Latest Updates in Genetic Testing for Children with Neurodevelopmental Disorders

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2020 Michael J. Bresnan Child Neurology Course

Learning Objectives

1. Appreciate the importance of etiology in neurodevelopmental disorders

2. Understand the basic genetic workup of neurodevelopmental disorders

3. Learn about the role of new technologies, especially whole exome sequencing, in diagnosing genetic causes of neurodevelopmental disorders

Conflicts of Interest

- None

Outline

PART I (PRE-EXOME SEQUENCING ERA)

- Context
- Background
- Workup of NDD in Pre-Exome Sequencing Era

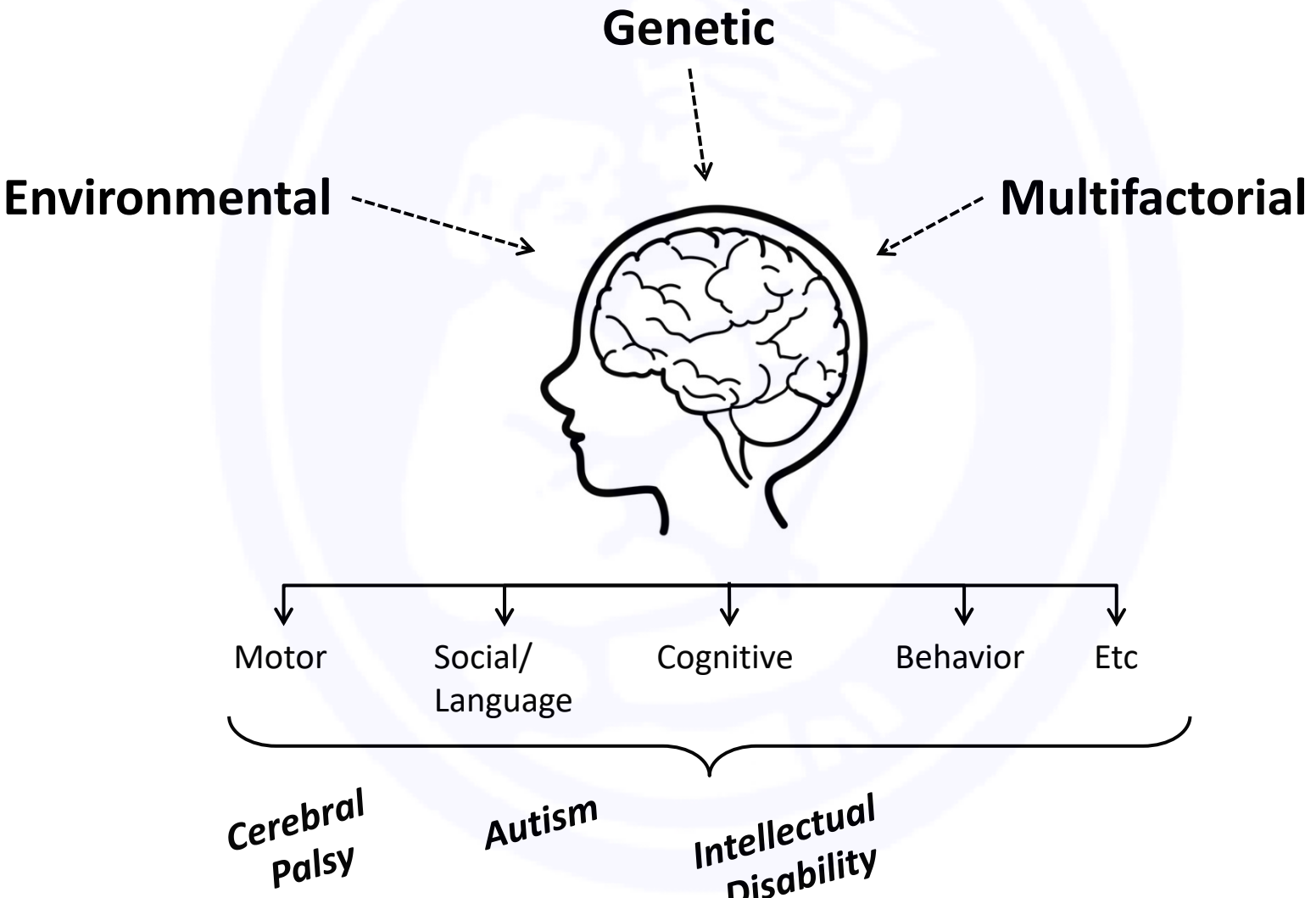
PART II (POST-EXOME SEQUENCING ERA)

- Whole Exome Sequencing
- Workup of NDD in Post-Exome Sequencing Era
- Cases
- Conclusions

PART I (PRE-EXOME SEQUENCING ERA)

Context

Neurodevelopmental Disorders (NDD)



Alec Hoon, Julie Cohen

Diagnosis

- Recurrence risk
- Psychosocial benefits
- Prognosis
- Medical management
- Treatment

Background

Neurodevelopmental Disorders (NDD)

- Intellectual Disability (ID)
- Autism Spectrum Disorder (ASD)
- Cerebral Palsy (CP)

Global Developmental Delay (GDD) / Intellectual Disability (ID)

GDD

- Significant developmental delay (DQ < 70%) in **at least two** major developmental domains (**cognition** included)
- Global delay is the precursor diagnosis to ID

ID

- Significant impairment in **cognition** (cognitive DQ < 70%, i.e. full scale IQ < 70) and **adaptive skills**, with onset before age 18 years

Autism Spectrum Disorder (ASD)

