Child Neurology FOUNDATION
Creating a Community of Support

Shortening the Diagnostic Odyssey

Child Neurology Foundation Symposium

Child Neurology FOUNDATION
Creating a Community of Support

Welcome

Scott L. Pomeroy, MD, PhD

President, **Child Neurology Foundation**
Bronson Crothers Professor of Neurology
Harvard Medical School
Chair, Department of Neurology
Neurologist-in-Chief
Boston Children's Hospital

Disclosures

Grant funding

Eunice Kennedy Shriver NICHD
National Institute of Child Health & Human Development

National Institute of Neurological Disorders and Stroke

National Cancer Institute

Children's Oncology Group
Dedicated to Discovery
Committed to Care

National Human Genome Research Institute

Editor Roles

- Elsevier
- UpToDate
- Wiley

50TH ANNUAL MEETING
CNS
SEPT 29-OCT 2, 2021
BOSTON • MASSACHUSETTS

Acknowledgements

STOKE THERAPEUTICS

GREENWICH BIOSCIENCES

BIOMARIN

ultragenyx pharmaceutical

PTC THERAPEUTICS

ucb Inspired by patients. Driven by science.

TAYSHA GENE THERAPIES

REGENXBIO

genome MEDICAL

ZOGENIX

illumina

Mallinckrodt Pharmaceuticals

Acknowledgements

TSC Alliance

SynGAP Research Fund
Collaboration. Transparency. Urgency.

NeurAbilities Healthcare

The Child Neurology Foundation mission is to serve as a collaborative center of education, resources, and support for children and their families living with neurologic conditions, and facilitate connection with medical professionals who care for them

Shortening the Diagnostic Odyssey Matters

- Average length of time from symptom onset to an accurate diagnosis of a rare disease is 5 years (1)
- 80% of rare diseases are caused by a faulty gene (2)
- 90 % of rare childhood disorders have major neurologic effects (3)

(1) Engel PA, et al. Physician and patient perceptions regarding physician training in rare diseases. Journal of Rare Disorders 2013: Vol. 1, Issue 2.
(2) Bavisetty S, et al. Emergence of pediatric rare diseases. Rare Diseases 2013, volume 1.
(3) National Institute of Neurological Disorders and Stroke (NINDS) Strategic Plan 2021- 2026

Shortening the Diagnostic Odyssey Matters

- A third of child neurologists report over 25% of their patients are undiagnosed
- 35-50% of families are still looking at 3+ years for a diagnosis
- 44% of caregivers have had to deal with a misdiagnosis

SOURCE: CNF 2021 CHILD NEUROLOGIST AND CAREGIVER NEEDS ASSESSMENTS

Putting it all together

Last year we discussed
how to facilitate and shorten the odyssey

This year we discuss
what to do when all the testing is done

Today's Agenda

- How a Whole Genome Sequencing Opportunity Impacted 25 Children, Caregivers and their Medical Providers
- Getting from Gene to Treatment and Disease-Specific Clinical Trials
- Possibilities with N-of-1 trials
- Participant Reflections and Break
- How to handle the various journeys
- Panel Discussion: How to effectively collaborate to get answers

Anup Patel, MD

Ohio State University College of Medicine
Nationwide Children’s Hospital

Child Neurology FOUNDATION
Creating a Community of Support

How a Whole Genome Sequencing Opportunity Impacted 25 Children, Caregivers, and their Medical Providers

Anup Patel, MD
Nationwide Children's
The Ohio State University College of Medicine

Disclosures

- Institutional research support: Encoded, Stoke
- Research support: NIH, PERF, and PCORI
- Scientific Advisory Group: Neurelis and Greenwich Biosciences

The diagnostic journey's burden on the healthcare system

Identifying all the known rare and ultrarare diseases can remain a challenge even for the most experienced clinical specialists

1. Rare Disease Impact Report: Insights from patients and the medical community. https://globalgenes.org/wp-content/uploads/2013/04/ShireReport-1.pdf.
2. Global Commission. Ending the diagnostic odyssey for children with a rare disease. 2019. globalrarediseasecommission.com.
3. Posada de la Paz M, Taruscio D, Groft SC. Rare diseases epidemiology: Update and overview. 2nd edition. Chapter 2. Springer 2017. Cham, Switzerland.
4. Soden SE, Saunders CJ, Willig LK, et al. Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. Sci Transl Med. 2014;6;265ra168.

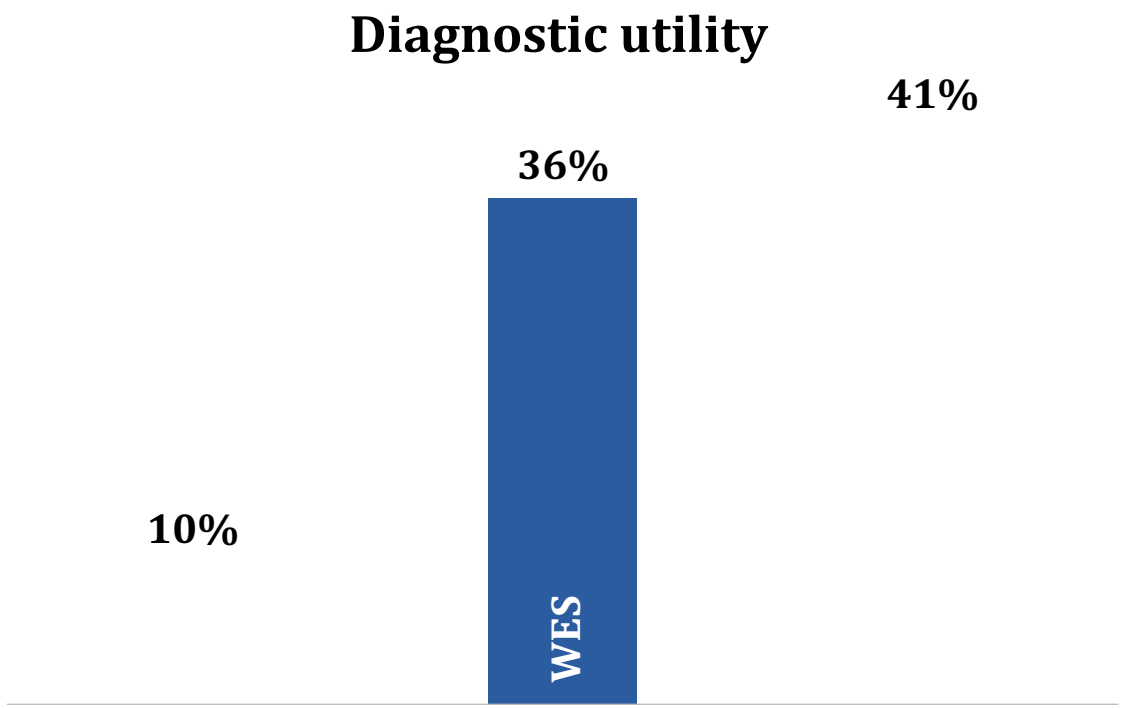
WGS Provides the Most Comprehensive Analysis of Genomic Variants Among All Genetic Testing Methods

	Sanger	Targeted NGS	PCR	FISH	Karyotype	CMA	WES	WGS
SNVs	✓	✓	✓				✓	✓
Indels	✓	✓	✓				✓	✓
CNVs			✓	✓		✓	Limited	✓
Repeat Expansions			✓					✓
Structural Variants				✓	✓		Limited	✓
Mitochondrial	✓	✓					✓	✓
Paralogs								✓
Mosaicism						✓		

WGS and WES Offer Significant Improvements in Diagnostic Success vs CMA in Select Patient Groups

8.3X GREATER ODDS OF DIAGNOSIS with WGS/WES*

In a meta-analysis of literature from January 2011 to August 2017, 37 studies comprising 20,068 children were included for review of diagnostic utility of 3 testing approaches: CMA, WES, and WGS



*95% CI: 4.7-14.9, *P*<0.0001.

CMA=chromosomal microarray; WES=whole-exome sequencing; WGS=whole-genome sequencing.

Reference: 1. Clark MM, Stark Z, Farnaes L, et al. Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected diseases. *NPJ Genom Med*. 2018 Jul 9;3:16. doi: 10.1038/s41525-018-0053-8

Highlights from ACMG Guideline

Exome and genome sequencing (ES/GS) for pediatric patients with congenital anomalies or ID/DD

- Strong recommendation for ES/GS as a first- or second-tier
- There is evidence of clinical utility of ES/GS in these indications
- Feasibility and acceptance of ES/GS have been demonstrated by relevant stakeholders

Manickam, K., McClain, et al. Exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability: an evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG).. Genet Med (2021) https://doi.org/10.1038/s41436-021-01242-6

CNF's WGS Program

39 Site Applications (104 Cases)

5 Sites Selected

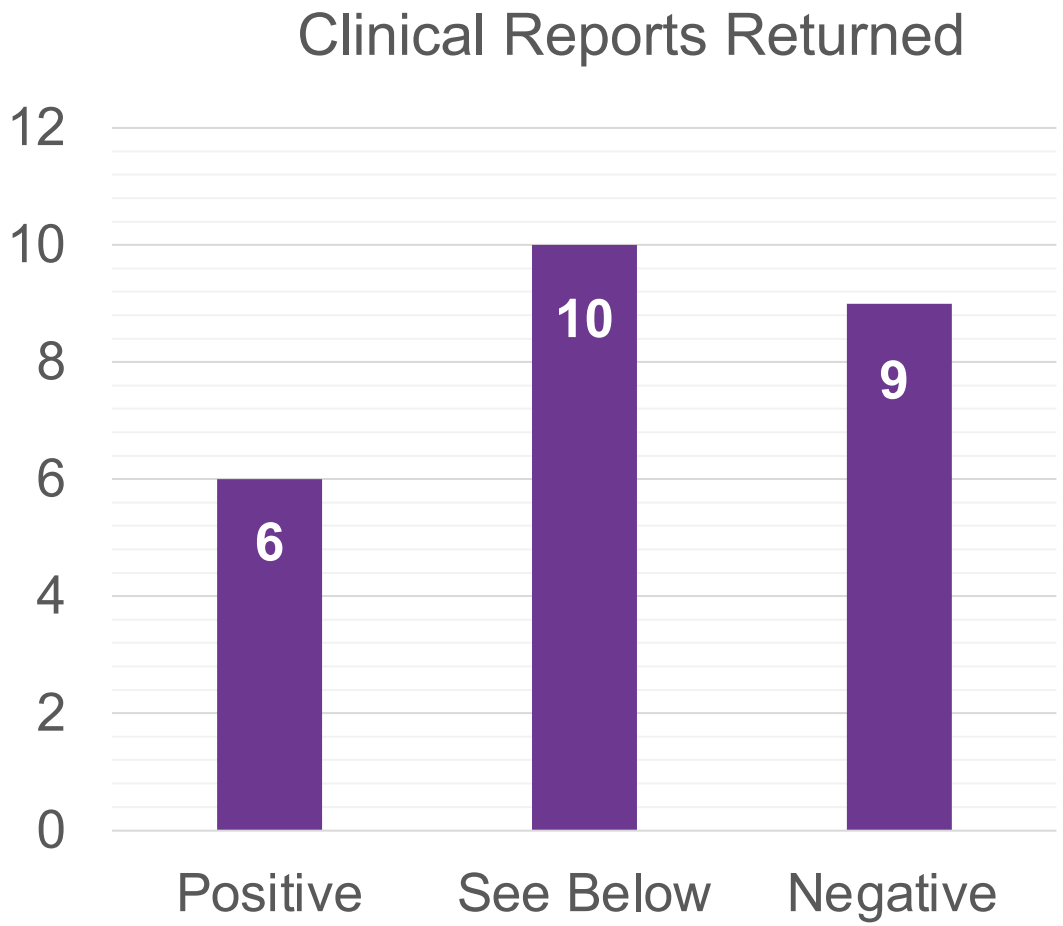
25 Children Received WGS at **no cost**

Thanks to the generous support of Illumina

Application Review Process

- **Applications accepted**
  - Mostly academic institutions applied
  - Did not have WGS available even for research purposes
- **Cases reviewed independently by two neurology providers**
- **Cases selected based on possibility of**
  - Diagnosis given previous genetic results
  - Potential treatment implications
  - Significant co-morbidities

Reporting Details



24% of Children Received a Diagnosis

Report labels

- **Positive**
  - Clinically significant (LP/P) variant in a gene with a gene-disease relationship that is at least moderate as based on the ClinGen Framework (PMID: 28552198)
- **See Below**
  - All VUS variants regardless of gene-disease relationship classification
  - Incidental findings
- **Negative**
  - No variants reported

Aggregate Data from Illumina Clinical Services Laboratory; Sarah Schmidt M.S. and Rady Children's Institute, Lisa M. Salz, MS, LCGC

5-year search ends with new care plan and connection

- Hiccups and jerking movements felt in utero
- Frequent myoclonic movement and hypotonia noted in infancy
- EEG showed encephalopathic pattern
- Unique facial features and VSD noted
- Extensive work-up (MRI, metabolic, genetic) unremarkable
- Diagnosed with a movement disorder and other chronic medical issues
- QWGS found diagnosis of PURA syndrome which brought relief and connection to support/advocacy group

14-year search ends with tailored prognosis and improved family planning

History of leukoencephalopathy, mild ID, spastic diplegia, short stature, progression sensorineural hearing loss, and retinitis pigmentosa

Repeat brain MRI showed progressive white matter lesions

Other work-up non-diagnostic

Two trio exomes 2013 and 2019 (separate labs) both non-diagnostic

WGS testing showed pathogenic variation in MORC2

Closure for family and relief

Closure for Family

- History of developmental epileptic encephalopathy
- Treatment resistant epilepsy
- Other work-up non-diagnostic
- WGS testing showed pathogenic variation in TATA-box binding protein associated factor 1 (TAF-1) gene
- X-linked recessive
- Mother asymptomatic carrier

“Families that didn’t get a diagnosis were not surprised; they have already done a lot of testing with no answers. They were grateful for the opportunity.”

“For the family with a diagnosis, having the answer was very positive, even though it was rare and there is currently no treatment.”

“Families are interested in the testing, and it can change care in meaningful ways such as giving them opportunities to connect with other families and to engage in research efforts.”

“WGS changed the child’s prognosis. We suspected a mitochondrial disorder, so he was getting a mitochondrial cocktail. His diagnosis is not mitochondrial, though still rare and some limits on prognosis, but it is better. It is non inherited, so the parents don’t have to worry about their other child and can plan for future children as well.”

Erika Augustine, MD, MS

Kennedy Krieger Institute

Child Neurology FOUNDATION
Creating a Community of Support

From Gene to Treatment and Disease-Specific Clinical Trials

Erika Fullwood Augustine, MD, MS

Kennedy Krieger Institute

Disclosures

Research Funding: NIH/NINDS, Batten Disease Support and Research Association

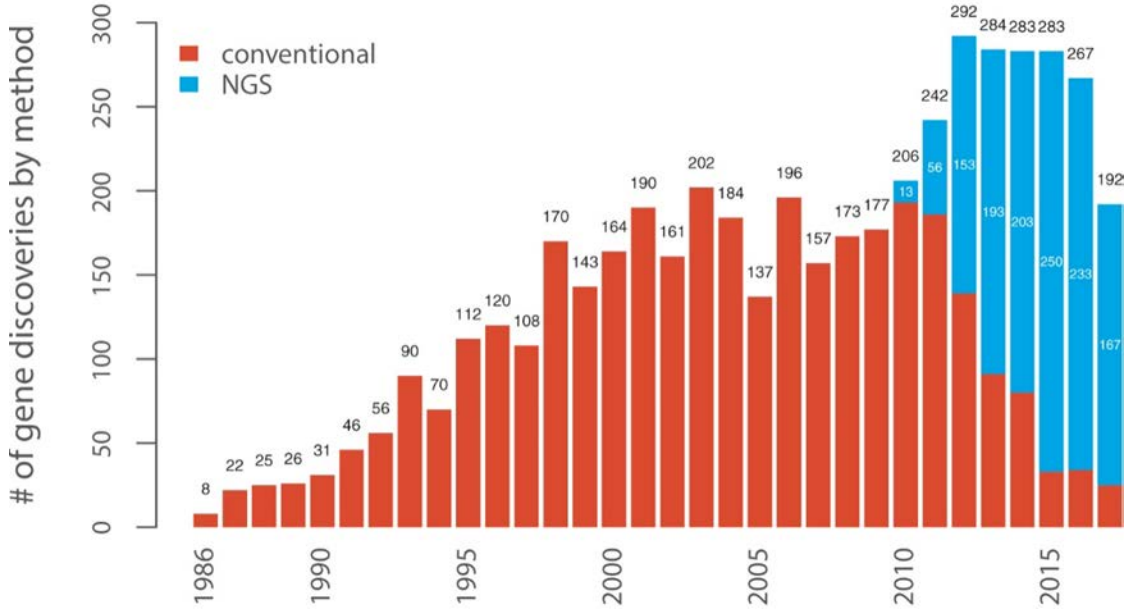
DSMB Member: PTC Therapeutics

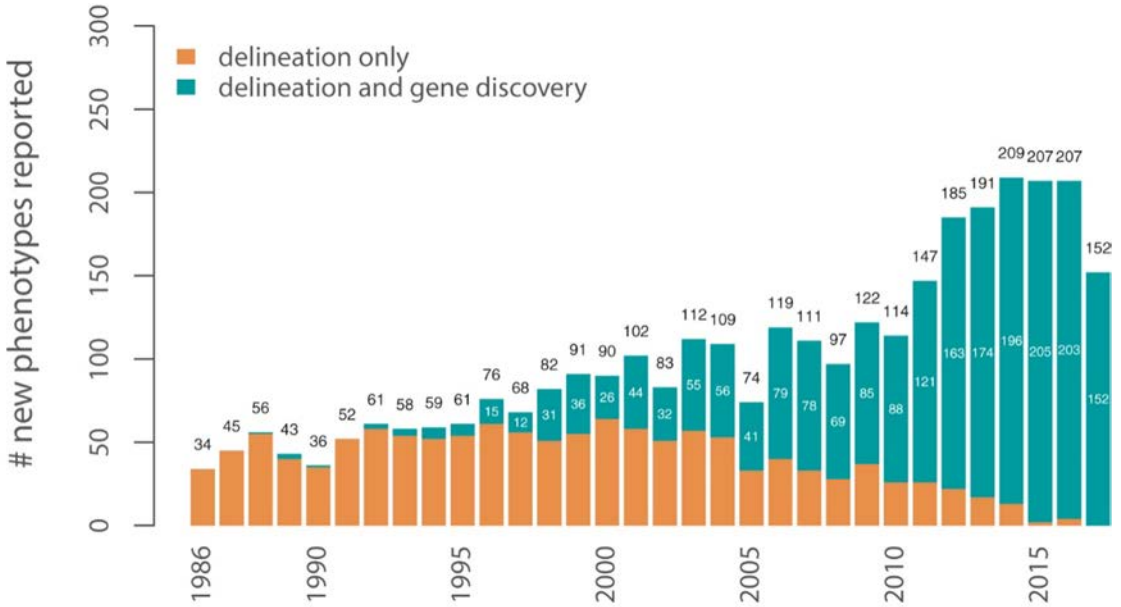
Consultant: Amicus Therapeutics, Beyond Batten Disease Foundation, Neurogene Inc, Signant Health, Taysha Gene Therapies

Objectives

- To understand factors that are enabling rapid advancement in development of novel therapies for orphan conditions
- To understand key importance of preparatory research to enable efficient and informative trials

