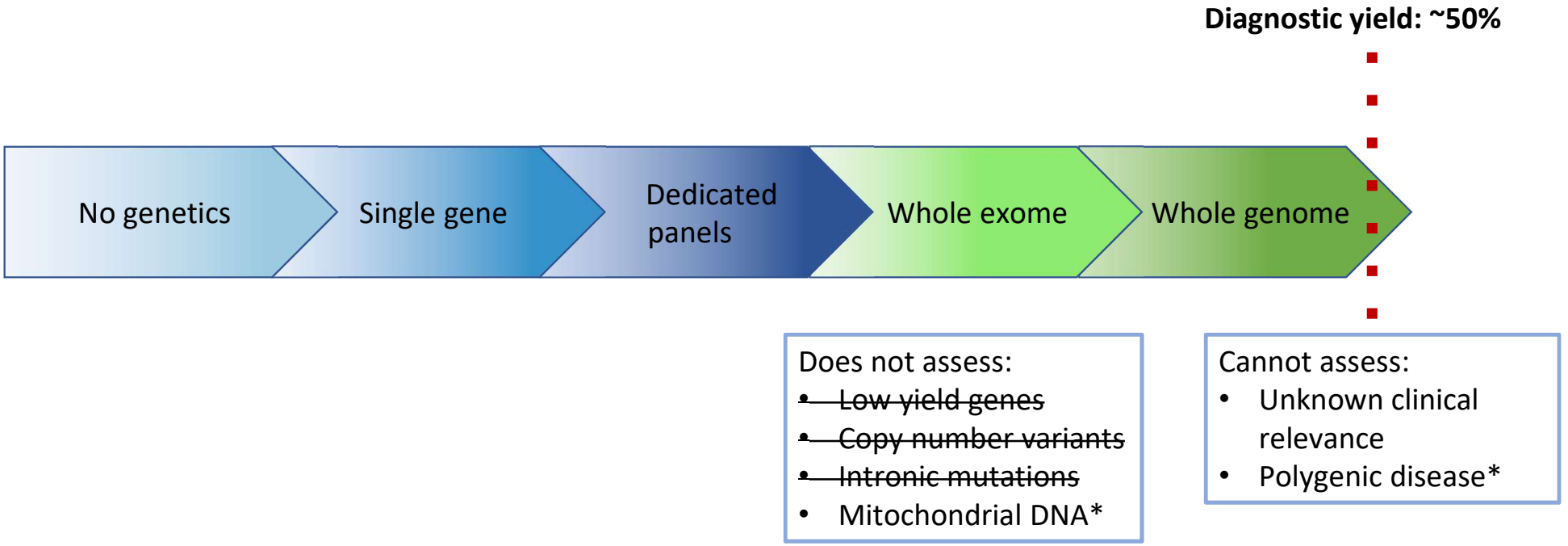
Limitations to genetic testing



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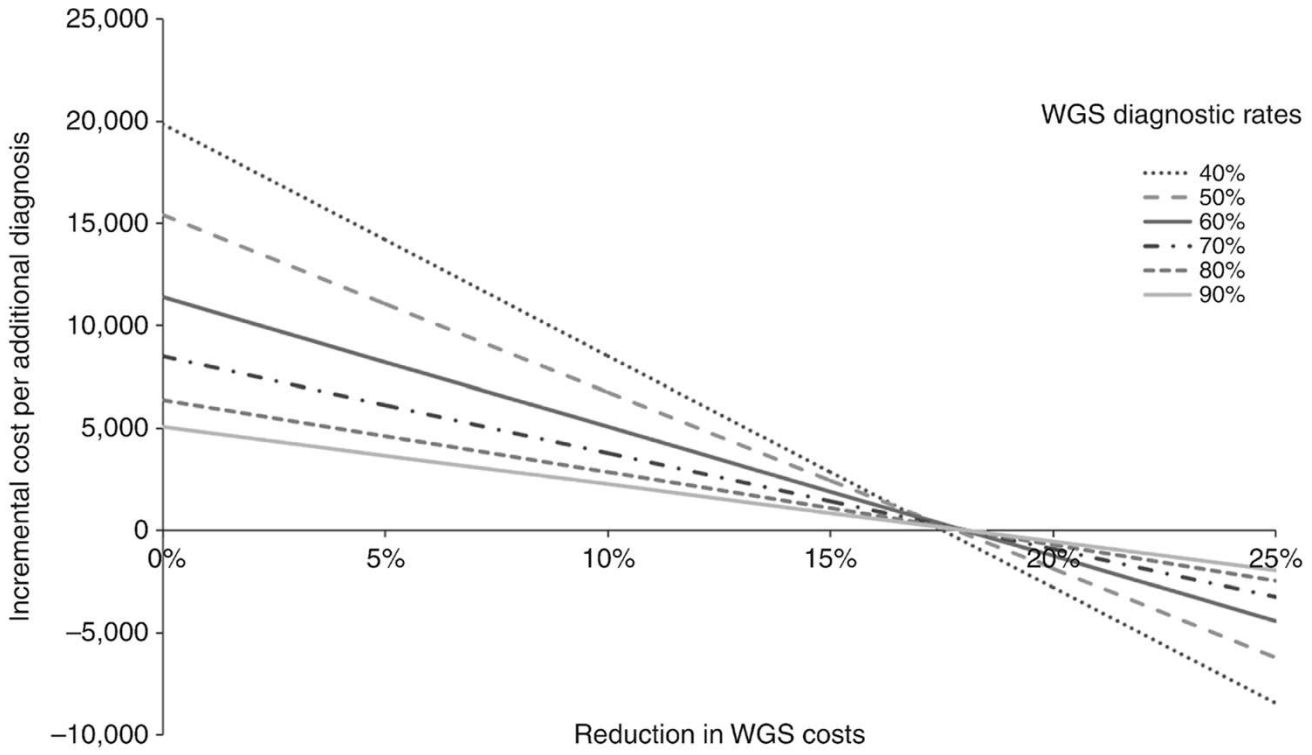
Cost effectiveness of whole genome vs whole exome



LJ Ewans et. al. Genet Med. 2018 Mar 29

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Controversy surrounding 3,4-DAP

Jacobus Pharmaceuticals (Princeton, NJ)	Catalyst Pharmaceuticals (Coral Gables, FL) - Firdapse®
Compassionate use since 1980s	EU approval for LEMS since 2009 FDA approval 11/28/18
	Orphan Drug Act with priority review and breakthrough therapy designation
	Firdapse® will have 7 year marketing exclusivity
	Ongoing trials in MuSK+ myasthenia gravis and SMA 3

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Lessons from the case

1. Importance of genetic diagnosis in congenital myasthenic syndromes
   - Periodic re-analysis of genetic testing may be indicated

2. Treatments are available but can worsen some conditions

2. CNS manifestations of presynaptic disorders are not well characterized

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Acknowledgements

The patient, his parents, and other physicians who have participated in his care

Jacinda Sampson, MD, PhD – Clinical Associate Professor of Neurology and Neurosurgery, Stanford University
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Sarada Sakamuri, MD – Associate Director Clinical Neurophysiology/EMG Fellowship, Stanford University
John W. Day, MD PhD – Director Neuromuscular Division and Clinics, Stanford University

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