Social-Communication

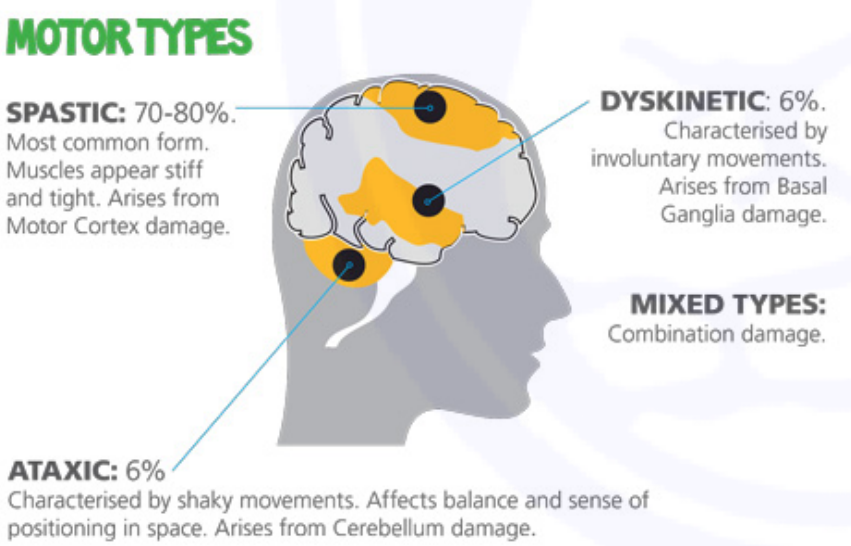
- Deficits in social emotional reciprocity
- Deficits in nonverbal communication
- Deficits in relationships

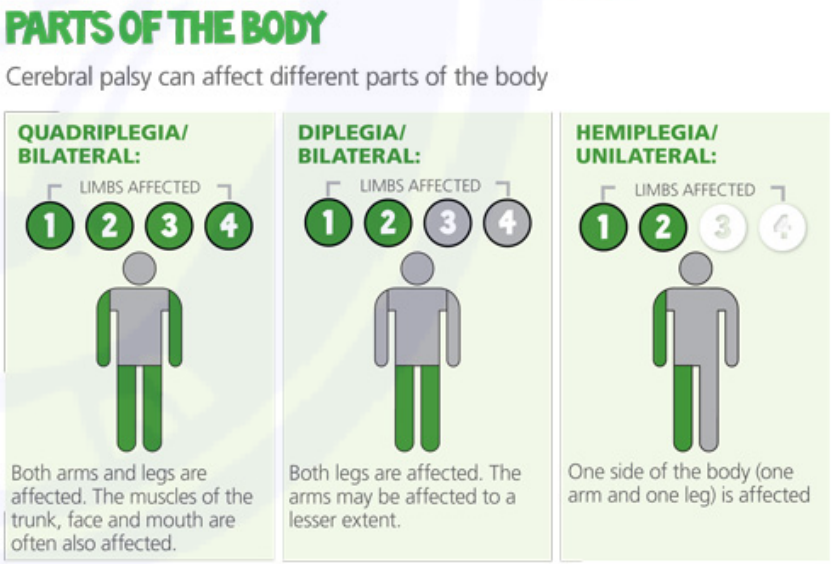
Restrictive Repetitive Behaviors

- Stereotyped/repetitive motor movements, use of objects, or speech
- Insistence on sameness, inflexible adherence to routines, or ritualized patterns of verbal or nonverbal behavior
- Highly restricted, fixated interests
- Hyper- or hypo-reactivity to sensory input or unusual interest in sensory aspects of the environment

Cerebral Palsy (CP)

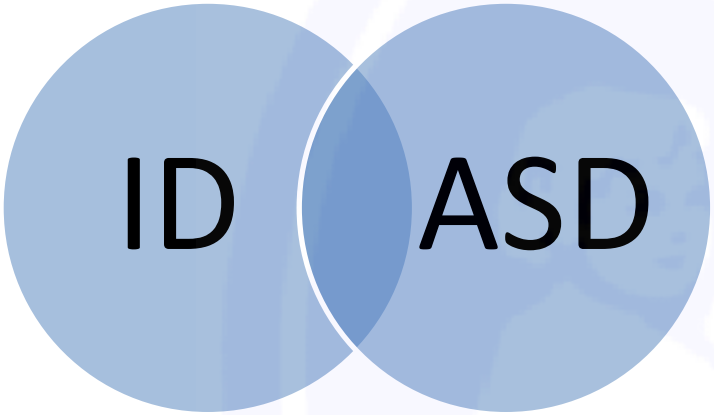
- Static encephalopathy
- Deficits in tone, movement, and posture