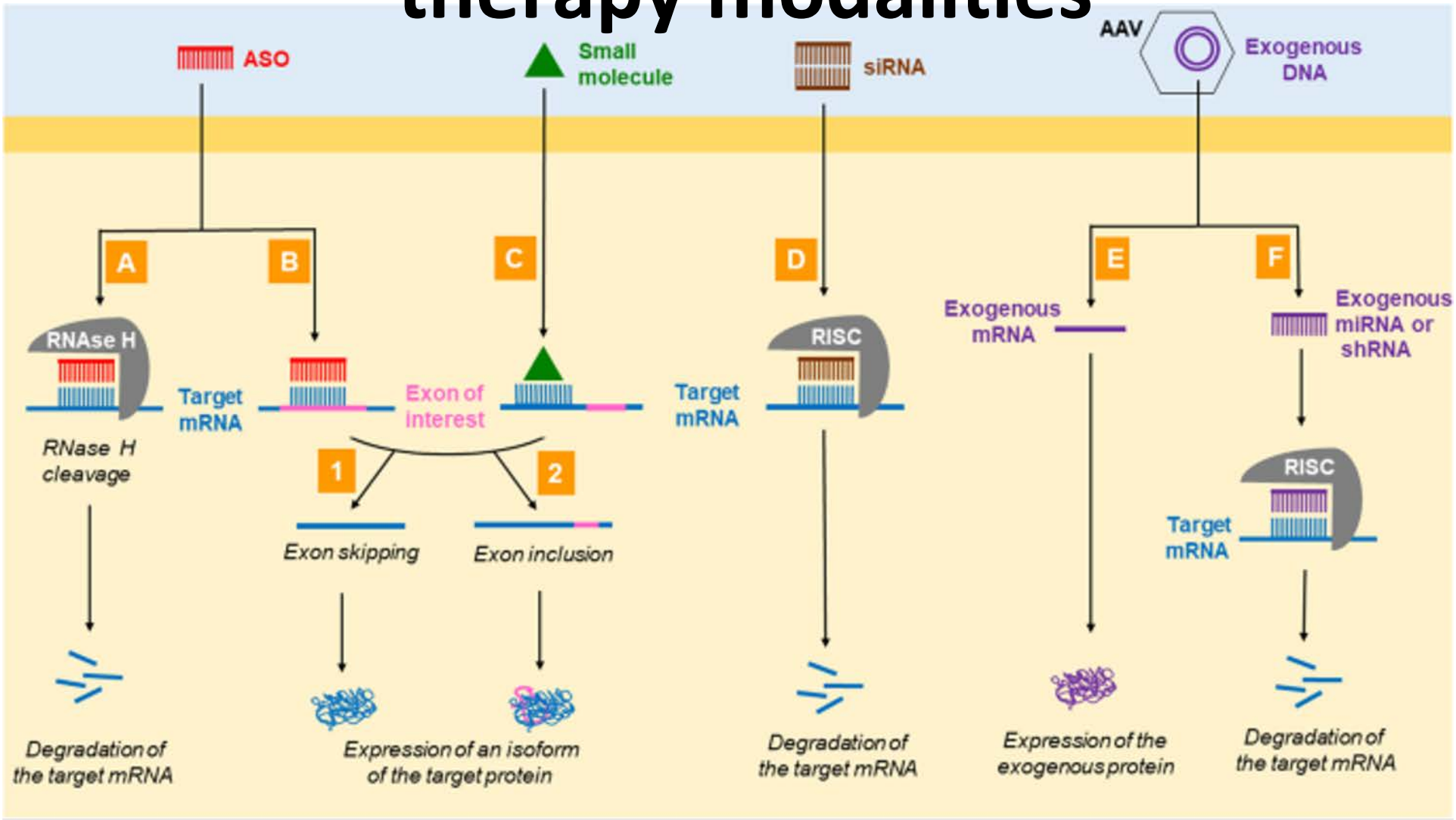
Rates of gene discovery and syndrome delineation are increasing





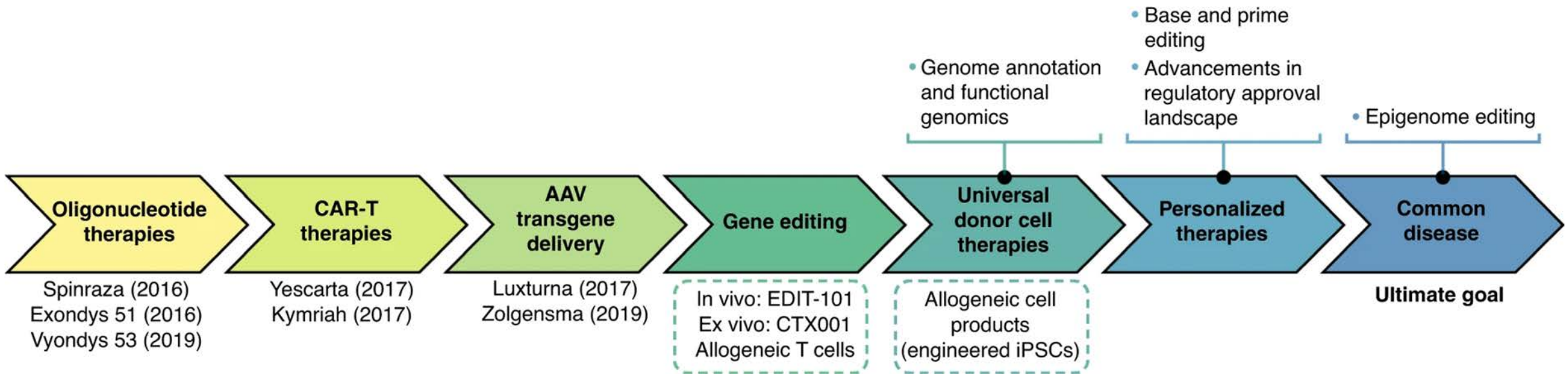
AJHG. 2019; 105: 448–455.

There are a growing number of gene-targeted therapy modalities



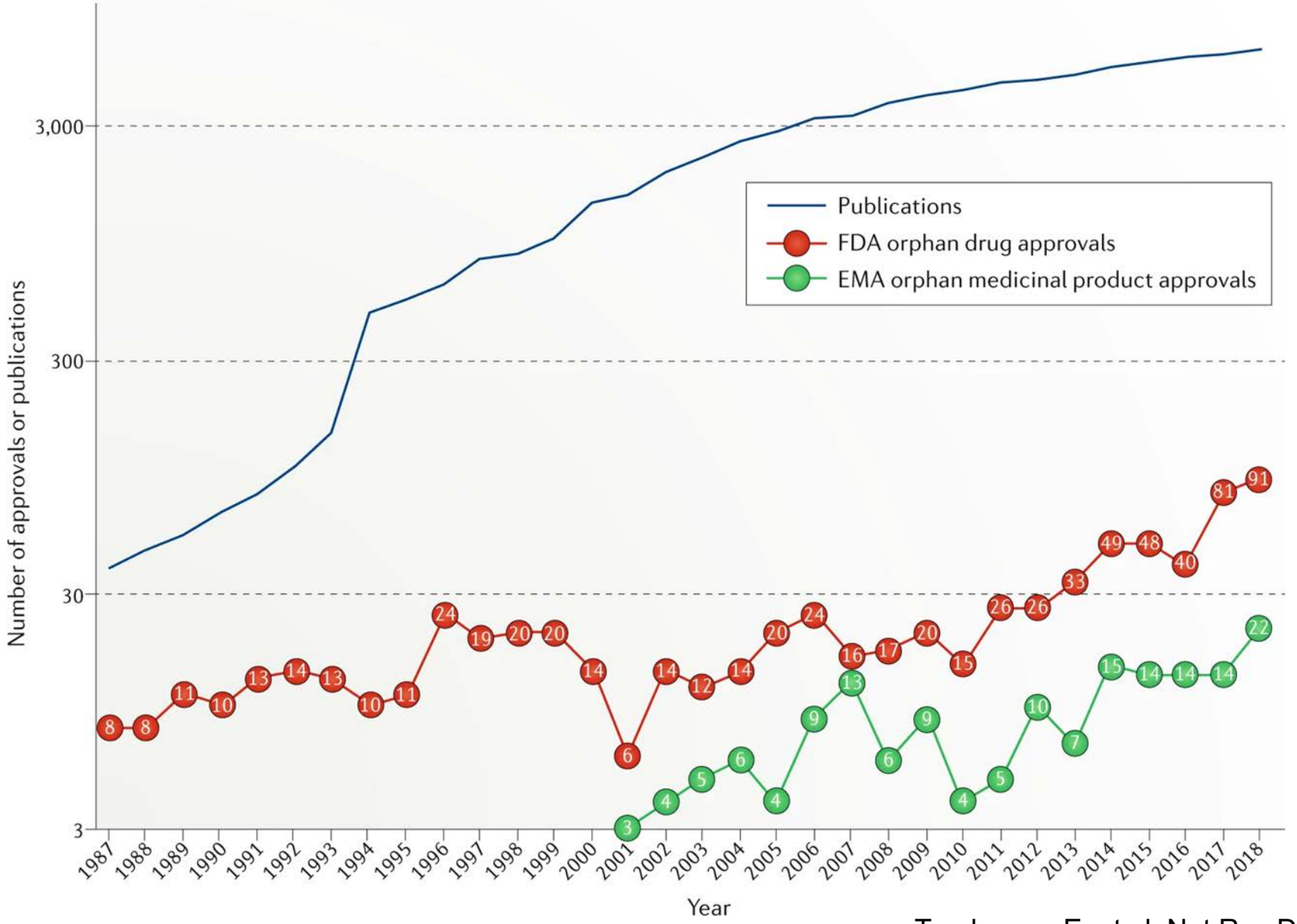
Brenner et al. Neurological Research & Practice. 2020; 2:25.

Milestones in the development of gene-targeted therapies



K Bulaklak, et al. Nat Commun. 2020; 11: 5820.

A translational gap remains and is widening



Tambuyzer E, et al. Nat Rev Drug Discov. 2020;19(2):93-111

Gene Discovery

Gene Targeted Therapy

Gene Discovery

Gene-Targeted Therapy

Pre-clinical Discovery

- Understand disease mechanisms
- Generate animal models that recapitulate human disease
- Identify therapeutic targets
- Processes of drug discovery
  - Compound screening/construct development
  - In vitro, in vivo analyses
    - Proof-of concept
    - Dose, safety, toxicity, efficacy

Gene Discovery

Gene-Targeted Therapy

Clinical Trial Readiness

- Understand natural history
- Identify important impacts for families
- Establish robust outcome measures & potential endpoints
- Biomarker development
- Identify experienced investigators
- Mobilize community

Gene Discovery

Gene-Targeted Therapy

Clinical Trials

- Adequate and appropriate trial design
- Consultation with regulatory authorities
- Strong community engagement
- Clinical trial programs that address a series of development questions
  - Safety
  - Dosing & route of administration
  - Target population & time of intervention
  - Efficacy

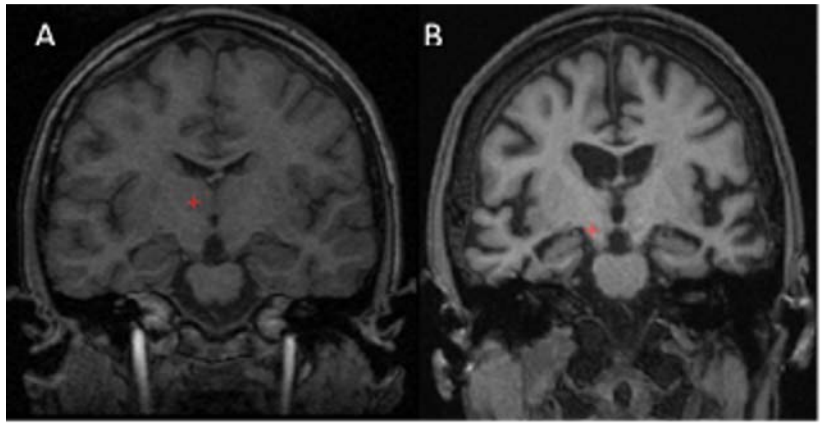
Gene Discovery

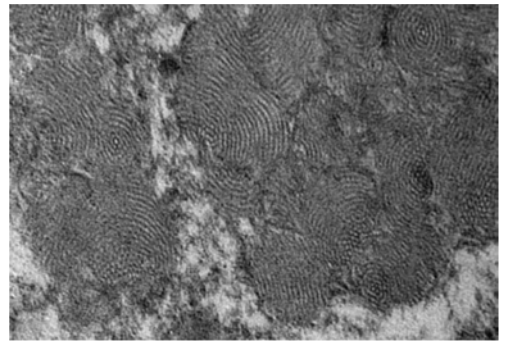
Gene-Targeted Therapy

Clinical Trial Readiness

Neuronal Ceroid Lipofuscinoses

- Most prevalent neurodegenerative disorder of childhood
- Group of lysosomal storage diseases
- Unifying clinicopathologic features
  - clinical symptoms
  - progressive neuronal loss
  - autofluorescent storage material





MRI image source – A. Schulz; Poyato, et al. JIMD. 2011; 34(5):1083-93.

Clinical Trial Readiness in CLN3 Disease

Expand network of collaborating investigators

Standardize Data Elements

Validate Outcome Measures

Train Evaluators

Validate Biomarkers

Identify methods for testing new therapies

Understand Natural History

NINDS (U01NS101946)

Unified Batten Disease Rating Scale (UBDRS)

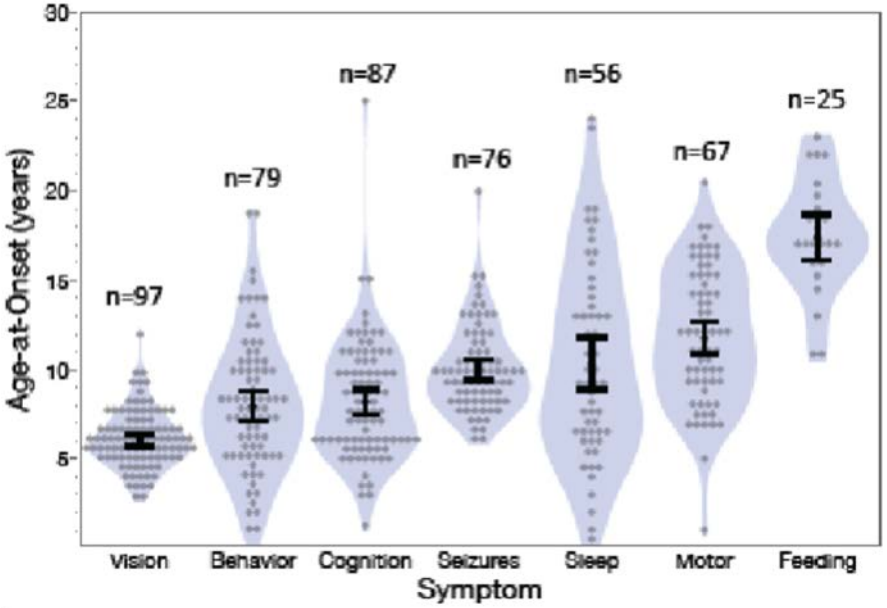
- Global Disease Assessment Tool
- Quantitative Ratings - 4 subscales
  - Physical Assessment (0-112) - 28 items
  - Seizure Assessment (0-54) - 12 items
  - Behavioral Assessment (0-55) - 9 items
  - Capability Assessment (0-14) - 5 items
- Sequence of Symptom Onset:
  - Vision, Behavioral, Cognitive, Motor, Seizures, Feeding, Sleep
- Clinical Global Impression of core symptom severity and change since previous evaluation

Marshall et al, Neurology 2005
DeBlieck, et al. Contemp Clin Trials 2013

UBDRS – Systematic approach to build clinical

UBDRS – Systematic approach to build clinical knowledge

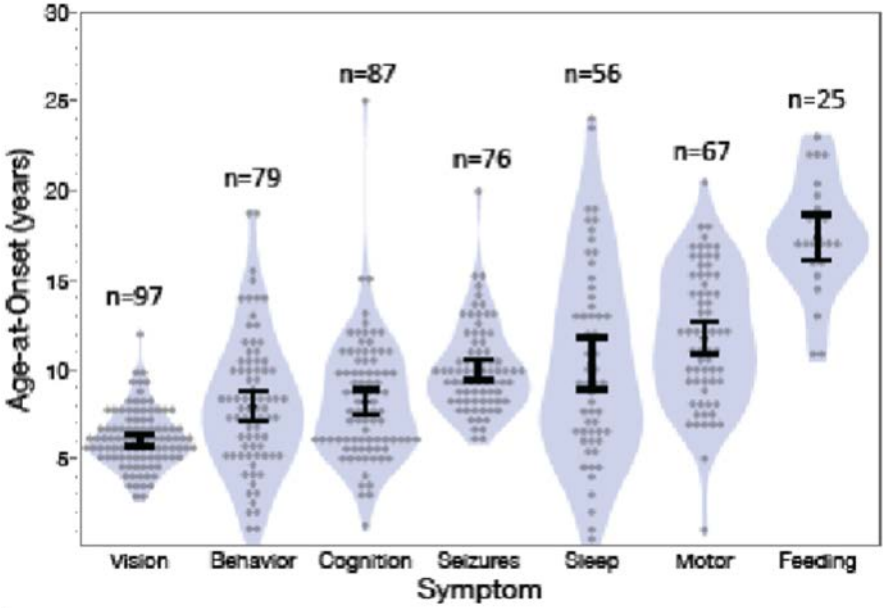
Sequencing Symptom Onset



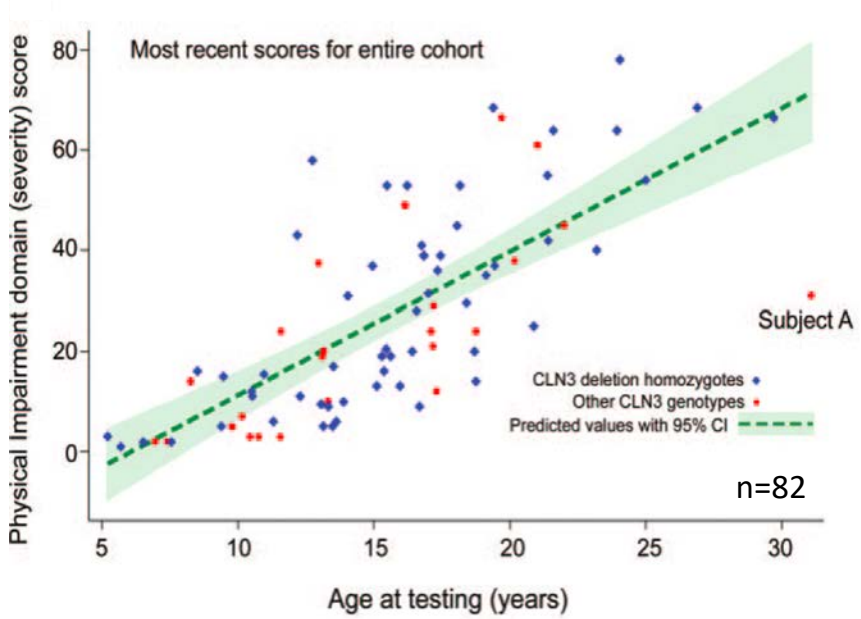
Unpublished data

UBDRS – Systematic approach to build clinical knowledge

Sequencing Symptom Onset



Quantifying Progression

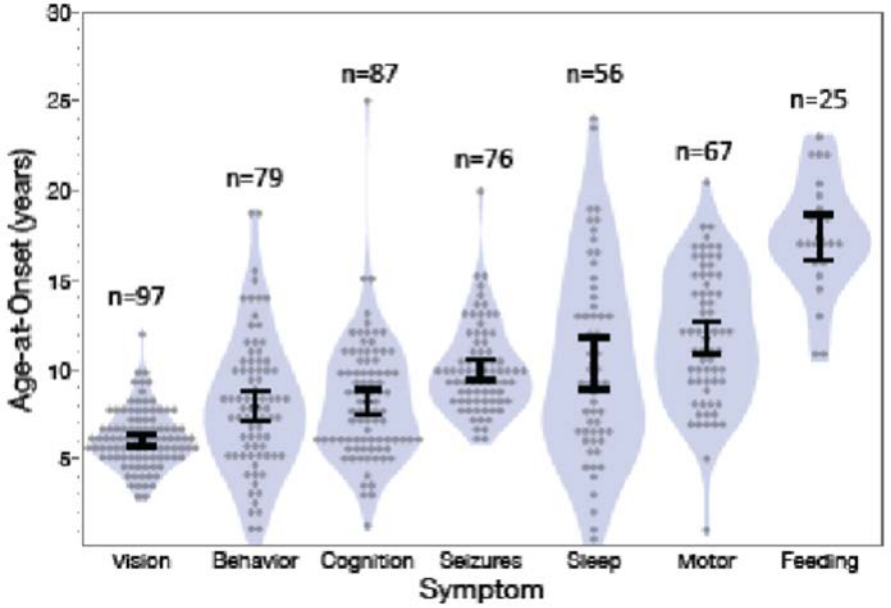


Unpublished data

Neurology 2011; 77(20): 1801-1807

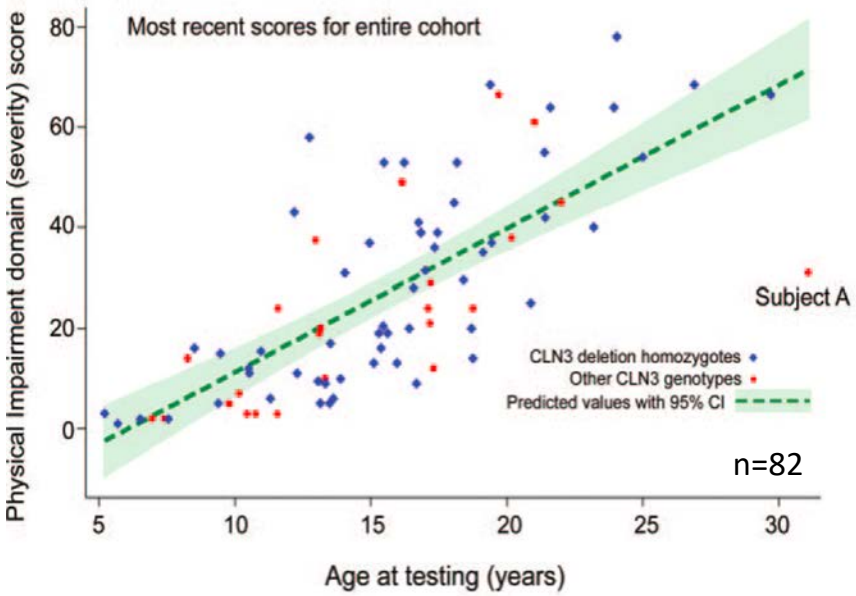
UBDRS – Systematic approach to build clinical knowledge