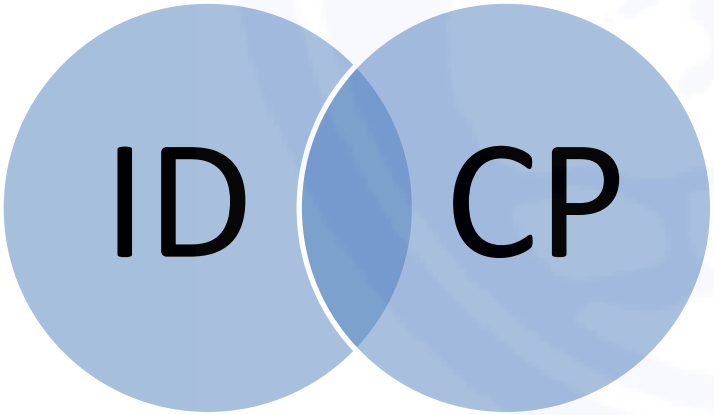


http://www.uni.edu/walsh/CPINFO.jpg

Overlap of NDD

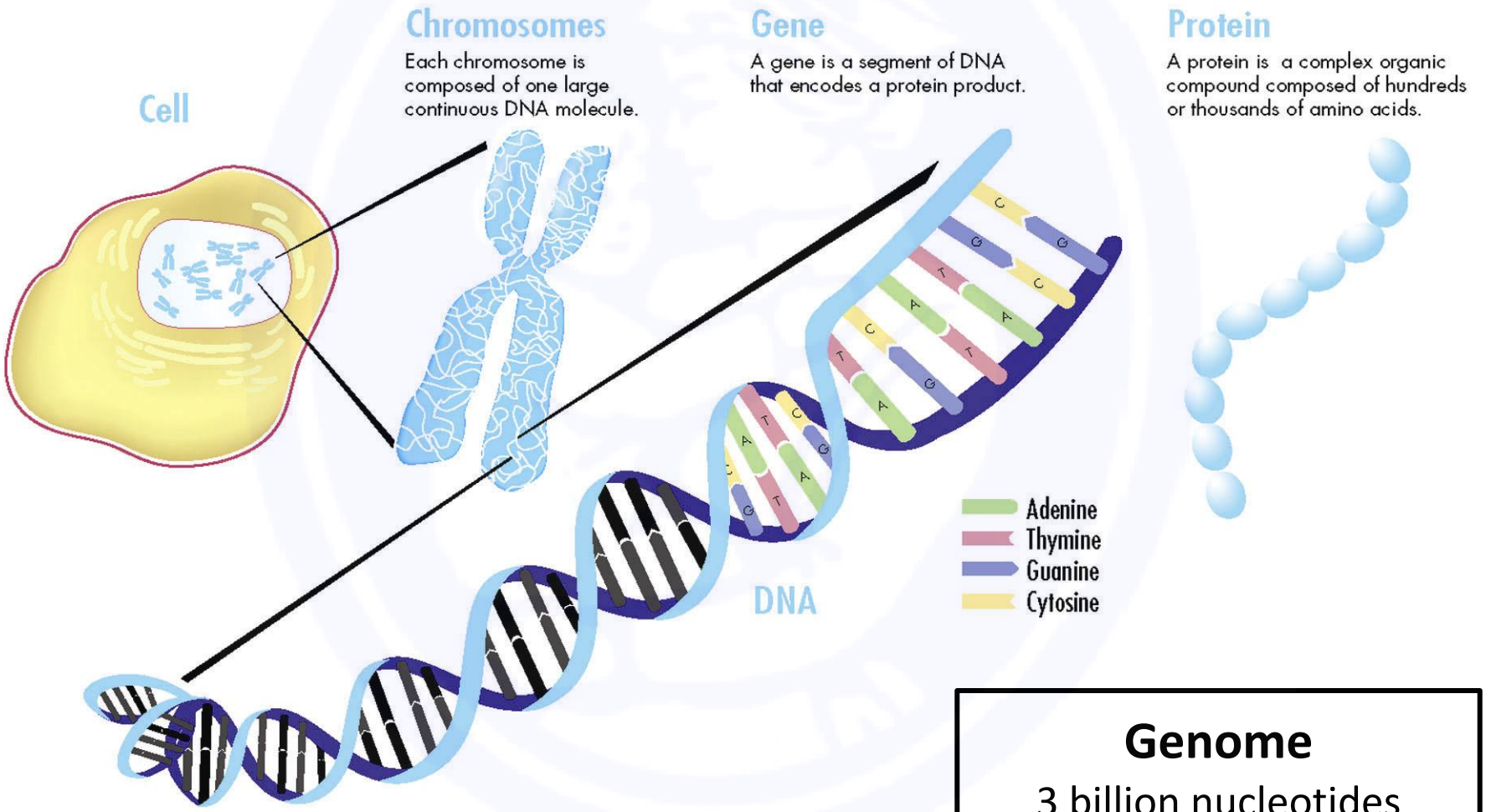


- 40% of ASD with ID



- 50% of CP with ID

Genetic Basics



Genome

3 billion nucleotides

>20,000 genes

http://hsc.ghs.org/wp-content/uploads/2016/02/Genetics-of-Autism-Nurturing-Developing-Minds-D.-Everman-2-16.pdf

Genetic Testing

- **Biochemical tests**
  - Screening assays
  - Diagnostic assays
- **Cytogenetic tests**
  - Karyotype
  - FISH
  - Chromosomal microarray
- **DNA tests**
  - Single gene analysis
  - Multi-gene panels
  - Whole exome/genome sequencing

Cytogenetic Testing

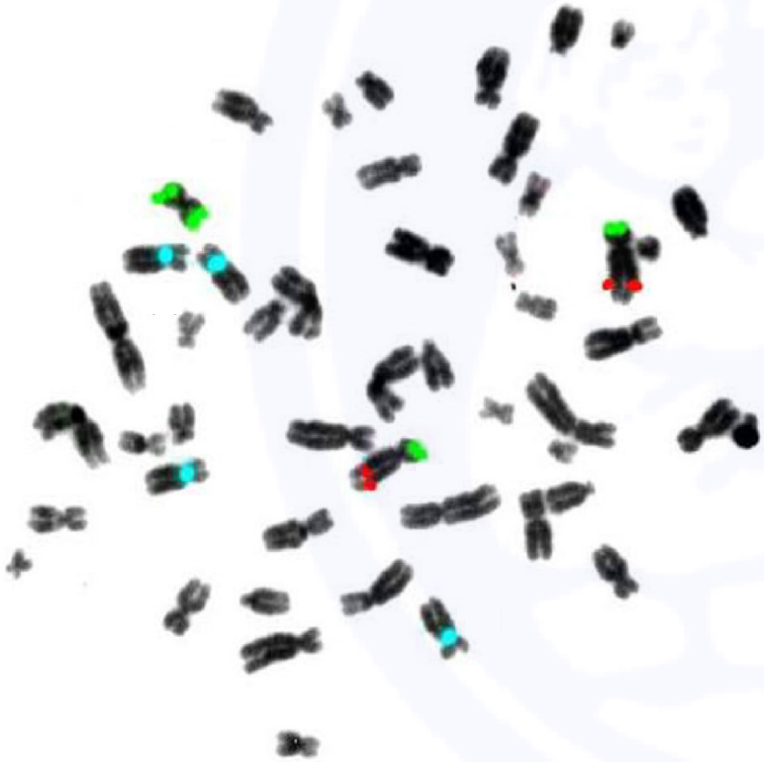
Karyotype

- Aneuploidy, rearrangements, translocations
- Large deletions/duplications > 5-10 megabasepairs