Sequencing Symptom Onset



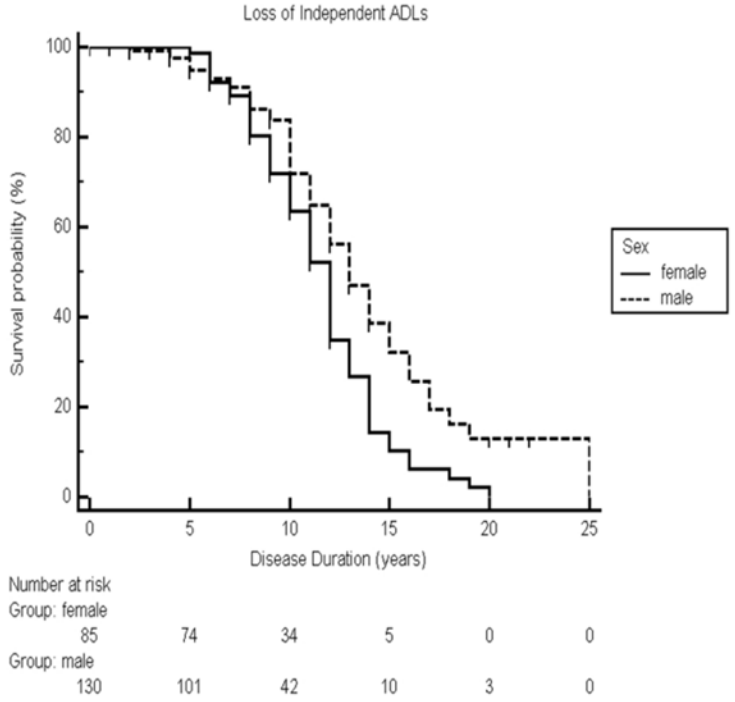
Unpublished data

Quantifying Progression



Neurology 2011; 77(20): 1801-1807

Examining Sex Differences



J Inher Met Dis 2012; 35(3): 549-555

Natural history tools may not = trial measures

	Natural History	Clinical Trials
Enrollment criteria	Broad	Strict
Disease stage	Full disease span	Early
Enrollment Period	Extended	Narrow
Sites	Multiple	Multiple
Assessments	Limited to comprehensive	Comprehensive
Assessment schedule	Flexible	Strict

Development of a diagnostic confidence system

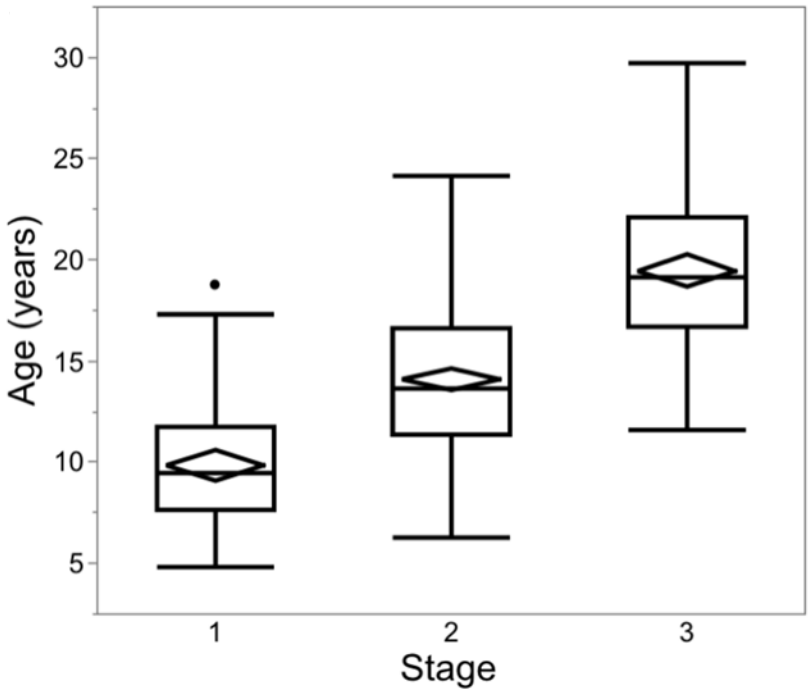
	Definite CLN3 Disease				Probable CLN3 Disease				Possible CLN3 Disease	CLN3 Disease PLUS
	1A	1B	1C	1D	2A	2B	2C	2D	3	CLN3-PLUS
	Syndromic CLN3 Disease	**Non-Syndromic CLN3 Disease**	**Undetermined Phenotype**	**Atypical CLN3 Disease**	**Syndromic CLN3 Disease**	**Non-Syndromic CLN3 Disease**	**Undetermined Phenotype**	**Atypical CLN3 Disease**	**Clinically Possible CLN3 Disease**	**CLN3 with additional medical disorder**
	Characteristic CLN3 Disease Phenotype	Vision Loss Only at Age 12 Years or Older	Vision Loss Only or Pre-symptomatic at Age <12 Years	Atypical Age-at-onset or Rate-of-Progression	Characteristic CLN3 Disease Phenotype	Vision Loss Only at Age 12 Years or Older	Vision Loss Only or Pre-symptomatic at Age <12 Years	Atypical Age-at-onset or Rate-of-Progression	Characteristic CLN3 Disease Phenotype	CLN3 Disease Phenotype plus non-NCL neuro features
+	Disease-causing Mutation on Both Alleles	Disease-causing Mutation on Both Alleles	Disease-causing Mutation on Both Alleles	Disease-causing Mutation on Both Alleles	Disease-causing mutation on one allele only **AND/OR** Fingerprint bodies / lymphocytic vacuoles **OR** Sibling with genetically confirmed CLN3	Disease-causing mutation on one allele only **AND/OR** Fingerprint bodies / lymphocytic vacuoles **OR** Sibling with genetically confirmed CLN3	Disease-causing mutation on one allele only **AND/OR** Fingerprint bodies / lymphocytic vacuoles **OR** Sibling with genetically confirmed CLN3	Disease-causing mutation on one allele only **AND/OR** Fingerprint bodies / lymphocytic vacuoles **OR** Sibling with genetically confirmed CLN3	Genetic testing not performed	Genetic or laboratory evidence for CLN3 disease
+	**OR** homozygous for common deletion without features of another disorder		**AND NOT** deletion homozygote						Microscopy not performed	
N	93	2	2	3	19	0	0	2	7	6

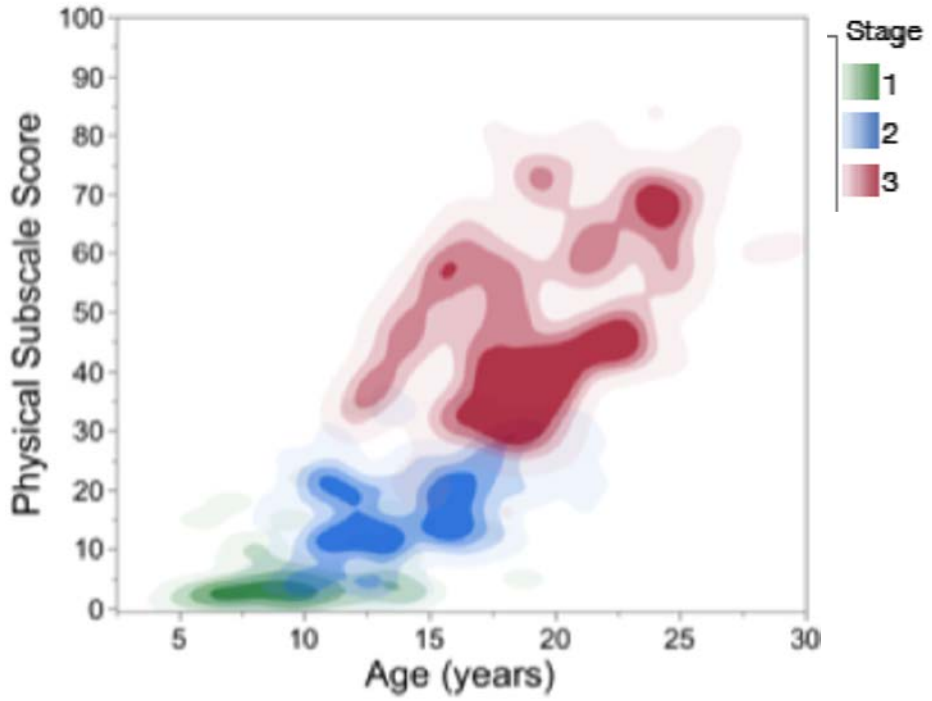
Masten, et al. Neurology; 2020. 9;94(23):e2436-e2440

Development of a clinical staging system

Disease Stage	Defining Event
0	Pre-Symptomatic
1	Vision Loss
2	Seizure Onset
3	Assistance for Ambulation

Justin Williams, MD Margaux Masten

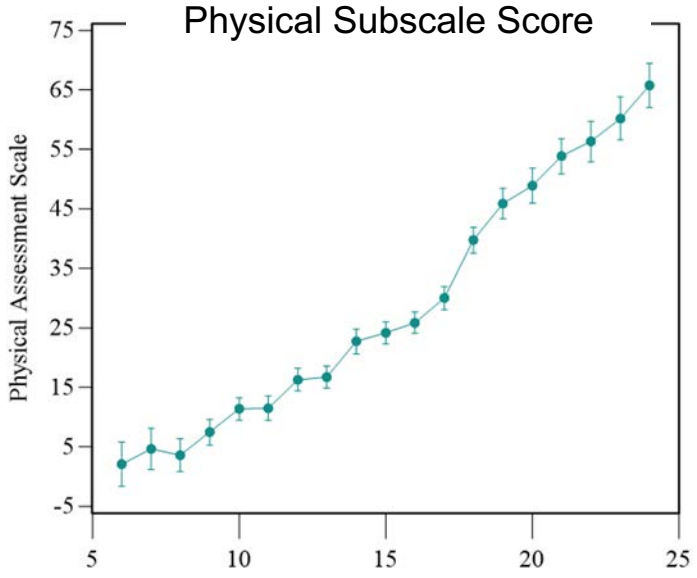


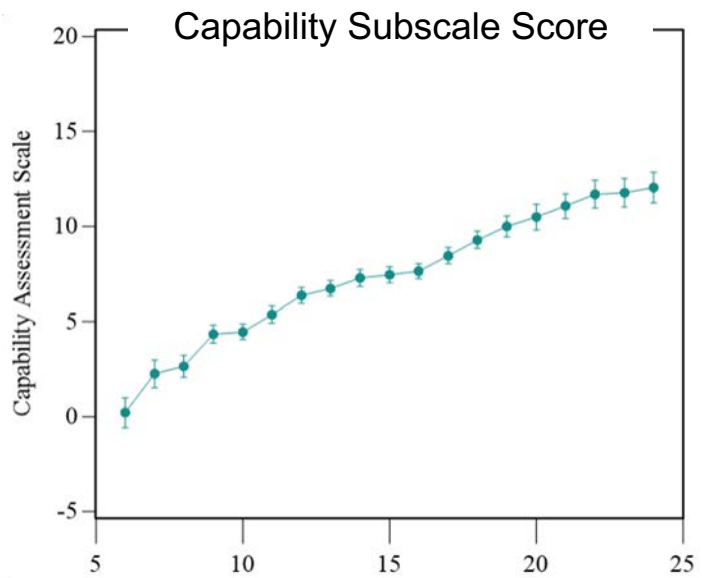


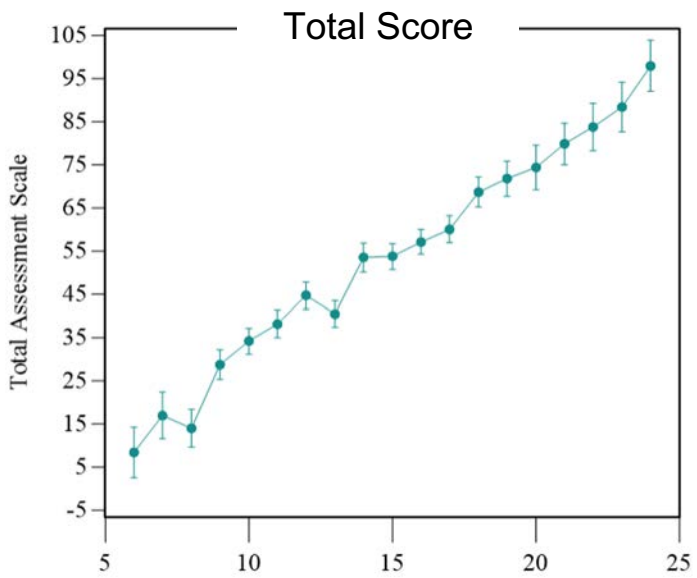
n=108 individuals; n=322 evaluations

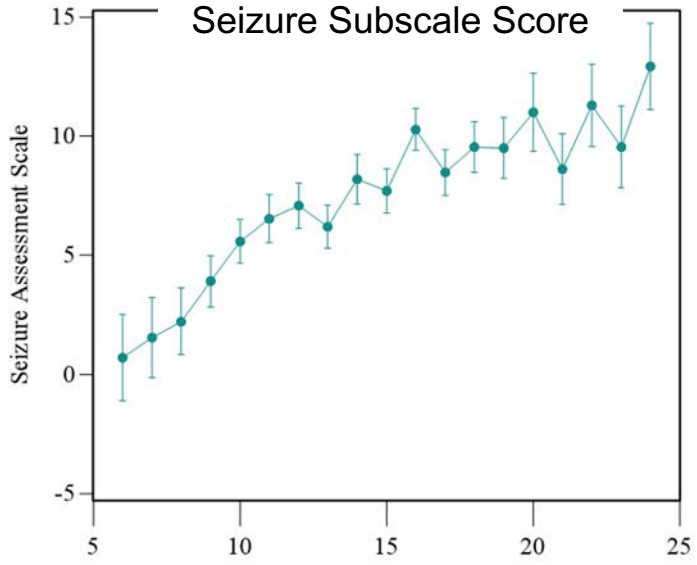
Masten, et al. Neurology; 2020. 9;94(23):e2436-e2440