Yield (excluding Down syndrome) ~3%

Cytogenetic Testing

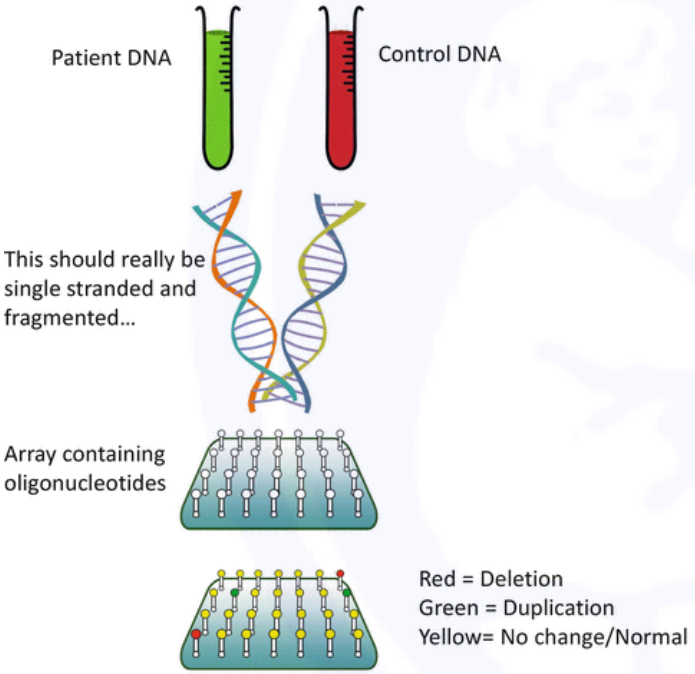
Fluorescence *In Situ* Hybridization (FISH)



- Known microdeletion regions or subtelomeres
  - Williams syndrome (7q11.2)
  - Smith-Magenis syndrome (17p11.2)
  - Prader-Willi & Angelman syndrome (15q11-q13)
  - DiGeorge syndrome (22q11)

Cytogenetic Testing

Chromosomal Microarray (CMA)



- Much higher resolution: as small as 20 kilobasepairs
- Different platforms, including SNP array
- Uses the binding of DNA probes, arranged on a chip in an orderly way, to assess Copy Number Variants

https://link.springer.com/referenceworkentry/10.1007%2F978-3-642-38482-0_10-1

Yield ~15-20% GDD/ID, ASD, major congenital anomalies

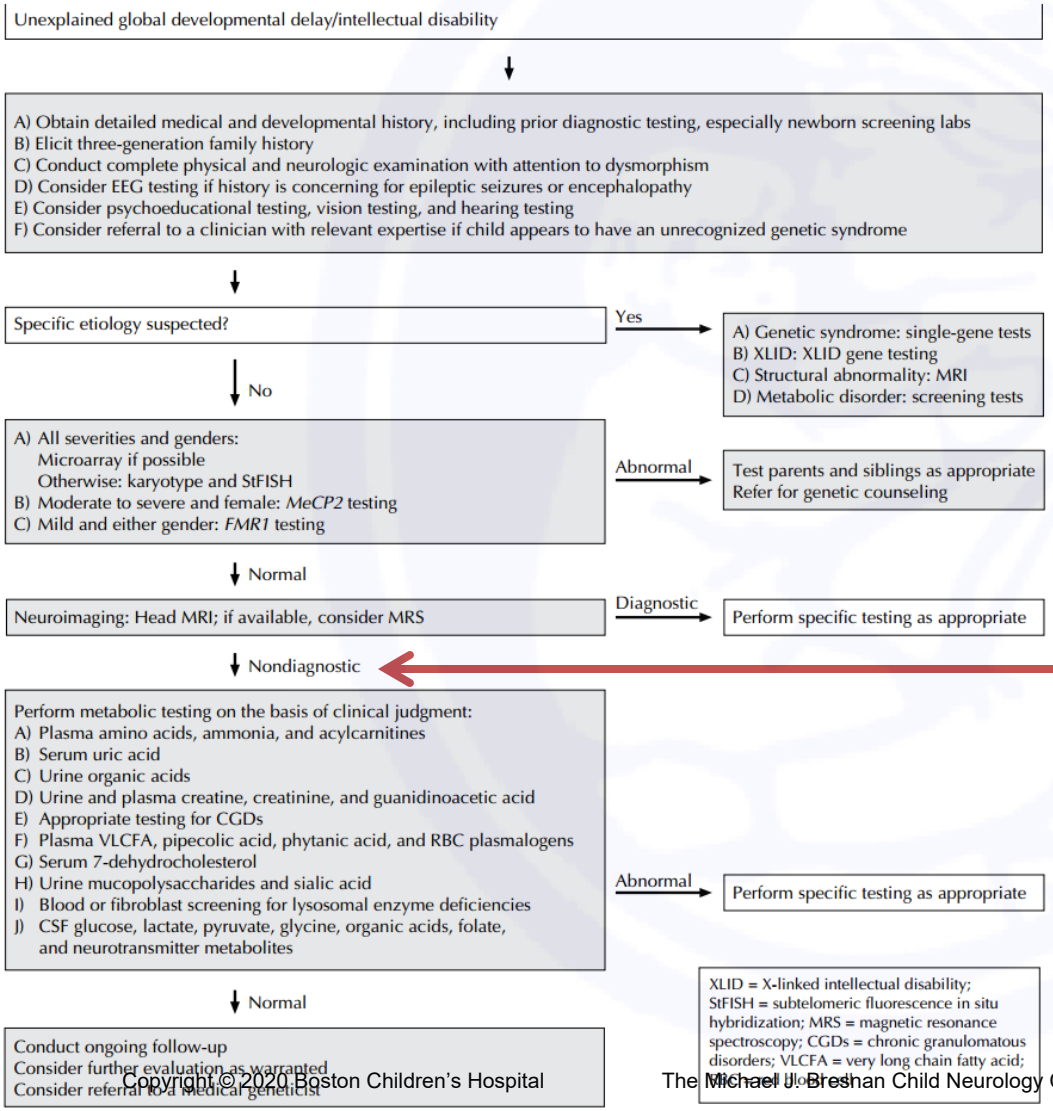
Warning

- Chromosomal microarray **will** detect:
  - ☑ Copy number variants (CNV) – gains / losses
  - ☑ Loss of heterozygosity due to UPD or consanguinity