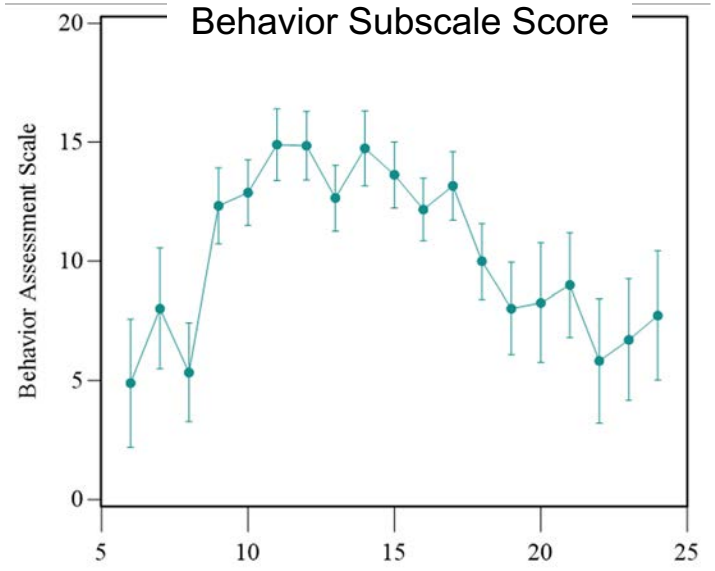
From natural history to trial measures







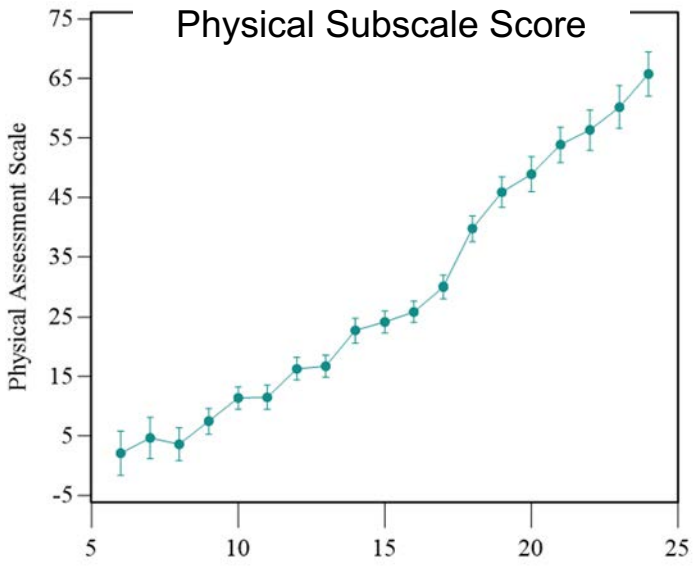


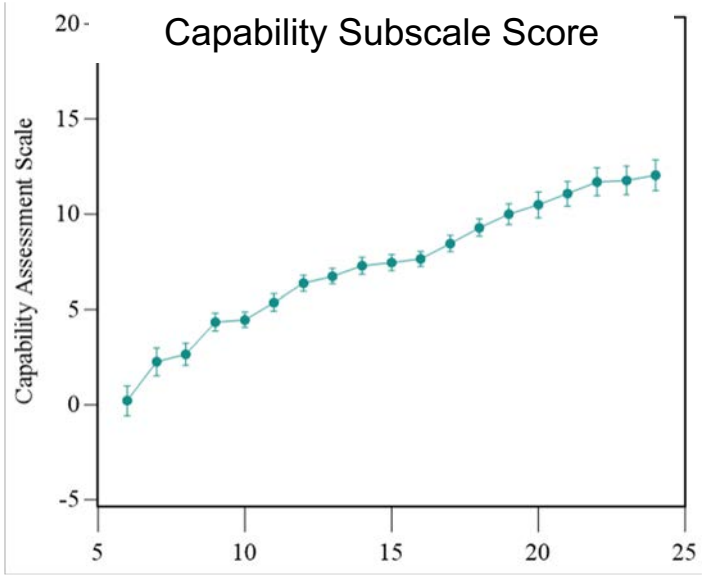


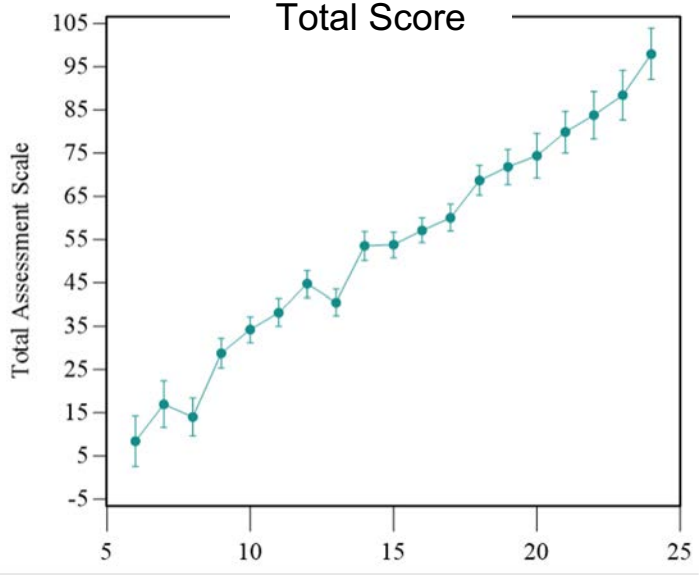
X-axis = age (years)
n=128 participants

Unpublished data

From natural history to trial measures



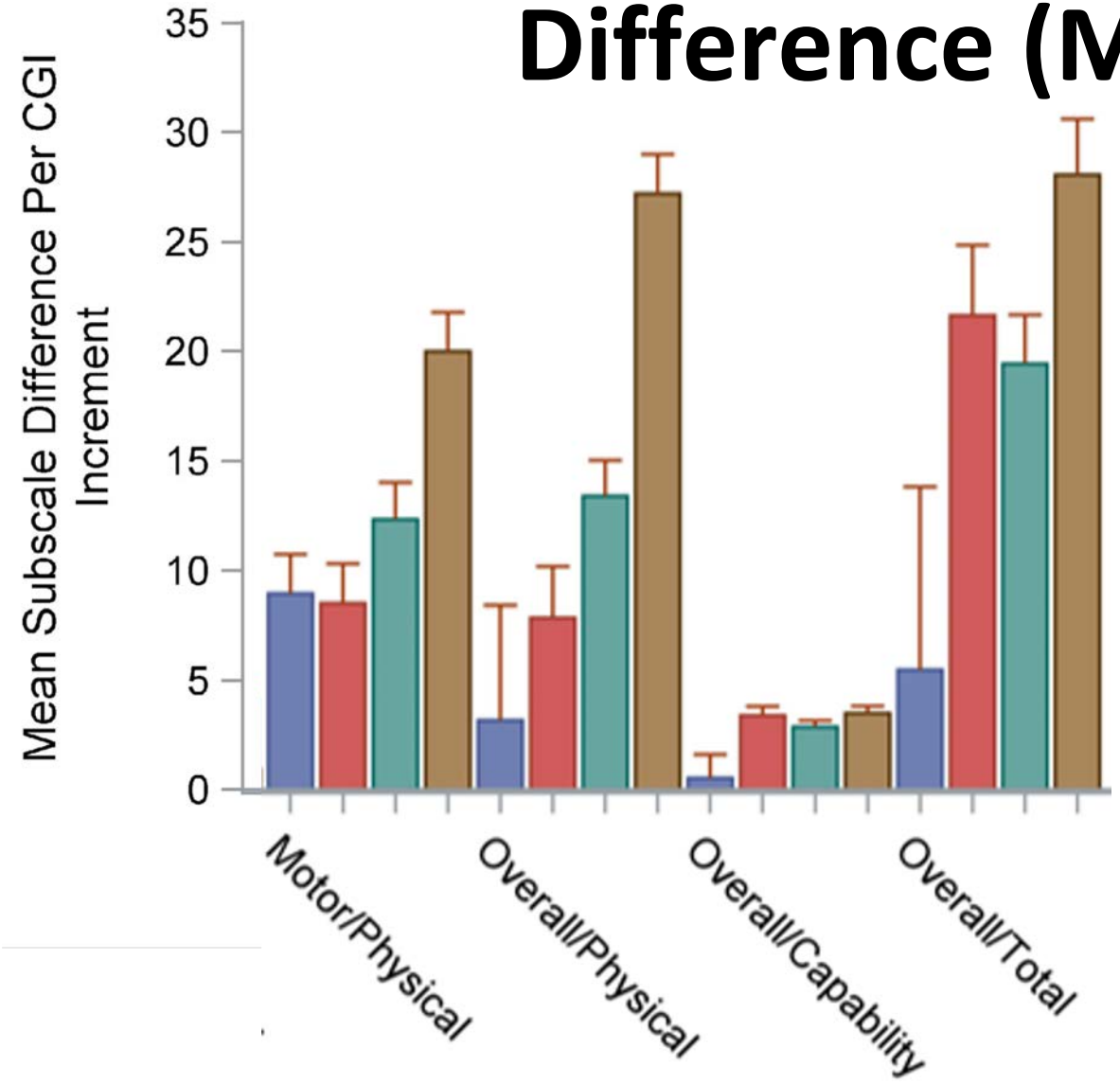




X-axis = age (years)
n=128 participants

Unpublished data

Determining Minimal Clinically Important Difference (MCID)



Clinical Global Impression of *Severity* (CGI-S)

1 = None
2 = Minimal
3 = Mild
4 = Moderate
5 = Severe

CGI 1 to 2 CGI 2 to 3 CGI 3 to 4 CGI 4 to 5

n=113 participants

Unpublished data

Determining Minimal Clinically Important Change (MCIC)

Clinical Global Impression of *Change* Since Last Visit (CGI-C)

1 = Much better
2 = Somewhat better
3 = About the same
4 = Somewhat worse
5 = Much worse

n=58 participants

Unpublished data

Conclusions

- Significant unmet therapeutic need in rare diseases
- Immense opportunity to expand application of platform therapies (gene-targeted therapies)
- Translational barriers prevent full realization of potential impact
  - Early and accurate diagnosis
  - Sufficient knowledge of natural history
  - Identification of responsive trial outcomes

Batten Research Group & Collaborators

Jonathan Mink	Heather Adams	Christopher Beck
Luke Gelinas	Alex Levin	Frederick Marshall
Scott McIntosh	Jen Vermilion	Amy Vierhile
Grace Zimmerman	Margaux Masten	Schulz Lab
Giovanni Schifitto	Arun Venkarataman	Laurie Seltzer
Astghik Baghinyan	Camille Corre	Anna Ecklund
Tom Dellaporta	Rochelle Vassell	Madalina Tivarus

Thank you - Families who participated in and supported this research

Research Funding: NIH/NINDS (U01NS101946), Batten Disease Support and Research Association, Batten Research Alliance

Kennedy Krieger Institute

batten@kennedykrieger.org

batten@urmc.rochester.edu

URBC
UNIVERSITY OF ROCHESTER BATTEN CENTER

Christelle Moufawad El Achkar, MD

Harvard Medical School

Boston Children's Hospital

Child Neurology FOUNDATION
Creating a Community of Support

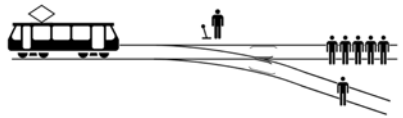
N-of-1 Trials (or precision medicine for 1): Possibilities, Pitfalls, and a Cautious Promise

Christelle Moufawad El Achkar, MD
Attending in Epilepsy and Neurogenetics
Division of Epilepsy and clinical Neurophysiology
Epilepsy Genetics Program
Boston Children's Hospital/Harvard Medical School

Disclosures

- No Financial disclosures
- Professional disclosures pertinent to this topic:
  - Site Principal Investigator and Co-Investigator for industry sponsored trials in rare genetic epilepsies
  - Principal investigator and Co-Investigator for n-of-1 anti-sense oligonucleotide (ASO) trials in neurodegenerative conditions and rare genetic epilepsies

How do you justify the risks? The cost? The effort?



What exactly are N-of-1 trials?

Too many sources of bias, how can we assess outcomes and risks?

Why are we bothering with N-of-1 trials and precision medicine?

But we do not even know if it works…

I think we should focus our efforts on the more common diseases, and help a larger number of people

This is just the “therapy du jour,” it is unlikely to be sustained

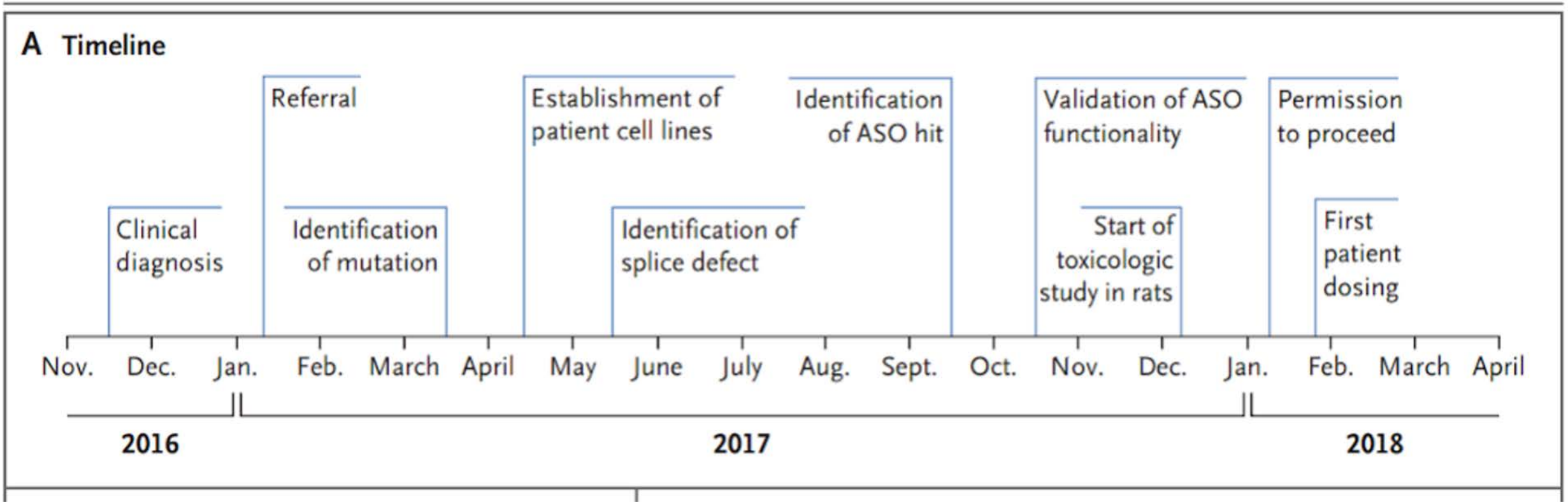
How soon can I start my patient on that therapy?

The story of a patient, a diagnostic odyssey, and the development of an N-of-1 therapy	
Definition of terms and concepts	
Historical perspective	
N-of-1 trials in Child Neurology	• Significance • Special considerations • Current landscape
The main variables	• Patient/disorder • Drug/target • Therapeutic goal/biomarkers
Pitfalls and special considerations	• Scientific capabilities, time, resources • Ethics
Possibilities and Promise of N-of-1 trials	

The story of a patient, a diagnostic odyssey, and the development of an N-of-1 therapy

- A healthy, bright 3 year old girl starts to have increased falls.
- She develops epilepsy.
- Over the span of 1-2 years, she starts to lose cognitive skills, speech, fine motor skills, ability to ambulate independently
- Serial MRIs show progressive atrophy involving the cerebellum
- There was still no diagnosis, despite extensive clinical genetic testing
- At age 6, research genome sequencing clenches the diagnosis, CLN7
- The race for a therapy begins…

Timeline of Milasen development

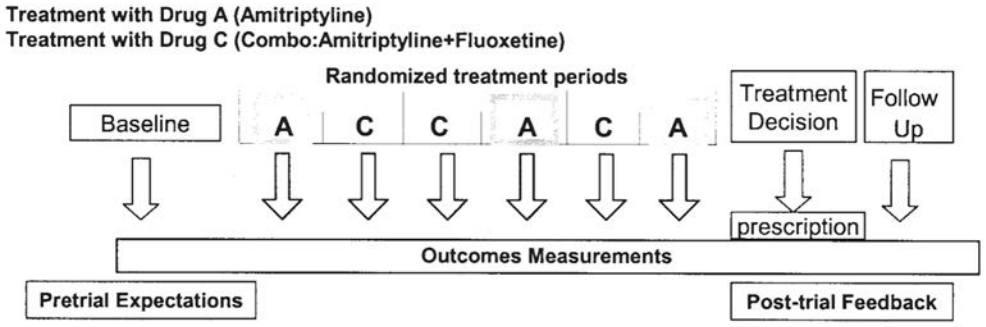


Kim J, Hu C., et. al, NEJM, 2019

What Exactly is an N-of-1 Trial? Some distinction is necessary

- According to Wikipedia, or the classic definition:

"An **N of 1 trial** is a clinical trial in which a single patient is the entire trial. A trial in which random allocation can be used to determine the order in which an experimental and a control intervention are given to a patient is an N of 1 randomized controlled trial."



Zucker et. al, JRheum, 2006

Design	Notes
A-B	Often the only possible method
A-A^1-A	Placebo design
A-B-A	Withdrawal design
A-B-A-B	Withdrawal design
A-B-A-B-A-B	Withdrawal design
A-B^1-B^2-B^3-B^n-A	effect of different versions of B

A= no treatment, A^1=placebo, B=treatment, B^n=treatment iterations

Conditions Necessary for N-of-1 Trials

Condition	Description
Nature of the disorder	Chronic, stable, slowly progressive, or frequently recurring/relapsing
Nature of the treatment(s)	Significant individual differences in treatment effects Uncertainty about best treatment Rapid onset of action Brief and safe washout periods
Outcome assessment	Validated, repeatable measures (e.g., biomarkers) of treatment effects
Stakeholders	Patients, health care providers, and health system willing to engage in N-of-1 trial effort

SOURCE: Adapted from Kravitz et al. 2014 [2]

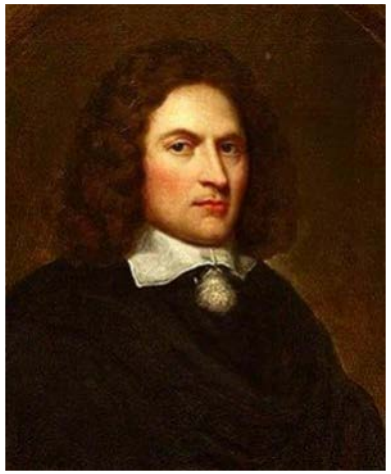
N-of-1 Trials in Our Current Context

- A single patient is the entire trial
- Therapy is **selected** or **designed** based on patient's particular disorder (e.g. genetic condition, +/- allele specific), and other physiological characteristics if applicable (e.g. pharmacogenomic profile)
- Patient is their own control (natural history of disorder is helpful, when available)
- While often patient specific, can be expanded to a small group (allele specific), or a relatively larger group (non-allele specific)
- An example of precision Medicine (used interchangeably with personalized medicine, no consensus on terminology)

"Precision medicine, sometimes known as "personalized medicine", is an innovative approach to tailoring disease prevention and treatment that takes into account differences in people's genes, environments, and lifestyles. *The goal of precision medicine is to target the right treatments to the right patients at the right time*"

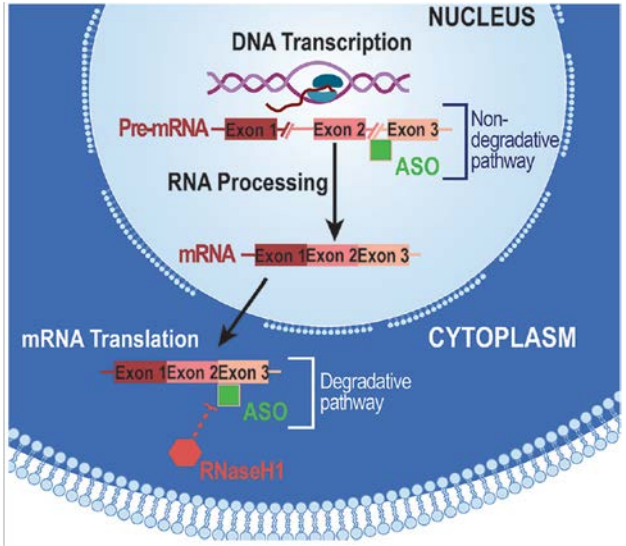
https://www.fda.gov/

Historical Perspective: From Laced Stockings to Allele-Specific ASO therapy



Richard Wiseman, surgeon to King Charles II, 1676

1950	Self recorded trials for “low-grade morbidity,” “efficacy of hypnotics”
1980 1990	• Concept brought to wider readership • Results of first trials published • User’s guide to N-of-1 trials
2000	• N-of-1 used in ADHD • Reporting guidelines, risk of bias
2010 2020	• Advances in genetic testing, possibility of precision therapies • Development of disease specific small molecule treatments • Pharmacogenomics • Gene Therapy • ASO (allele and non-allele specific) • Development of specific FDA regulatory pathways • NIH precision medicine initiative

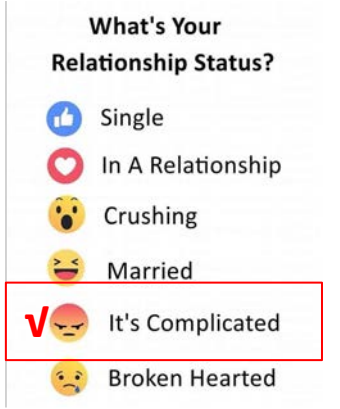


Van Laar A, Van Laar V et. al, Practcial Neurology, 2019

N-of-1 trials and Child Neurology: A Match Made in Necessity

- Put together, “rare” genetic disorders are very common in child neurology
- Several conditions do not respond to, or do not tolerate available therapies (e.g., epilepsy is refractory in about 30% of children)
- Neurodegenerative conditions (SMA, PME, NCL, CMT)
- Many disease mechanisms are unknown (or can only be targeted through genetic modification or chemical modification of mRNA)

N-of-1 trials and Child Neurology: A Match Made in Necessity, but…



- Many conditions are inherently static (e.g., brain malformations, developmental epileptic encephalopathies
- In neurodegenerative conditions, clinical symptoms and/or diagnosis lag behind irreparable neuronal loss (e.g. ALD)
- Drug delivery and target: blood, particular organ, spine, brain
- And then within the brain: differential distribution?
- Study design itself: use of placebos or cycles not ideal in neurodegenerative conditions
- Patient assent often impossible due to age, cognitive level, neurological regression

Very limited interventional window

N-of-1 Trials in Child Neurology: Current Landscape

- Examples from neurodevelopmental disorders, epilepsies, and neurodegenerative disorders
- Levels of precision in therapeutic target of N-of-1 therapies

N-of-1 Trials: Neurodevelopmental disorders

Systematic Review of N-of-1 Studies in Rare Genetic Neurodevelopmental Disorders

The Power of 1

Annelieke R. Müller, MSc, Marion M.M.G. Brands, MD, PhD, Peter M. van de Ven, PhD, Kit C.B. Roes, PhD, Martina C. Cornel, MD, PhD, Clara D.M. van Karnebeek, MD, PhD, Frits A. Wijburg, MD, PhD, Joost G. Daams, MA, Erik Boot, MD, PhD, and Agnies M. van Eeghen, MD, PhD

Correspondence
Dr. van Eeghen
a.m.vaneeghen@amsterdamumc.nl

Neurology® 2021;96:529-540. doi:10.1212/WNL.0000000000011597

Criteria used:

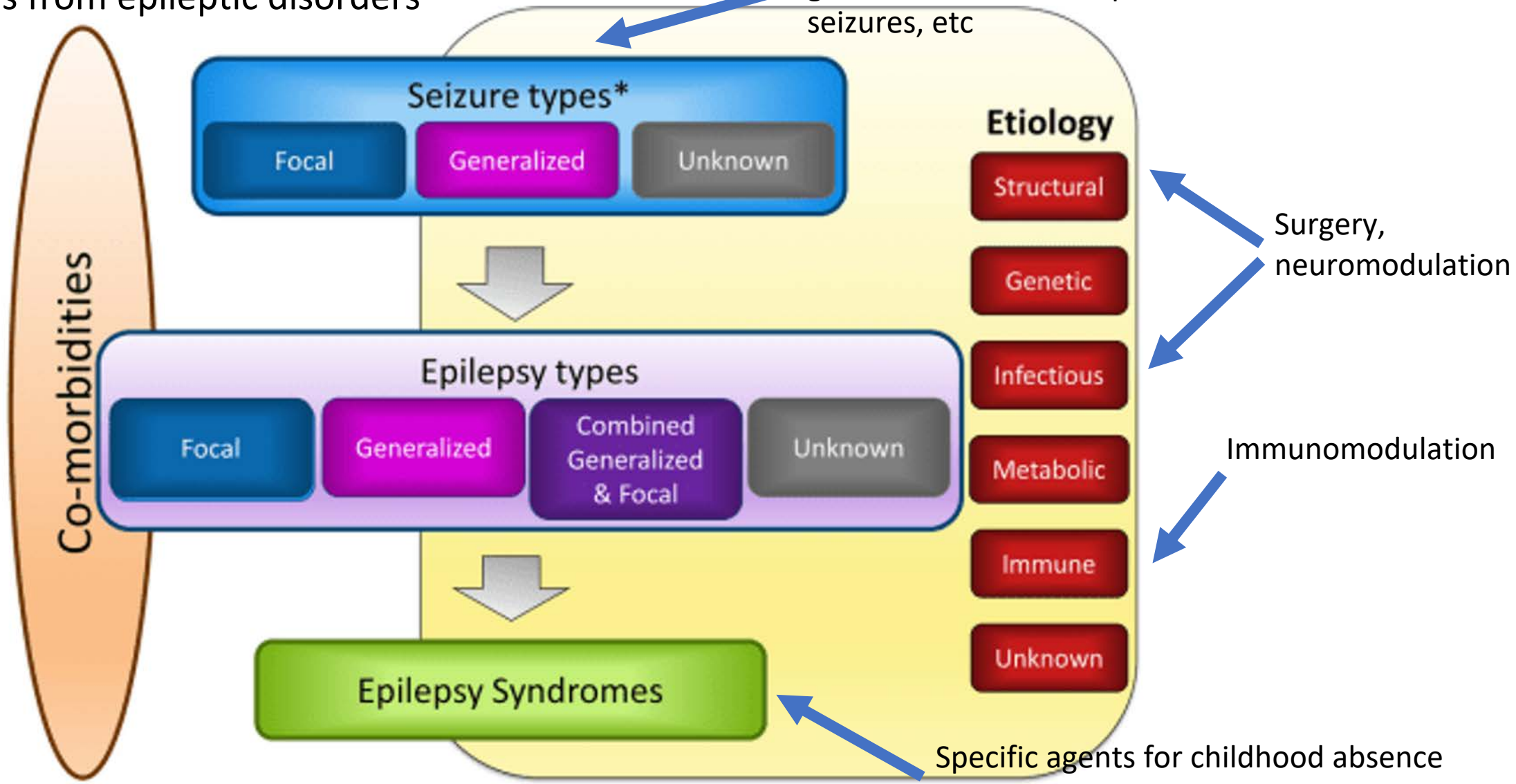
- Peer reviewed studies
- At least 3 controlled episodes of treatment or comparator
- Interventions targeting neurobehavioral symptoms

Table 2 Characteristics of N-of-1 Studies in Rare Genetic Neurodevelopmental Disorders

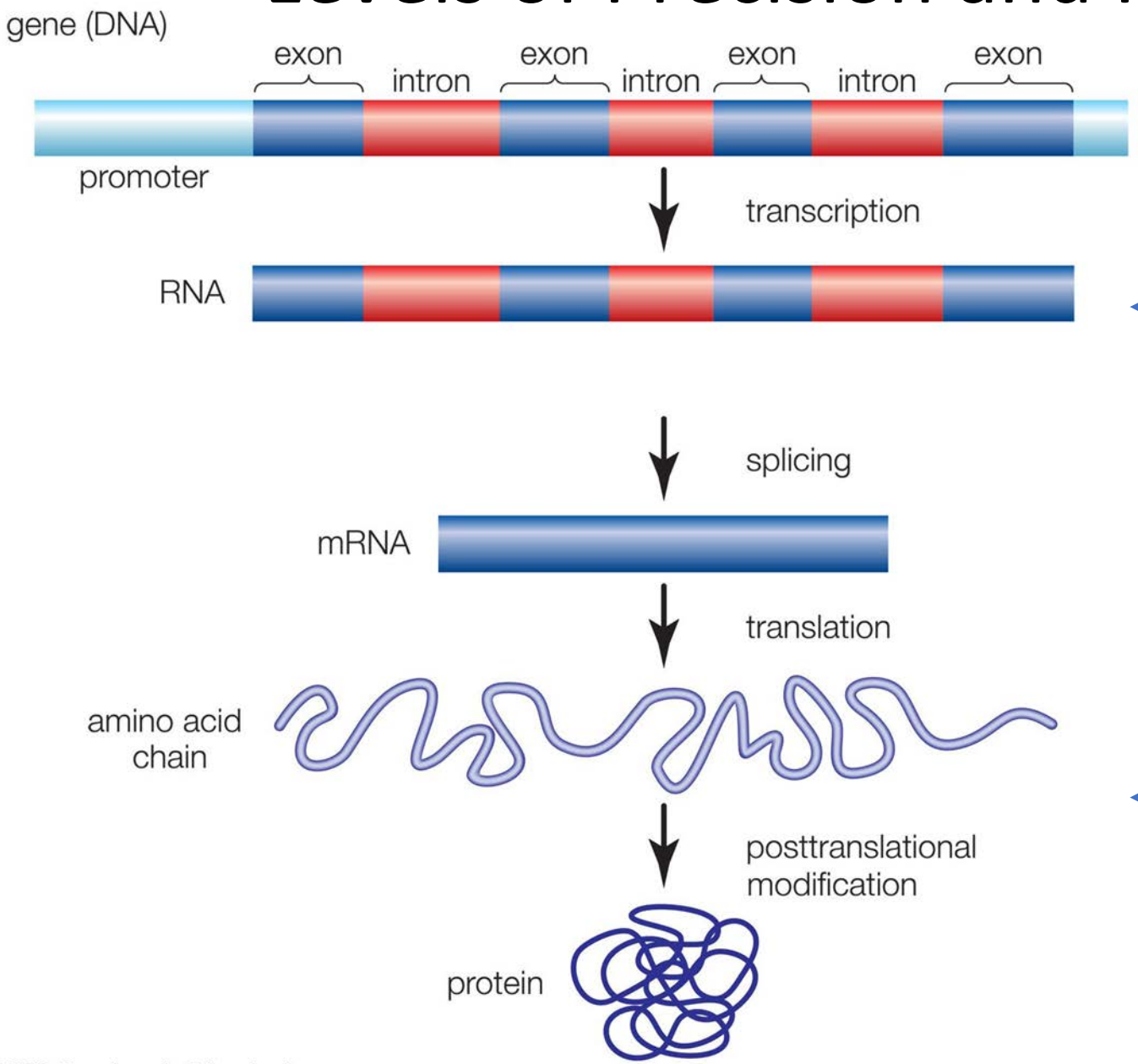
Study	Diagnosis	No. of participants	Average age of participants (range); y	Intervention	Primary and secondary outcome measures[a]	Assessed by
Bawden et al.[55]	Williams syndrome	4	11 (9–13)	Methylphenidate	Child Behavior Checklist, Conners Parent/Teacher Questionnaire, Side Effects Questionnaire, and cognitive psychometric measures	Caregiver
Byiers et al.[56]	Rett syndrome	3	30 (15–47)	Functional communication training	*Communicative behavior*	Investigator
Camfield et al.[21]	Cerebellar hypoplasia tapetoretinal degeneration syndrome	6	7 (3–13)	Melatonin	Average number of hours asleep per 24 h and the number of awakenings and nights without arousals	Caregiver and parents
Crook et al.[57]	Down syndrome	5	59 (55–63)	Cognitive stimulation therapy	*Dementia Care Mapping*	Caregiver
Fisch et al.[26]	Fragile X syndrome	6	8 (3–15)	Folic acid	*Vineland Adaptive Behavior Scales, Autistic Descriptors Checklist*, questionnaire about noticed changes in behavior,and red blood cell folate levels	Caregiver and parents
Giffin et al.[27]	Phenylketonuria	3	15 (9–21)	Phenylalanine restriction	*Visual attention*, plasma phenylalanine, and tyrosine levels	Investigator
Hackett et al.[28]	Ornithine transcarbamylase deficiency	1	48	L-arginine	*Quality of life/mood assessment questionnaire*, plasma glutamine, and arginine levels	Patient and investigator
Khasnavis et al.[30]	Lesch-Nyhan disease	9	10 (6–22)	Ecopipam	*Behavior Problems Inventory*, Clinical Global Impression scale, and adverse events	Caregiver and study staff
Luciano et al.[29]	Myoclonus-dystonia syndrome	2	29 (28–31)	Tetrabenazine	Global Dystonia rating scale, Fahn-Marsden rating scale, and Unified Myoclonus Rating Scale	Investigator
Marholin et al.[25]	Phenylketonuria	6	36 (19–53)	Low phenylalanine diet and behavior modification	Social and motor behavior and serum phenylalanine levels	Investigator
Simacek et al.[20]	Rett syndrome	3	3 (3–4)	Functional communication training	*Idiosyncratic responses and augmentative and alternative communication requests*	Investigator
Tierney et al.[31]	Smith-Lemli-Opitz syndrome	10	11 (5–20)	Cholesterol—easy eggs liquid egg yolks	*Aberrant Behavior Checklist (ABC)*	Caregiver

[a] Italics when indicated as a primary outcome measure by the authors.

Different levels of precision medicine:
Examples from epileptic disorders



Levels of Precision and Intervention



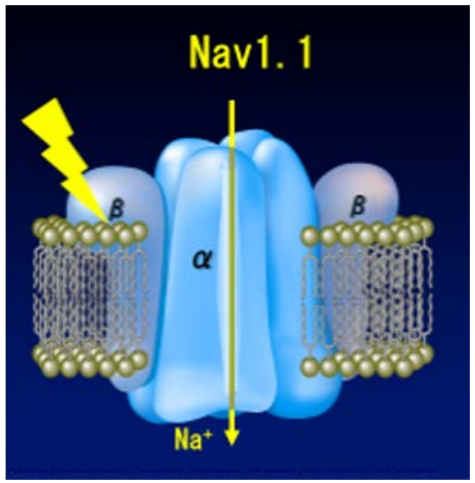
Targeted gene therapy.
CRISPR-Cas9

Anti-sense oligonucleotide (ASO) Therapy (allele specific v/s non-allele specific)

Targeting the effect of the abnormal protein including:

-Enzyme replacement
-Metabolite replacement
-Channel modulators, inhibitors, activators
-Pharmacogenomics

Common examples in epilepsy: Intervention at the protein function level

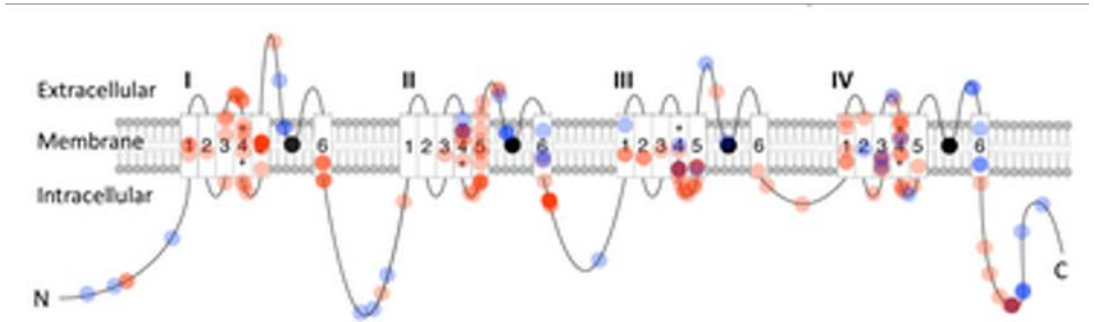


(SCN1A.NET)

Avoid further sodium channel blockade

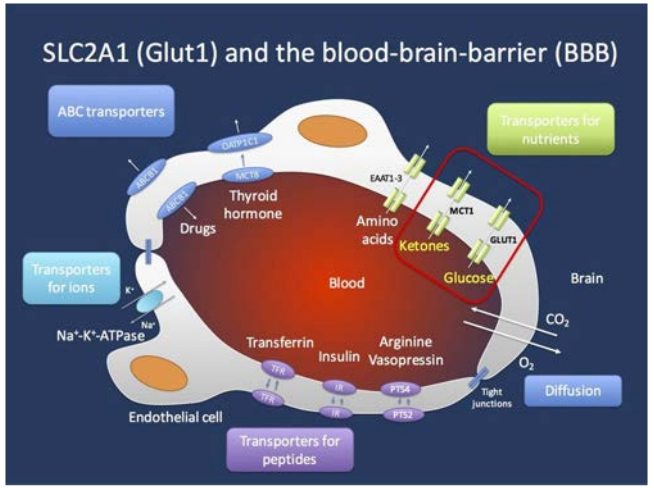
Several ASMs are recommended with various, non-specific mechanisms

ASO currently in phase I/II trial



(Simonsfoundation.org)

SCN2A: majority with gain of function
Sodium channel blockers recommended



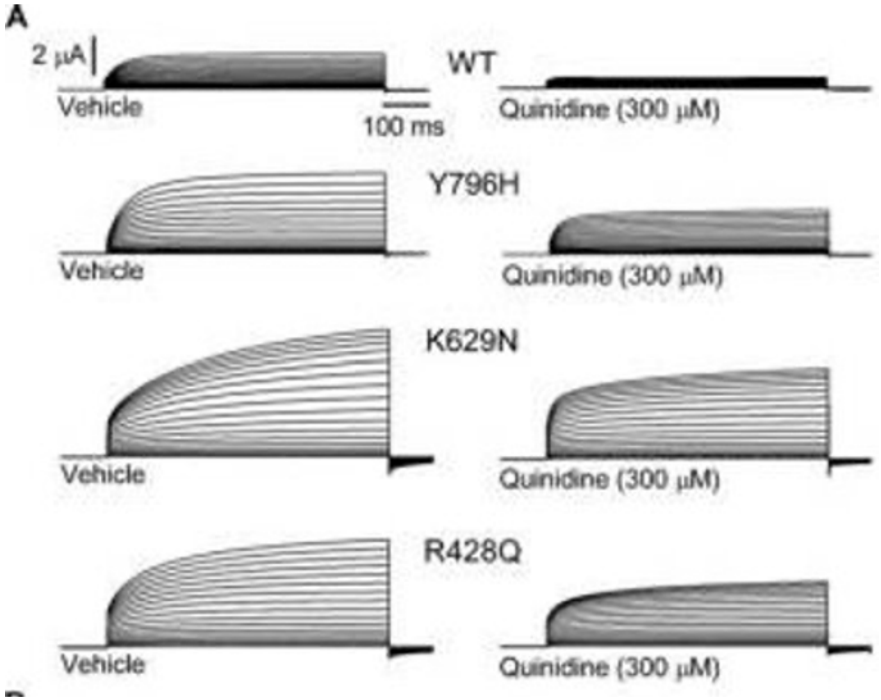
(epilepsygenetics.net)

Overcome the glucose transporter deficiency:

Ketogenic diet

Toward more precision:
Effect of specific variants on protein function

KCNT1 variant	In vitro response	Age at administration	Phenotype	Effect of quinidine on seizures
p.Y796H	Relatively mildblockade	11 years	Early onsetADNFLE	No significant response
K629N	Strong blockade	3 years	EIFMS	80% decrease
R428Q	Strongest blockade	3 years	EIFMS	>90% decrease



Mikati et. Al, Ann Neurol, 2016
Bearden et. Al, Ann Neurol, 2014

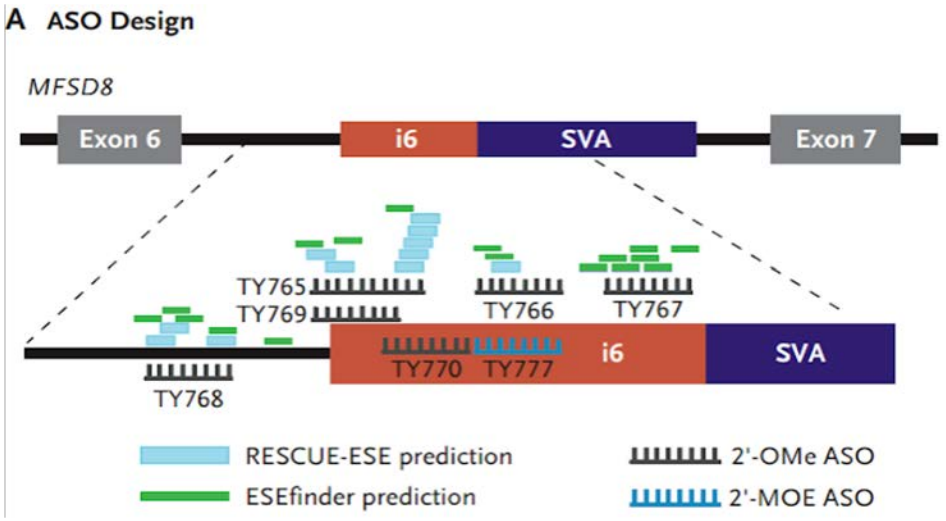
Toward even more precision: the Milasen example. Intervention at the mRNA level, with an allele specific ASO

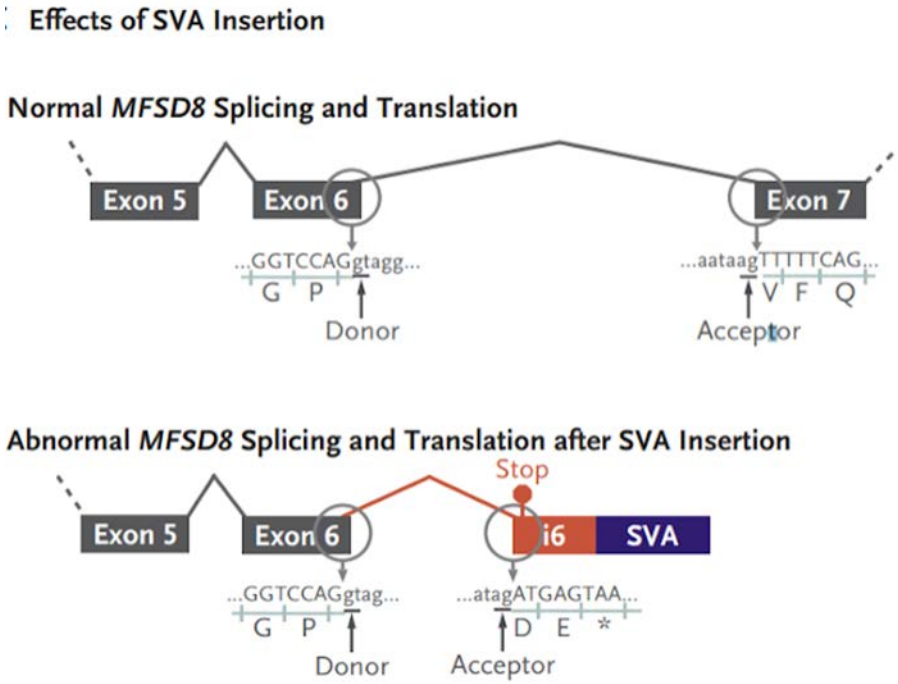
The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Patient-Customized Oligonucleotide Therapy for a Rare Genetic Disease

J. Kim, C. Hu, C. Moufawad El Achkar, L.E. Black, J. Douville, A. Larson, M.K. Pendergast, S.F. Goldkind, E.A. Lee, A. Kuniholm, A. Soucy, J. Vaze, N.R. Belur, K. Fredriksen, I. Stojkovska, A. Tsytsykova, M. Armant, R.L. DiDonato, J. Choi, L. Cornelissen, L.M. Pereira, E.F. Augustine, C.A. Genetti, K. Dies, B. Barton, L. Williams, B.D. Goodlett, B.L. Riley, A. Pasternak, E.R. Berry, K.A. Pflock, S. Chu, C. Reed, K. Tyndall, P.B. Agrawal, A.H. Beggs, P.E. Grant, D.K. Urion, R.O. Snyder, S.E. Waisbren, A. Poduri, P.J. Park, A. Patterson, A. Biffi, J.R. Mazzulli, O. Bodamer, C.B. Berde, and T.W. Yu





N-of-1 Trials and Patient Selection

- Established genetic diagnostic
- Known effect of genetic variant
- Failure, or non-existence, of other established therapeutic modalities
- Plausible mechanism for the therapy to lead to improvement/stabilization
- Availability of safety data
- Benefit outweighs risk
- Informed consent
- Clear, measurable outcomes and expectations

Other important factors: Patient selection in the context of resource allocation

- Severity of clinical state *(treat less v/s more severe?)*
- Stage of disease *(treat advanced v/s early/asymptomatic?)*
- Natural course of disorder *(prioritize neurodegenerative conditions v/s static epilepsies?)*
- Patient age *(treat younger v/s older?)*

Drug target: Anti-Sense Oligonucleotides (ASOs) as a model

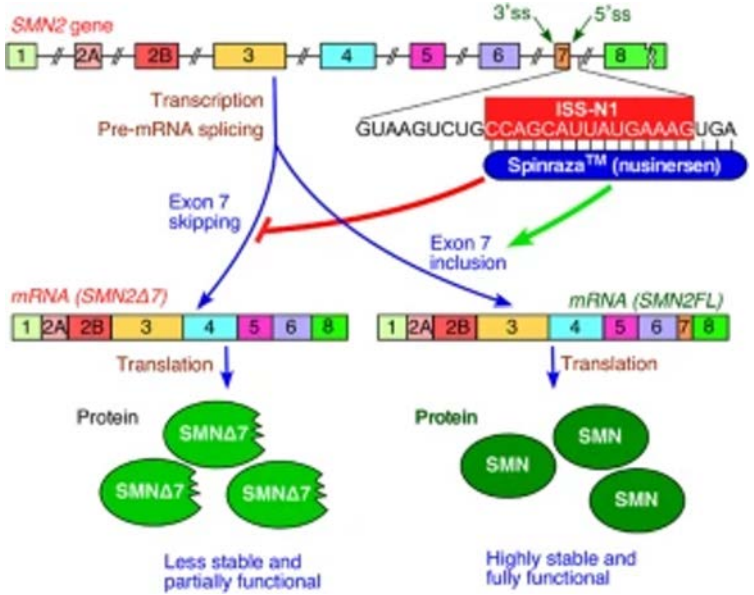
- Development and use for other neurological conditions was encouraged by the success of Nusinersen in children with SMA
- Why ASO's in N-of-1 therapies?
- Can be designed to target specific mRNA sequences and regions to affect how a particular gene is "read"
- Different techniques can lead to translation of a more functional protein, or change in the amount of a specific protein
- Can be allele specific, or non-allele specific (broadly affects overall function, e.g targeting the GoF or LoF pathogenicity)
- Therefore, it can be tailored to 1 patient or a small group of patients

Examples of ASO targets

- SMA: Splice modulating/exon inclusion of the otherwise skipped material (Allele specific- but very common allele)

  Dravet Syndrome: Alternative splicing/exon exclusion of the non-productive isoform (Non-allele specific, but must be a loss of function variant)

  CLN7, Milasen: splice correction affecting transposon unique to one of the patient's alleles, to produce a more functional MFSD8 protein



Singh NN et. al, Gene Therapy, 2017

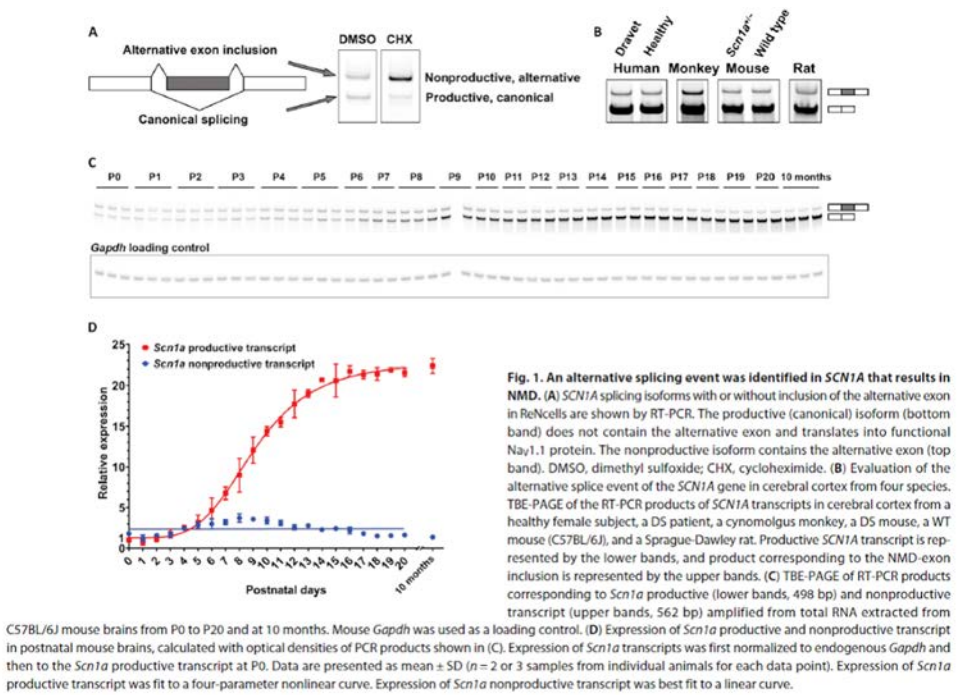
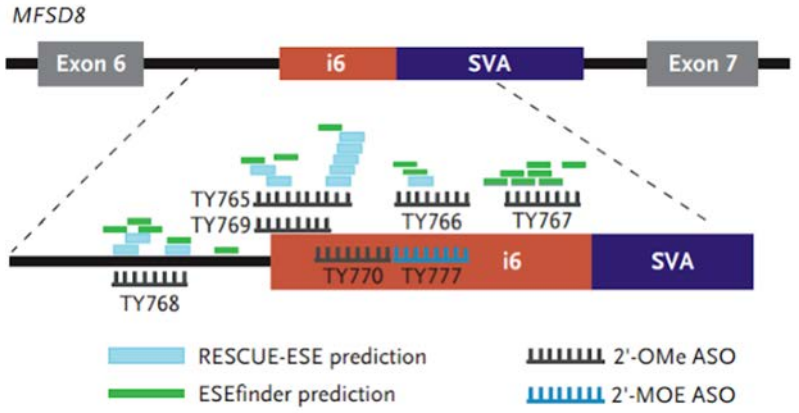


Fig. 1. An alternative splicing event was identified in *SCN1A* that results in NMD. (**A**) *SCN1A* splicing isoforms with or without inclusion of the alternative exon in ReNcells are shown by RT-PCR. The productive (canonical) isoform (bottom band) does not contain the alternative exon and translates into functional $Na_V1.1$ protein. The nonproductive isoform contains the alternative exon (top band). DMSO, dimethyl sulfoxide; CHX, cycloheximide. (**B**) Evaluation of the alternative splice event of the *SCN1A* gene in cerebral cortex from four species. TBE-PAGE of the RT-PCR products of *SCN1A* transcripts in cerebral cortex from a healthy female subject, a DS patient, a cynomolgus monkey, a DS mouse, a WT mouse (C57BL/6J), and a Sprague-Dawley rat. Productive *SCN1A* transcript is represented by the lower bands, and product corresponding to the NMD-exon inclusion is represented by the upper bands. (**C**) TBE-PAGE of RT-PCR products corresponding to *Scn1a* productive (lower bands, 498 bp) and nonproductive transcript (upper bands, 562 bp) amplified from total RNA extracted from C57BL/6J mouse brains from P0 to P20 and at 10 months. Mouse *Gapdh* was used as a loading control. (**D**) Expression of *Scn1a* productive and nonproductive transcript in postnatal mouse brains, calculated with optical densities of PCR products shown in (C). Expression of *Scn1a* transcripts was first normalized to endogenous *Gapdh* and then to the *Scn1a* productive transcript at P0. Data are presented as mean ± SD ($n = 2$ or 3 samples from individual animals for each data point). Expression of *Scn1a* productive transcript was fit to a four-parameter nonlinear curve. Expression of *Scn1a* nonproductive transcript was best fit to a linear curve.

Han et. al, Science Translational Medicine, 2020



Kim J, Hu C., et. al, NEJM, 2019

ASO development for neurodegenerative conditions

Table ASO therapeutics for neurodegenerative disease

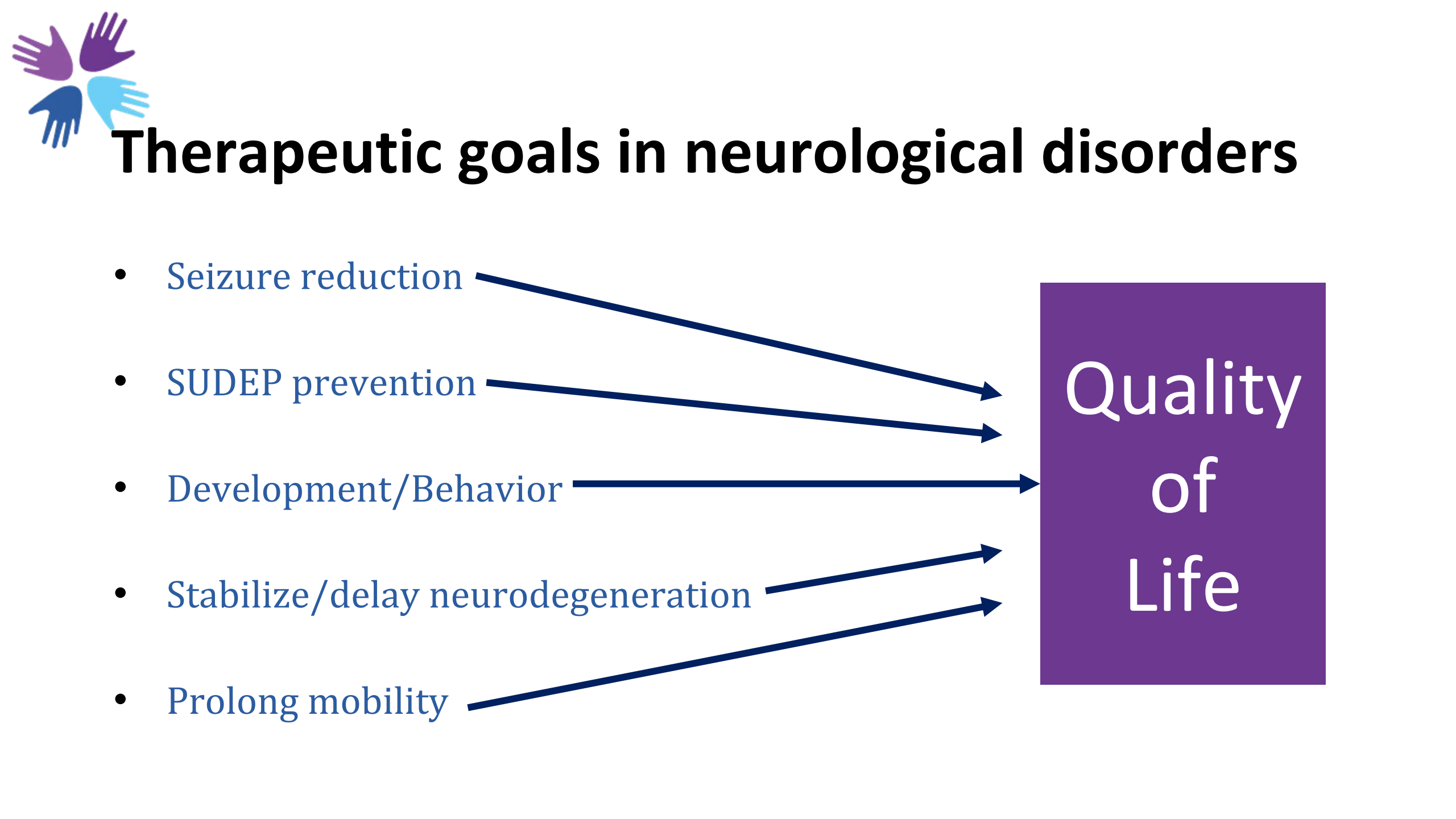
Drug	Indication	Target	ASO chemistry	Status
Nusinersen	SMA	*SMN2*, exon-7 inclusion	ASO, full 2′-MOE	FDA approved
Eteplirsen	DMD	*DMD*, exon-51 skipping	Morpholino	FDA approved
Inotersen	FAP	*TTR* expression	ASO MOE gapmer	FDA approved
WVE-210201	DMD	*DMD*, exon-51 skipping	Stereopure ASO	Phase 1 clinical trial
RG6042	HD	*HTT* expression	ASO MOE gapmer	Phase 3 clinical trial
WVE-120101	HD	*HTT* expression	Stereopure ASO	Phase 1/2 clinical trial
WVE-120102	HD	*HTT* expression	Stereopure ASO	Phase 1/2 clinical trial
IONIS-MAPTRx	AD	*Tau* expression	ASO MOE gapmer	Phase 1/2 clinical trial
BIIB078	ALS	*C9ORF72* expression	ASO MOE	Phase 1 clinical trial
IONIS-SOD1Rx	ALS	*SOD1* expression	ASO MOE gapmer	Phase 1 clinical trial
ATXN2 ASO	SCA2	*ATXN2* expression	ASO MOE gapmer	Preclinical development[38]
ATXN3 ASO	SCA3	*ATXN3* expression	ASO MOE gapmer	Preclinical development[42]

Abbreviations: AD = Alzheimer disease; ALS = amyotrophic lateral sclerosis; ASO = antisense oligonucleotide; DMD = Duchenne muscular dystrophy; FAP = familial amyloid polyneuropathy; FDA = Food and Drug Administration; HD = Huntington disease; MOE = methoxyethyl; SCA = spinocerebellar ataxia; SMA = spinal muscular atrophy.

Scoles DR. et. al, Neurol Genet, 2019

Therapeutic goals in neurological disorders

- Seizure reduction
- SUDEP prevention
- Development/Behavior
- Stabilize/delay neurodegeneration
- Prolong mobility



Selecting and defining an outcome measure

Clinical measures

- e.g: seizure frequency, developmental trajectory, mobility assessment

Biomarkers

- Neuroimaging (rate of atrophy, volumetric analysis, signal analysis)
- Neurophysiology (EMG/NCS, EEGs with analysis of frequency/continuity)
- Molecular (Byproducts, levels of normal or abnormal proteins in serum and CSF)

Pitfalls and special considerations: Scientific limitations

- Animal models, with fair replication of human phenotype, are not easily available
- Biomarkers for target engagement and standardized outcome measures not always available

The problem with outcomes and biomarkers

Clinical measures

e.g: seizure frequency, developmental trajectory, mobility assessment

Subjective, variable, lack of detailed natural history studies, patient-to-patient variability

Biomarkers:

- Neuroimaging (rate of atrophy, volumetric analysis, signal analysis)

  Variable, lack of controls
- Neurophysiology (EMG/NCS, EEGs with analysis of frequency/continuity)

  Largely unknown significance
- Molecular (Byproducts, levels of normal or abnormal proteins in serum and CSF)

  Unknown significance as surrogate for clinical function, and most of the time unavailable

Therapy related challenges

- Novel therapy: proof of concept, Pre-clinical (safety data, animal models and their limitations), manufacturing, resources, cost
- Existing therapy: drug availability, acquisition, cost
- Drug administration: oral, intravenous, intrathecal, intraventricular
- Drug properties: pharmacokinetics, pharmacodynamics, blood brain barrier permeability
- Side effects and risks, which are largely unknown

Ethical considerations: Resource allocation

- Finite resources
- How does the cost of treatment get covered?
- Should therapies impacting the maximum number of people be prioritized over those impacting fewer people?
- But where would that leave patients with rare/orphan diseases?

Inherent bias in precision medicine: Genomic disparity

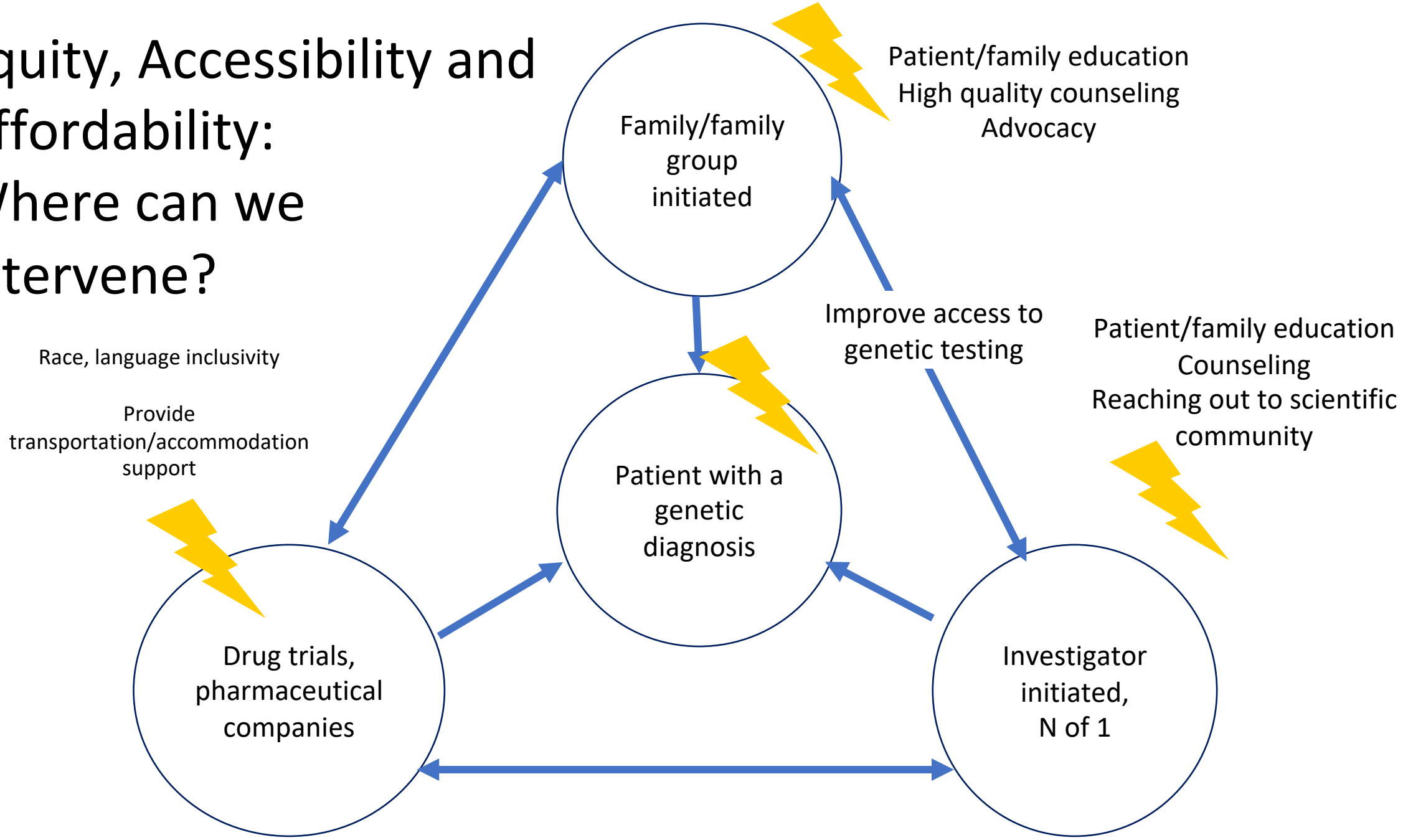
- Reliance on high quality data/genetic information
- Most population samples (e.g. genome-wide association studies) 80% of participants are of European ancestry, 3-5% African and Hispanic
- Disparities extend to other under-represented groups including LGBTQ communities, undocumented, uninsured individuals, etc.

Lee S., The American Journal of Bioethics, 2020

Ethical consideration: The patient

- Patient selection
- Patient wishes/assent
- Measuring quality of life through caregivers

Equity, Accessibility and Affordability: Where can we intervene?



Future possibilities through N-of-1 trials and precision medicine

- High need for such therapies in rare neurogenetic conditions
- Drug selection and development should be highly evidence based
- N-of-1 trials can lead to larger scale trials and advance understanding of pathogenic mechanisms and drug development
- Need for strict regulatory mechanisms, independent ethical and scientific oversight

Drug Regulation in the Era of Individualized Therapies

Janet Woodcock, M.D., and Peter Marks, M.D., Ph.D.

N Engl J Med, 2019

If such individualized interventions become common, and some are successful, the questions of regulatory approval and sustainability of production also become pertinent. Some investigational products, such as snake antivenins, have remained investigational for decades, maintained by various nonprofit or governmental organizations. Approvals as variations on a well-characterized archetypal product might be feasible if the interventions are closely related. Finally, finding sustainable funding for such interventions may prove challenging, because the cost of production can be quite substantial, particularly for gene therapies. In the upcoming months, these issues will need to be addressed at the FDA with input from academic, patient advocate, pharmaceutical industry, and other stakeholders.