- Chromosomal microarray **will NOT** detect:
  - ☒ Truly balanced chromosome rearrangements
  - ☒ Low-level mosaicism
  - ☒ DNA sequence mutations (i.e., single-gene disorders)
  - ☒ Triplet repeat disorders (e.g., Fragile X syndrome)

Workup of NDD (in Pre-Exome Sequencing Era)

ID Workup: Practice Parameter

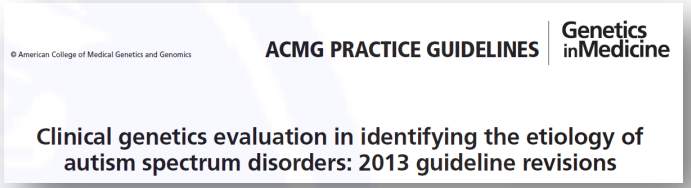


For pediatricians: consider referring at this point

[21956720]

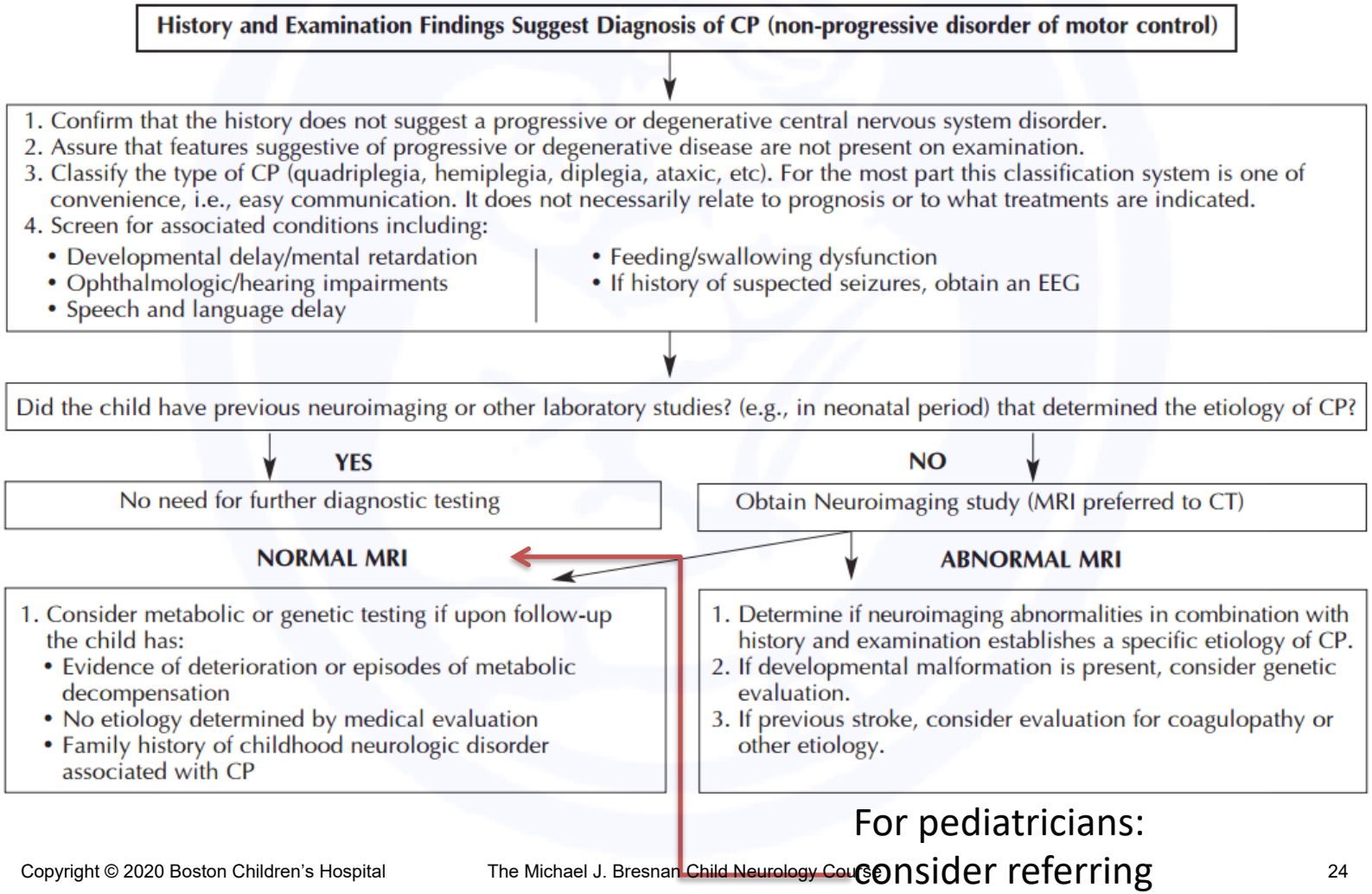
ASD Workup: Practice Parameter

- First tier
  - Three-generation family history with pedigree analysis
  - Initial evaluation to identify known syndromes or associated conditions
    - Examination with special attention to dysmorphic features
    - If specific syndromic diagnosis is suspected, proceed with targeted testing
    - If appropriate clinical indicators present, perform metabolic and/or mitochondrial testing (alternatively, consider a referral to a metabolic specialist)
  - Chromosomal microarray: oligonucleotide array-comparative genomic hybridization or single-nucleotide polymorphism array
  - DNA testing for fragile X (to be performed routinely for male patients only)[a]
- Second tier
  - *MECP2* sequencing to be performed for all females with ASDs
  - *MECP2* duplication testing in males, if phenotype is suggestive
  - *PTEN* testing only if the head circumference is >2.5 SD above the mean
  - Brain magnetic resonance imaging only in the presence of specific indicators (e.g., microcephaly, regression, seizures, and history of stupor/coma)



[23519317]

CP Workup: Practice Parameter



For pediatricians: consider referring at this point

[15037681]

Workup of NDD (in Pre-Exome Sequencing Era)

Unified, Stepwise Approach to Workup of Child with Neurodevelopmental Disorder (ID ± ASD ± CP)

*** NOTE: THIS APPROACH WILL BE DIFFERENT IN PART 2!! ***

Step 1: Does the child have CP?

YES

Make sure you get brain MRI

Ask yourself these questions:

- Does the presumed cause of CP match with child's motor and cognitive presentation? (e.g., ex 32weeker, spastic diplegia, PVL)
- Is the clinical course non-progressive?
- Is the MRI abnormal in a way that explains the child's presentation? (e.g., perinatal stroke, hemiplegia)

If any of the answers are **NO**, then refer to neurogenetics and **STOP**

If all of the answers are **YES**, the continue expectant management and **STOP**

NO

Go to Step 2

Step 2: Does the child have ID or ASD?

YES

Perform a detailed history/physical with the following in mind:

Component	Considerations
Clinical History	Birth history? Seizures?
Developmental History	How delayed? Regression?
Family History	Other affected family members?
General Exam	Dysmorphisms? Ophthalmic findings? Cardiac findings? Organomegaly? Skin findings?
Neurological Exam	Nystagmus? Hypotonia? Spasticity? Dystonia? Hyperreflexia? Ataxia? Focal findings?
Ancillary Studies	Hearing/vision testing

If you can't establish a diagnosis, or do not suspect a diagnosis, go to **Step 3**

NO

Genetic testing is not warranted

Step 3: Is there evidence of metabolic/mitochondrial dysfunction?

- Symptoms
  - Cyclical vomiting
  - Developmental regression associated with illness/fever
- Multisystem involvement
  - Dermatological changes (alopecia, hypertrichosis)
  - Gastroparesis
  - Cardiac, hepatic, renal problems
- Labs
  - Acid/base or electrolyte disturbances
  - Anemia with elevated MCV
  - Lactic acidosis
  - Unknown/unclear newborn screen results

YES Refer to neurogenetics and **STOP**

NO Go to **Step 4**

Step 4: Tier 1 Workup

- For all patients, send:
  - Microarray
  - Fragile X trinucleotide repeat analysis (except female + ASD + normal IQ)

- If you can't establish a diagnosis, go to **Step 5**

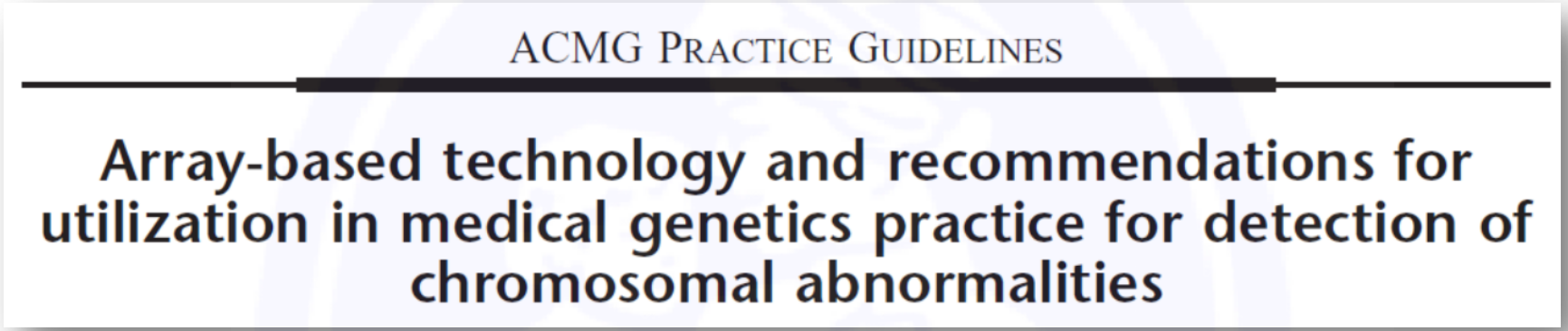
Step 5: Tier 2 Workup

- For females with either ASD or moderate-severe ID, consider *MECP2* testing
- For children with ASD + macrocephaly, consider *PTEN* sequencing
- Send brain MRI if clinically indicated:
  - Micro/macrocephaly
  - Seizures
  - Regression
  - History of coma/encephalopathy
- Send EEG if:
  - Suspected seizures
  - Loss of social/communication function
- If you can't establish a diagnosis, refer to neurogenetics and **STOP**