In the room

Discuss with a nearby colleague

Online

Make a few notes to yourself

What's standing out for you from the presentations you've just heard?

Scan this…

…or visit this URL

bit.ly/2XHR8Xo

Enter a question or comment

What's one thing you'd like to know more about?

Include your email to get all the presenters' responses

Heather Mefford, MD, PhD

St. Jude Children's Research Hospital

Child Neurology FOUNDATION
Creating a Community of Support

Getting a genetic diagnosis… or not. What's next?

Heather C. Mefford, MD, PhD
St. Jude Children's Research Hospital
Center for Pediatric Neurological Disease Research
@hcmefford

Disclosures

St. Jude Pediatric Translational Neuroscience Initiative

Center for Pediatric Neurological Disease Research

- Basic & Translational research
- Recruiting: FACULTY POSITIONS

Center for Experimental Neurotherapeutics

- Clinical research, clinical trials
- Recruiting: FACULTY POSITIONS

Mefford Lab

50TH ANNUAL MEETING
CNS
SEPT 29-OCT 2, 2021
BOSTON • MASSACHUSETTS

Genetic Diagnosis – why does it matter?

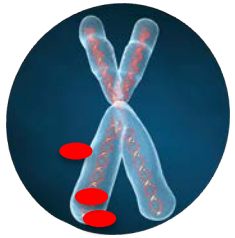
- Improve prognosis counseling
- Enable discussion of recurrence risk
- May affect choice of medications
- Provide research opportunities
- Connect families with the same diagnosis
- Goal: Implement targeted therapy

50TH ANNUAL MEETING
CNS
SEPT 29-OCT 2, 2021
BOSTON • MASSACHUSETTS

Genetic testing options in pediatric neurology

Chromosome array

Deletions, duplications, unbalanced translocations



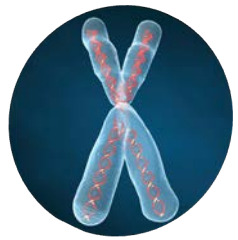
Gene Sequencing

Gene Panel

Dozens to hundreds of genes known to cause a disorder or group of disorders

Exome

All ~20,000 genes in human genome (~4,600 known to cause disease)



Whole Genome Sequencing

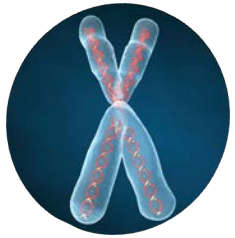
100% of DNA

RNA Sequencing

Genetic testing: Diagnostic yield matters

Chromosome array

5-30%



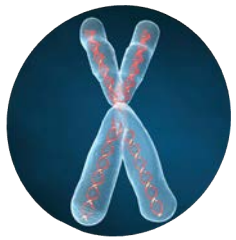
Gene Sequencing

Gene Panel

Up to ~20-30%

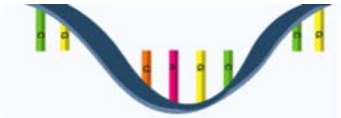
Exome

~25-50%

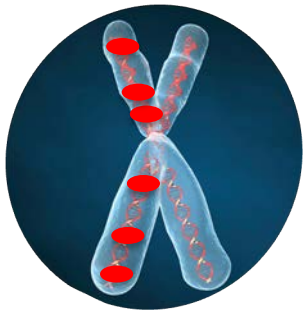


Whole Genome Sequencing

???



Genetic testing: Early-onset epilepsy



Exome
~25-50%

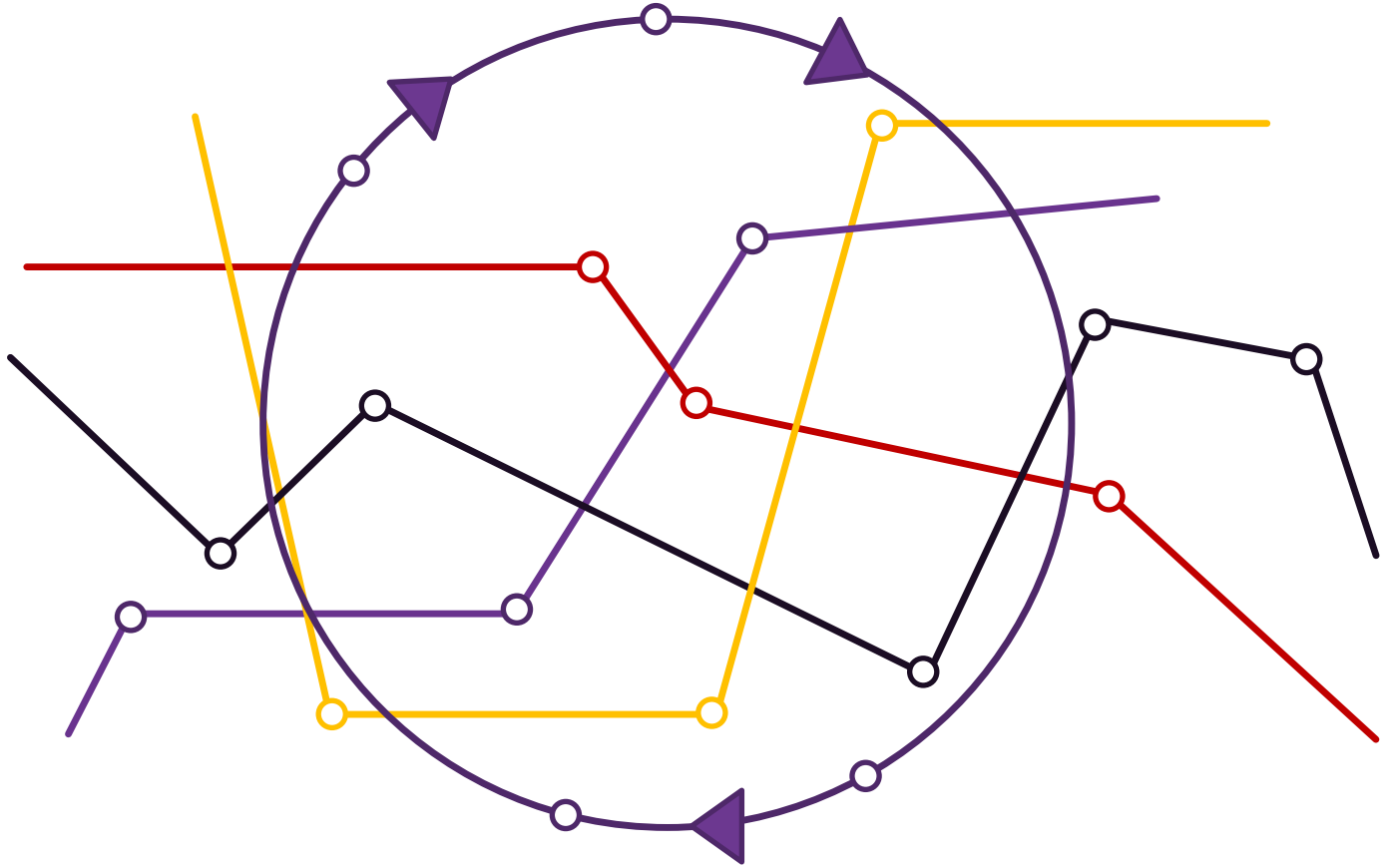
>

Gene Panel
Up to ~20-30%

>

Array
5-10%

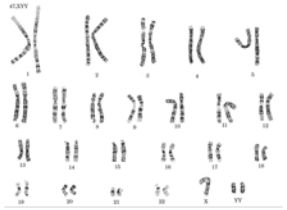
Diagnostic odysseys take many forms



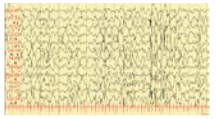
Diagnostic Odyssey #1

6 mo
? delays

9 mo
array
47,XXY



8 mo



+ lots of normal tests

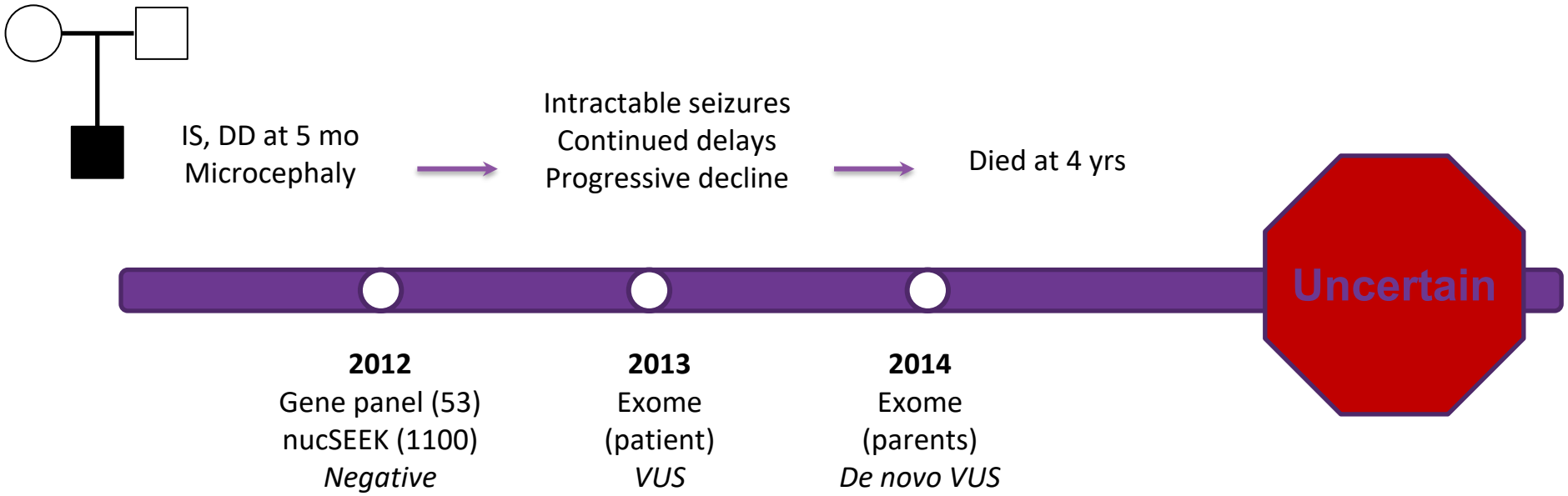
15 mo - 327
gene panel sent
Result: VUS (x4)

20 mo – parental testing
VUS x4 inherited

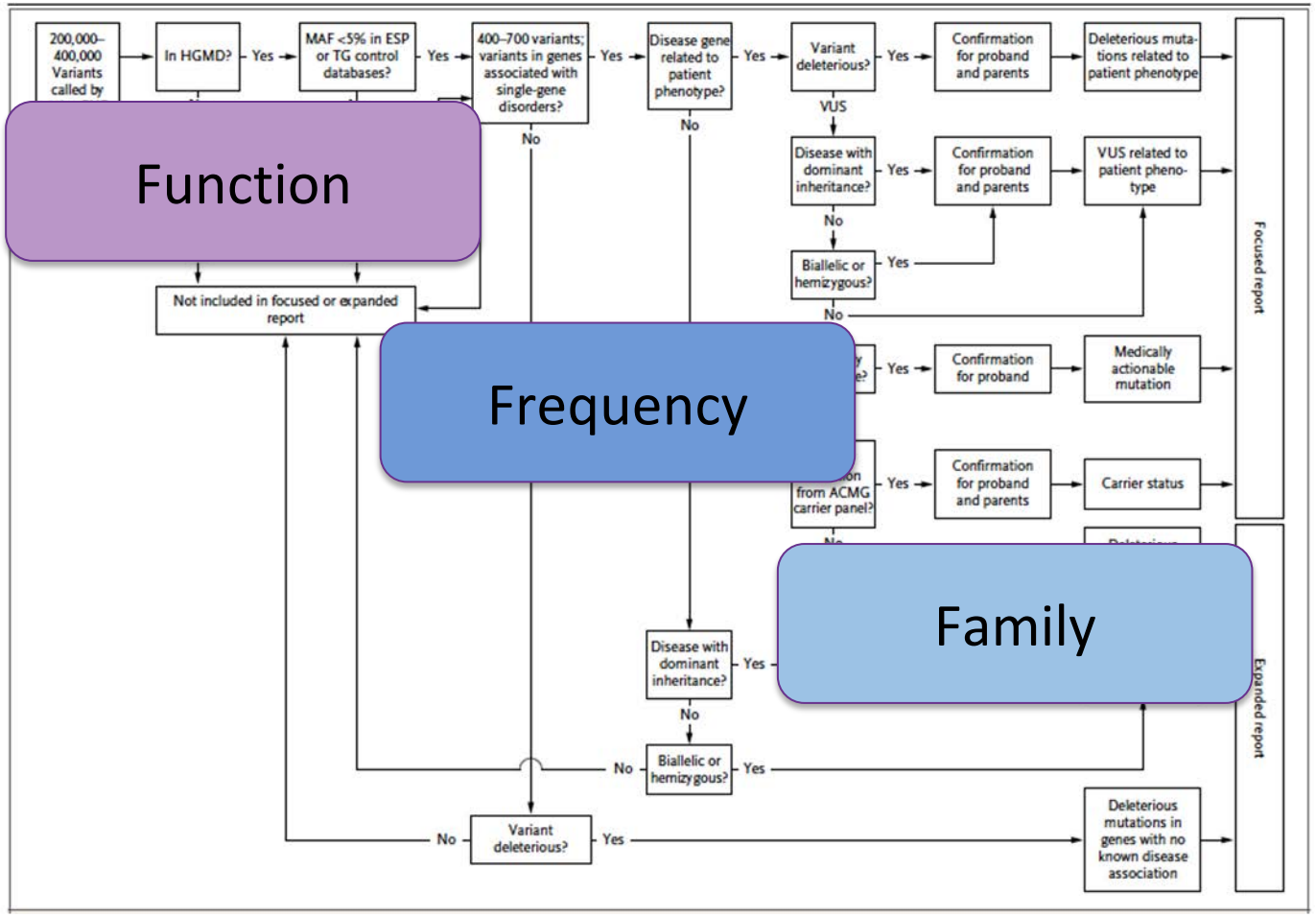
Metabolic genetics
Additional testing
(Nondiagnostic)

No Diagnosis

Diagnostic Odyssey #2



What goes into variant interpretation?



Function

Impacts protein

- Missense, splice, frameshift

Predicted to be deleterious

- Polyphen, SIFT, MutationTaster, CADD….

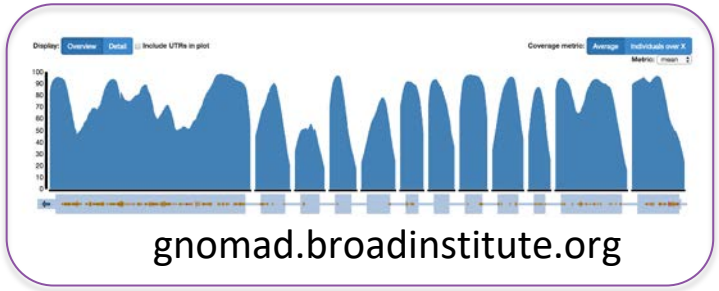
Consistent with disease mechanism

- Loss vs gain of function, location of mutation

Frequency

Severe, rare, sporadic disease

- *De novo* dominant: Disease-causing variants should be absent or ultra-rare in unaffected individuals

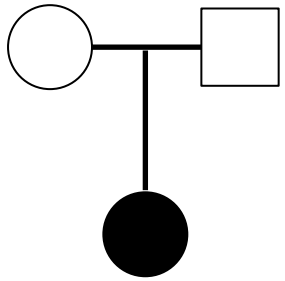

gnomad.broadinstitute.org

Rare, recessive disease

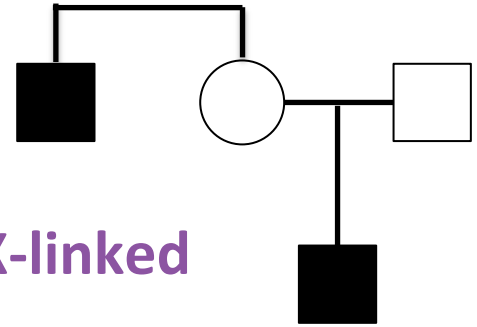
- Carriers may be present in the population
- Frequency will still be “rare”

ClinGen
Clinical Genome Resource

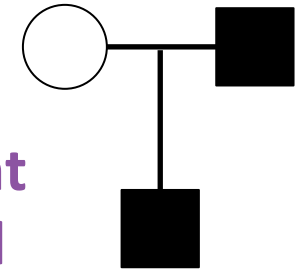
Family



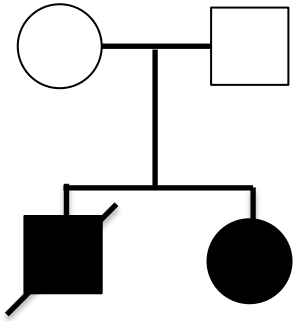
De novo



X-linked



Dominant inherited



Autosomal recessive

Genetic testing – interpretation

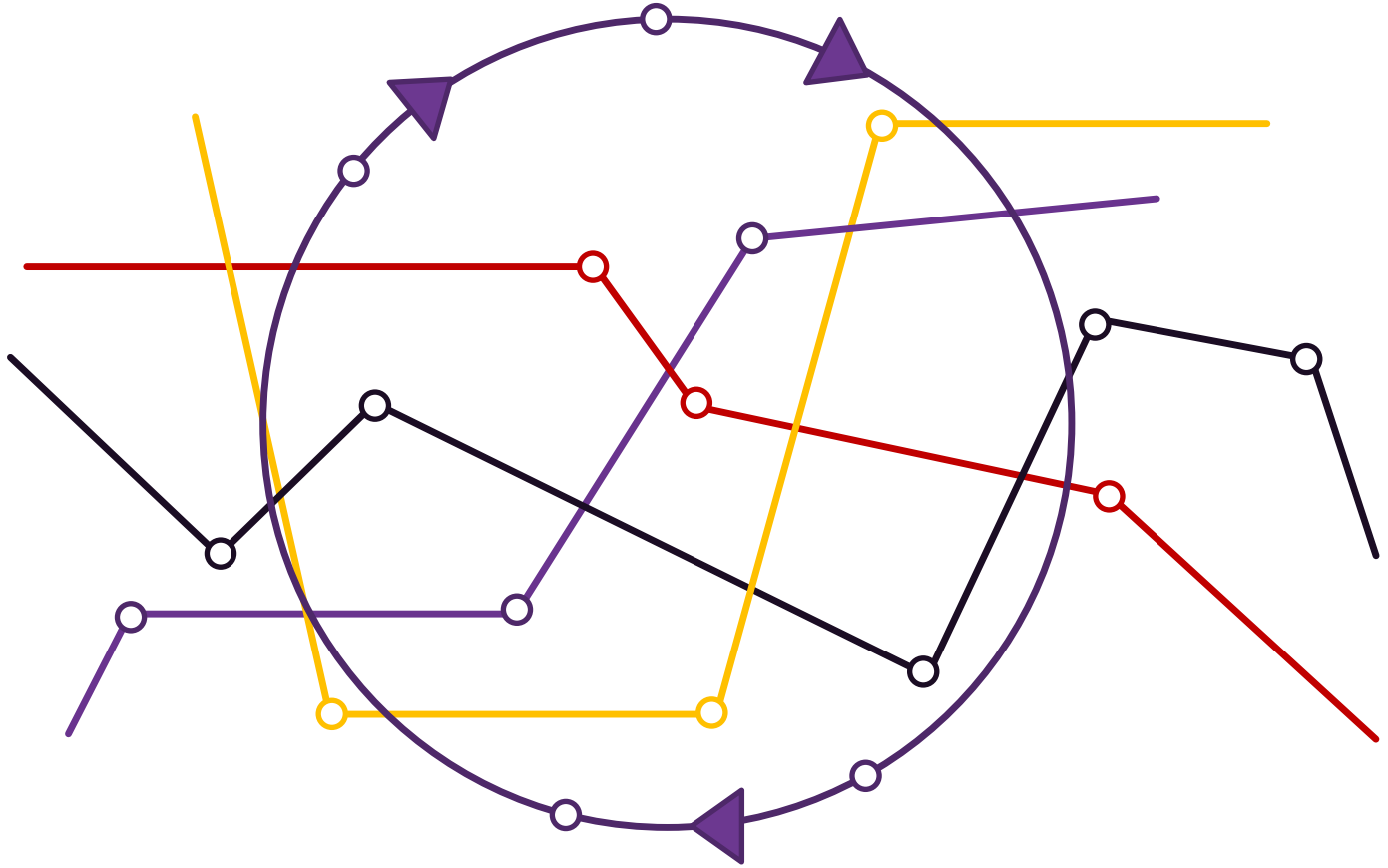
VARIANT INTERPRETATION

- Benign / likely benign
- Variant of Uncertain Clinical Significance (VUS)
- Likely Pathogenic
- Pathogenic