Workup of NDD

Rationale

CMA in Clinical Practice



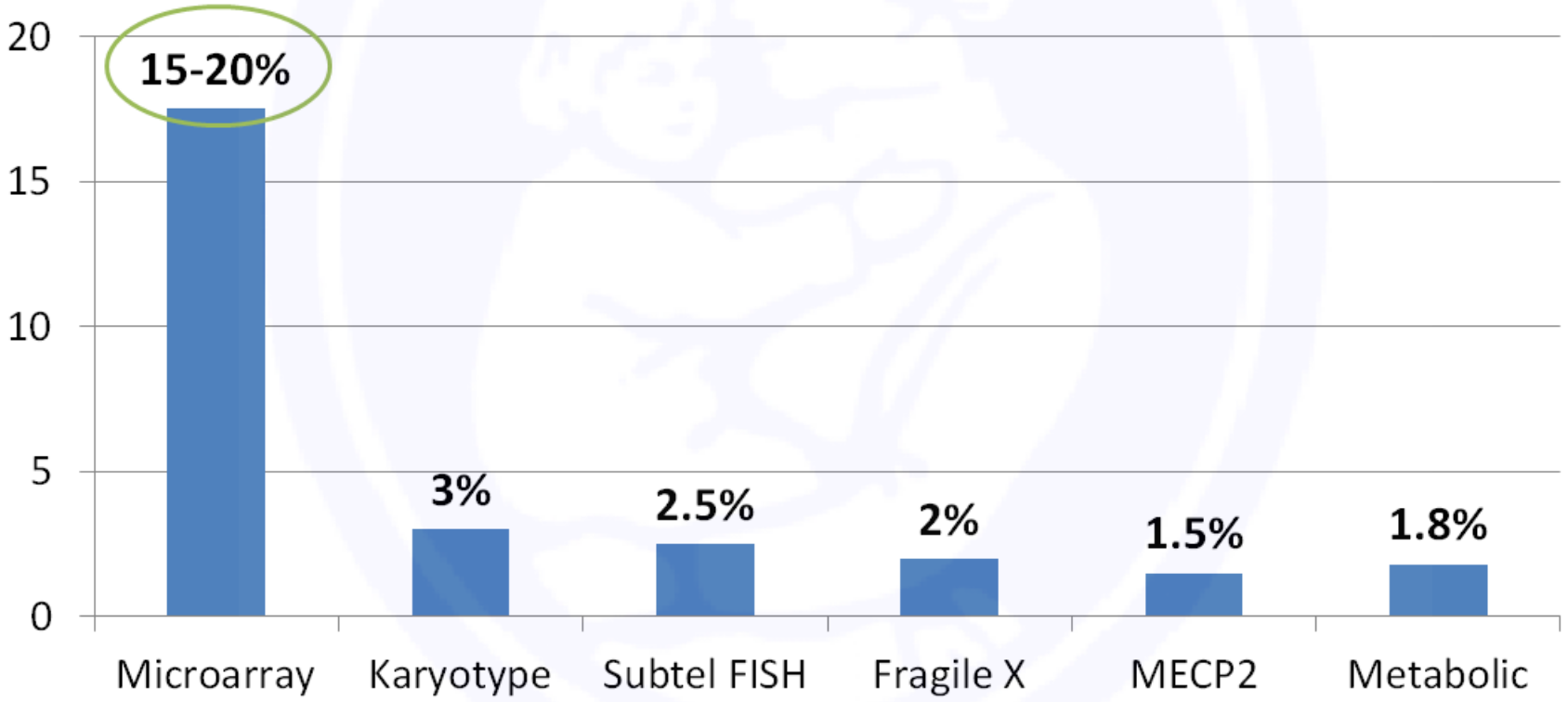
CMA was *previously* recommended as a first-line test in the initial evaluation of individuals with

- ID
- ASD
- Multiple congenital anomalies

[20962661]

Rationale for CMA

High Diagnostic Yield



[20466091]

Rationale for CMA

Changes Management

Study	Results
Ellison *et al*. 2012 (n = 46,298)	5% of all cases and 35% of patients with abnormal results had a medically-actionable CMA diagnosis
Riggs *et al*. 2014 (n = 28,526)	7% of all cases and 46% of patients with abnormal results had a medically-actionable CMA diagnosis
Henderson *et al*. 2014 (n = 1,780)	55% of patients with abnormal results underwent management changes

[23071206]; [23347240]; [24625444]

Yield of Other Testing in ID

Test	Gender	NDD	Yield
Fragile X testing	M, F	Mild ID	2%
MECP2 testing	F	Mod/severe ID	1.5%
MECP2 testing	M	ID	0.5%

[21956720]

Yield of Other Testing in ASD

Test	Gender	NDD	Yield
Fragile X testing	M, F	ASD	1-5%
MECP2 testing	F	ASD	4%
PTEN testing	M, F	ASD, HC > 2.5 SD	5%

[23519317]