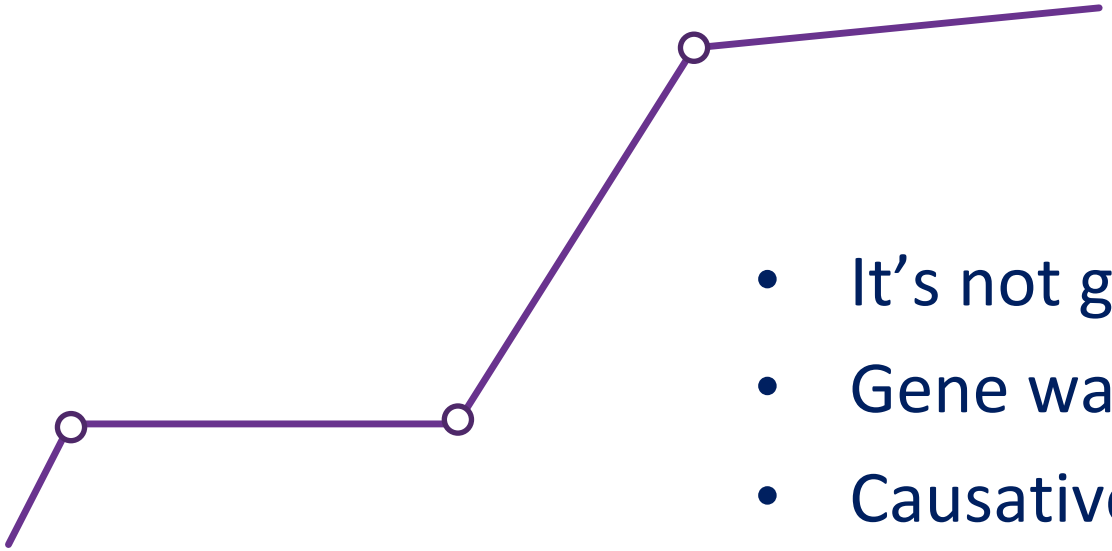
TEST INTERPRETATION

- Non-diagnostic / negative
- Diagnostic / positive

Diagnostic odysseys take many forms

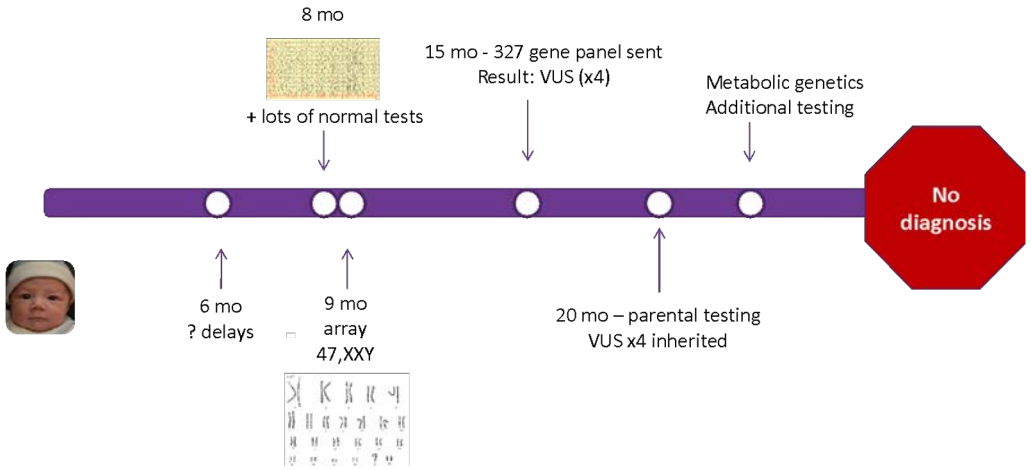


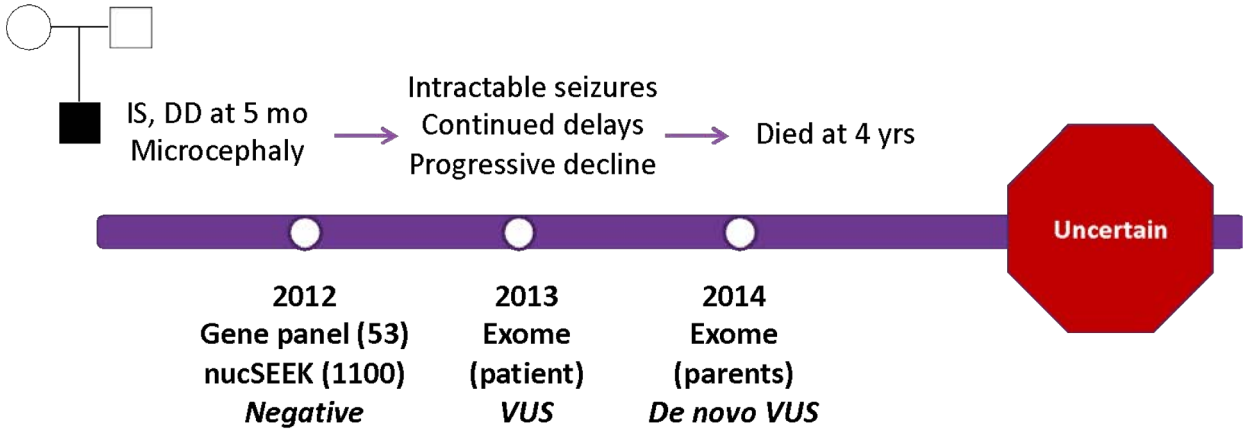
~~Diagnostic~~ odysseys take many forms



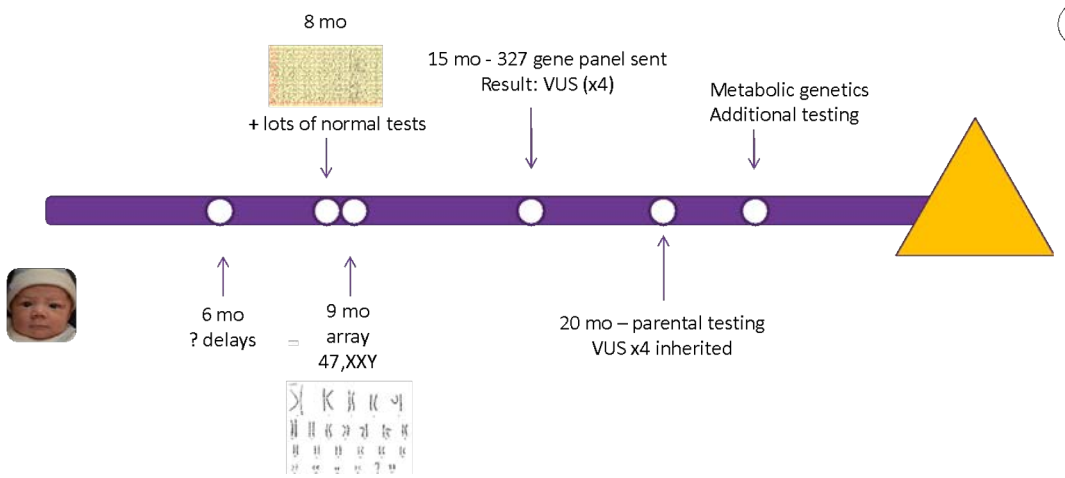
- It’s not genetic
- Gene wasn’t on the panel
- Causative gene not discovered yet
- Variant missed for technical reasons
- Variant misinterpreted

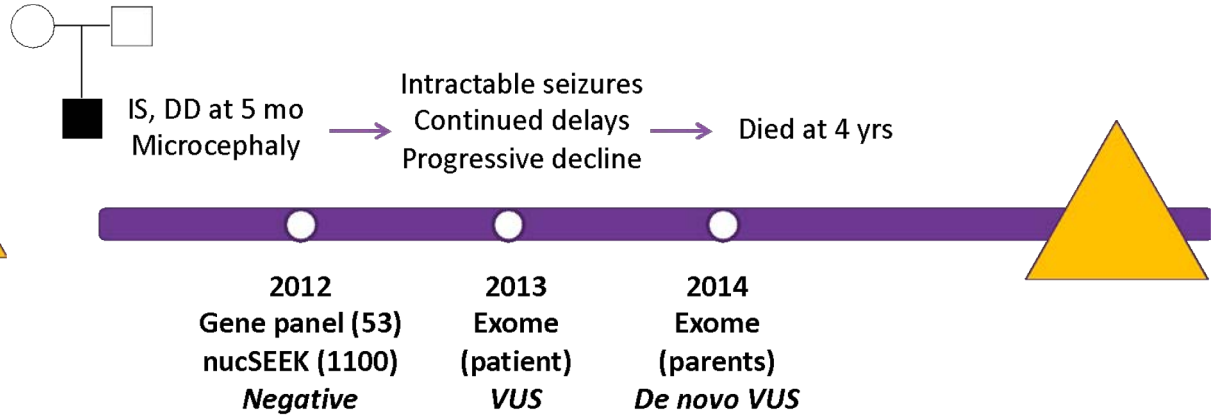
End of the line?



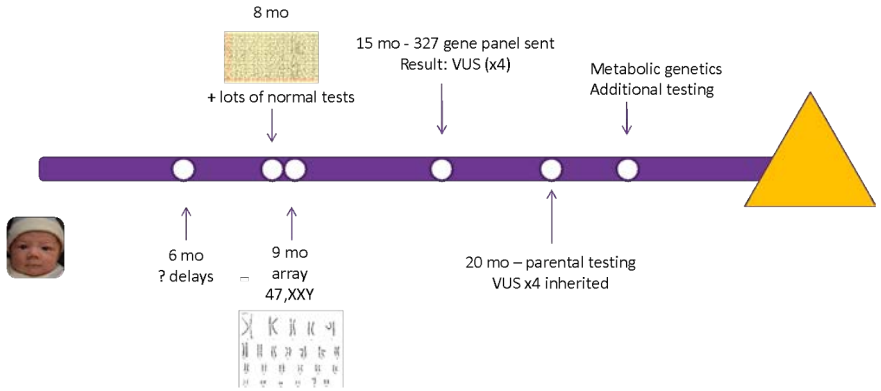


Only a speed bump!





So what's next?



Engage genetics MD / genetic counselor

Follow up and review testing to date

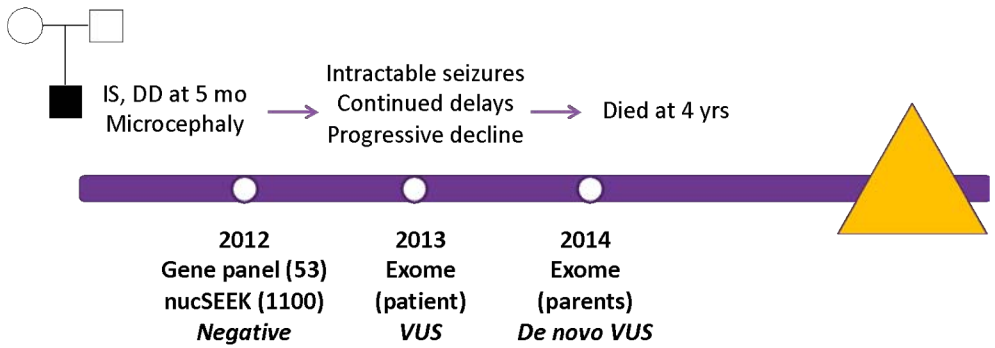
- Are new tests available?
- Is there new clinical or family information?
- Timeline: 1yr…?

Ask for reanalysis

- Exome can be reanalyzed to incorporate new information

Consider research

Uncertainty



Ask the experts

- Is anyone studying the (uncertain) gene?
- Use ‘matchmaking’ databases

Follow up and review testing to date

- Are new tests available?
- Is there new clinical or family information?

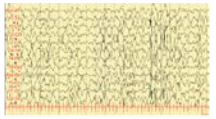
Ask for reanalysis

- Exome can be reanalyzed (after >1yr)

Consider research

Nevertheless…the geneticist persisted!

8 mo



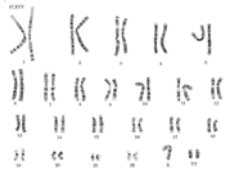
+ lots of normal tests

15 mo - 327 gene panel sent
Result: VUS (x4)

Metabolic genetics
Additional testing
(Nondiagnostic)

6 mo
? delays

9 mo
array
47,XXY



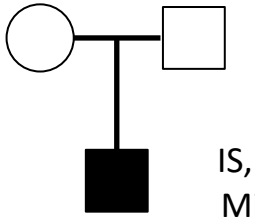
20 mo – parental testing
VUS x4 inherited

MEF2C added to panel

24 mo – whole exome seq
26 mo – results
DIAGNOSIS!
de novo MEF2C

30 mo

Case example



IS, DD at 5 mo
Microcephaly

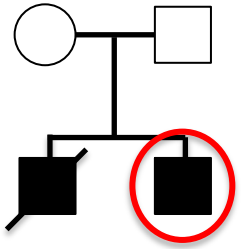
→

Intractable seizures
Continued delays
Progressive decline

→

Died at 4 yrs

→



IS, DD at 10 mo
Microcephaly

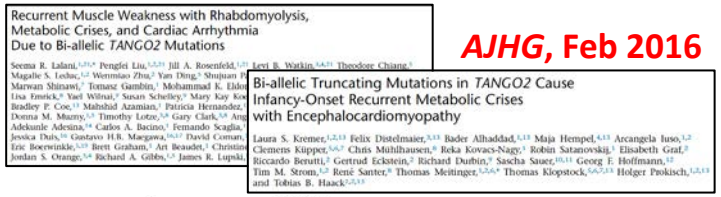
2012
Gene panel (53)
nucSEEK (1100)
Negative

2013
Exome
(patient)
VUS

2014
Exome
(parents)
De novo VUS

2016
Exome
(brother)
Diagnosis: *TANGO2*

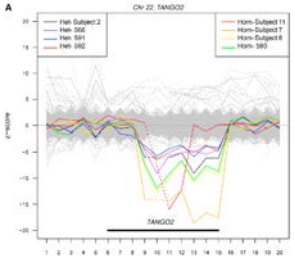
New knowledge →


Recurrent Muscle Weakness with Rhabdomyolysis, Metabolic Crises, and Cardiac Arrhythmia Due to Bi-allelic *TANGO2* Mutations

Bi-allelic Truncating Mutations in *TANGO2* Cause Infancy-Onset Recurrent Metabolic Crises with Encephalocardiomyopathy

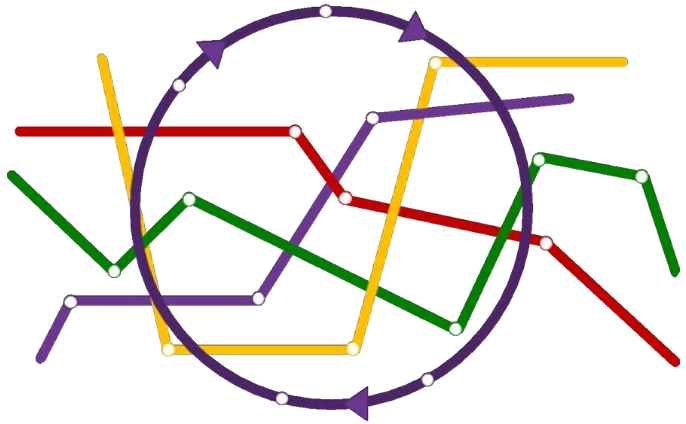
***AJHG*, Feb 2016**

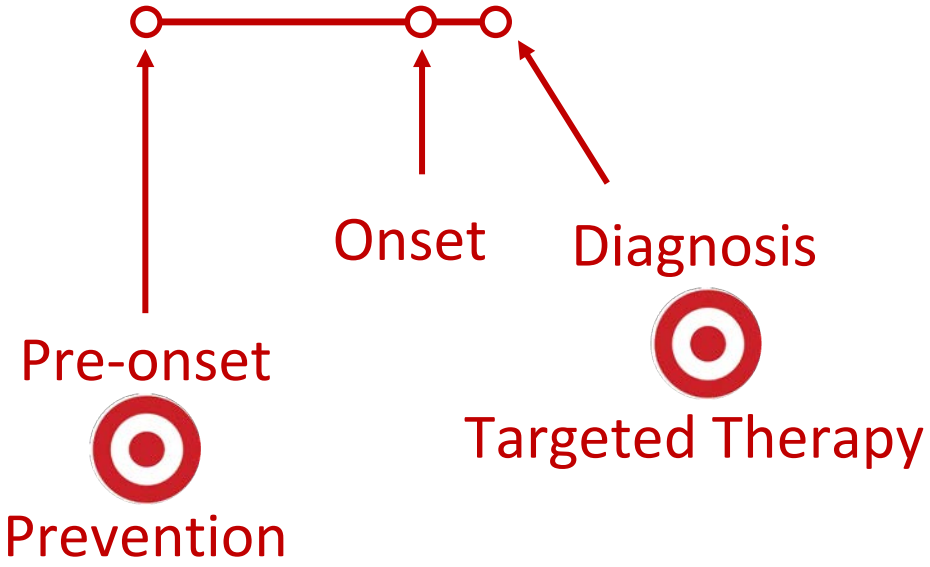
New technology →



50TH ANNUAL MEETING
CNS
SEPT 29-OCT 2, 2021
BOSTON • MASSACHUSETTS

The future of the diagnostic odyssey





The future of the diagnostic odyssey

- Early diagnosis is important
- Know your highest-yield test and start there!
- Exome > sequencing + large CNV
- Whole genome sequencing increasingly available
- Future: Whole genome sequencing in newborns?

St. Jude Pediatric Translational Neuroscience Initiative

Center for Pediatric Neurological Disease Research

- Basic & Translational research
- Recruiting: FACULTY POSITIONS

Center for Experimental Neurotherapeutics

- Clinical research, clinical trials
- Recruiting: FACULTY POSITIONS

Mefford Lab

50TH ANNUAL MEETING
CNS
SEPT 29-OCT 2, 2021
BOSTON • MASSACHUSETTS

Panel Discussion: Effective Collaboration

Moderated by

Annapurna Poduri, MD, MPH

Harvard Medical School
Boston Children's Hospital

Anne T. Berg, PhD

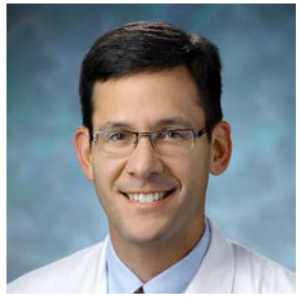
Ann & Robert H. Lurie
Children's Hospital of Chicago
Neurology

Louise Bier, MS, CGC

Columbia's Institute for
Genomic Medicine

Krista Harding

National Multiple Sclerosis
Society



Adam Hartman, MD

National Institute of Neurological
Disorders and Stroke

Disclosures

Anne Berg, PhD
- None

Louise Bier, MS, CGC
- None

Krista Harding
- None

Adam L. Hartman, MD
- Consultant: Teladoc®c®
- Editorial Board, *Neurology*

Annapurna Poduri, MD, MPH
- Consultant: Teladoc®
- Strategic Advisory Board: TevardBio and Syngap Research Fund
- Precision Medicine Board: Eisai

In the room

Discuss with a nearby colleague

Online

Make a few notes to yourself

What's standing out for you from the presentation & panel discussion you've just heard?

Scan this…

…or visit this URL

Enter a question or comment

What's one thing you'd like to know more about?

Include your email to get all the presenters' responses

Child Neurology FOUNDATION
Creating a Community of Support

50TH ANNUAL MEETING
CNS
SEPT 29-OCT 2, 2021
BOSTON • MASSACHUSETTS

@cnfoundation @childneurologyfoundation @child_neurology childneurologyfoundation.or