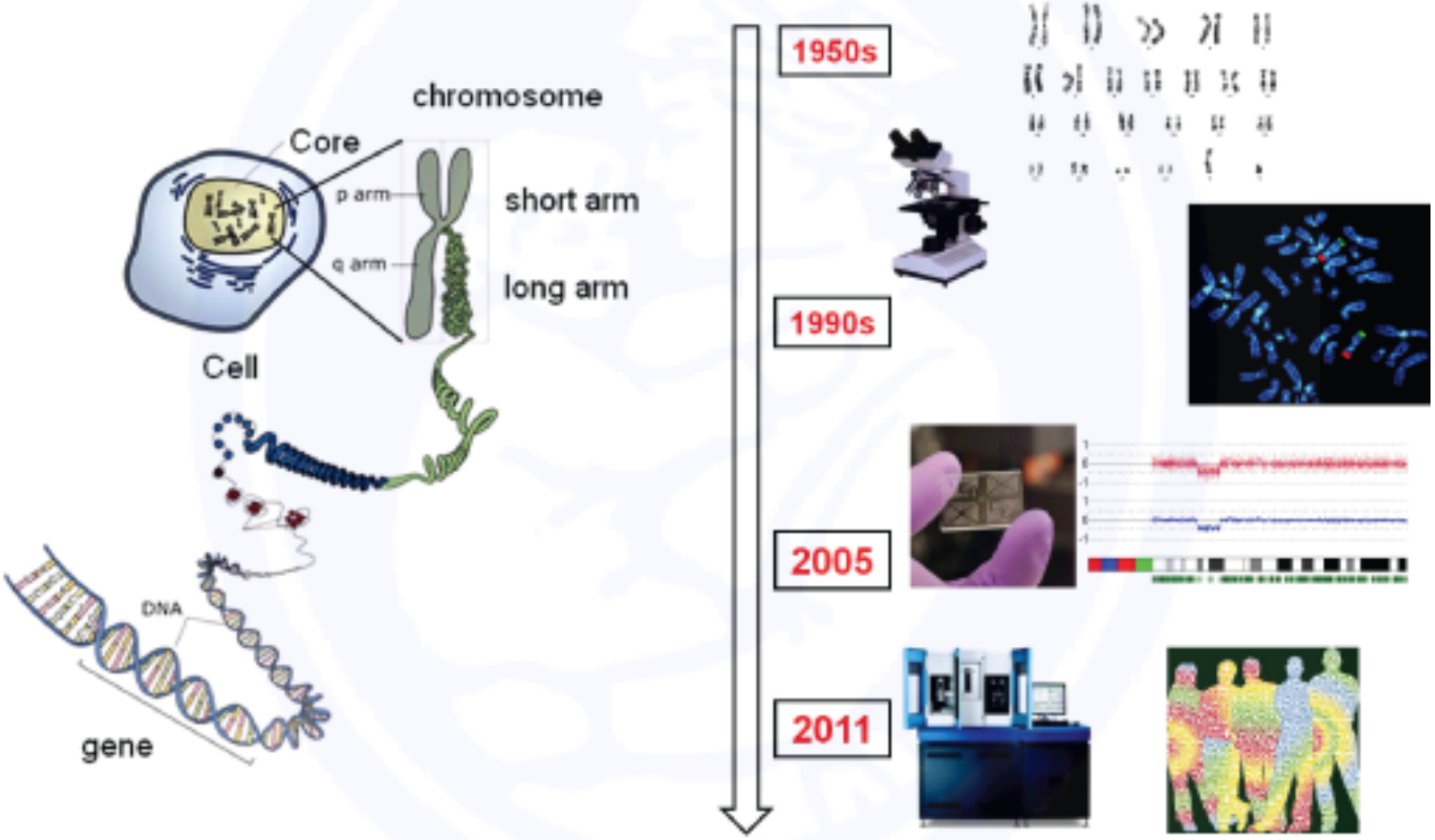
Yield of Metabolic Testing in ID

Test	Gender	NDD	Yield
Plasma amino acids, urine organic acids, etc.	M, F	GDD/ID	0.2-4.6%
Carbohydrate deficiency transferrin analysis	M, F	GDD/ID	1.4%
Urine/plasma creatine, creatinine, guanidinoacetic acid	M, F	GDD/ID	2.8%

- Low yield, high impact
- http://www.treatable-ID.org (free iPhone app)

[21956720]

Making Headway



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PART II (POST-EXOME SEQUENCING ERA)

Welcome to the Genomic Era

Single Gene Disorders

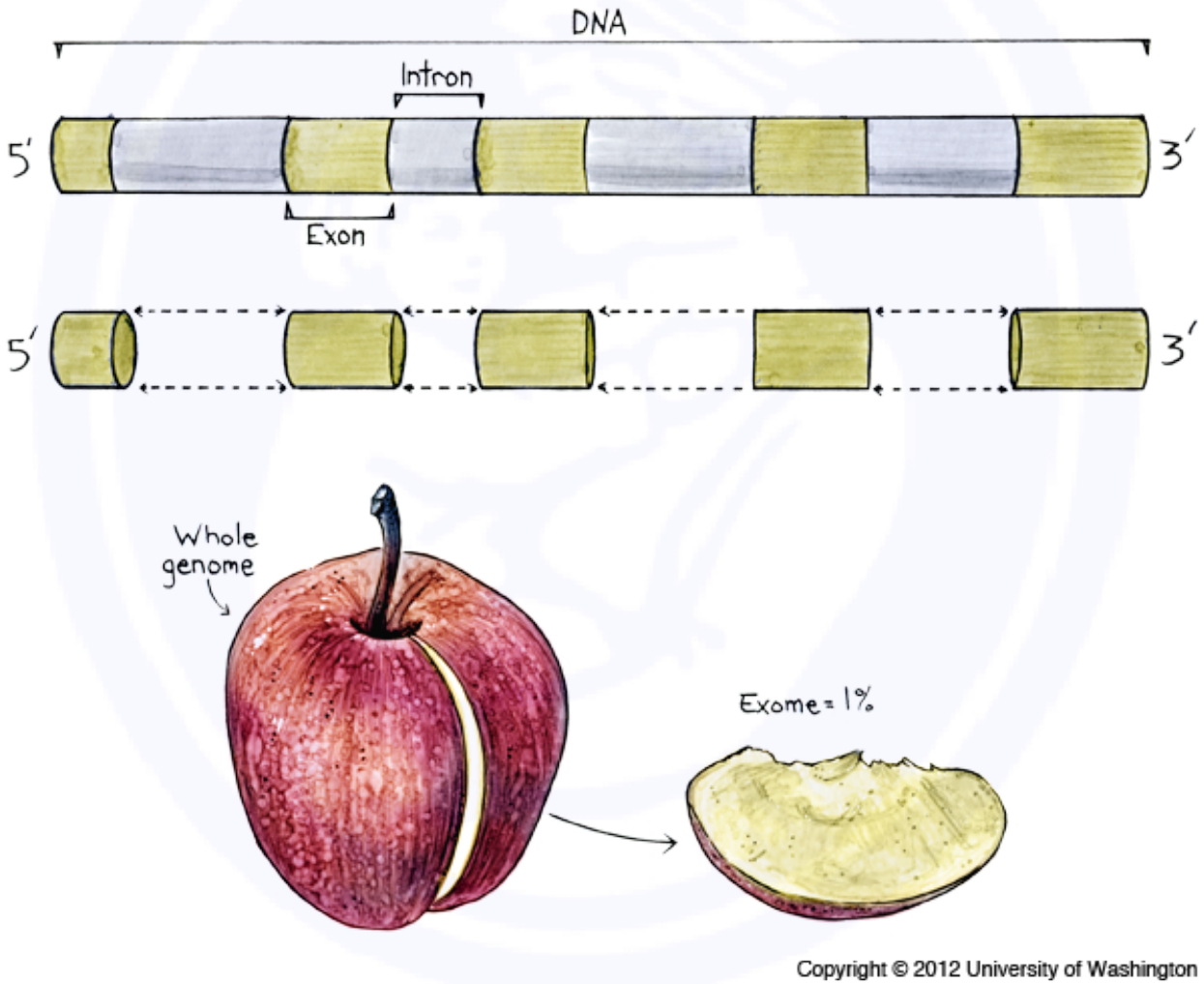
OMIM Gene Map Statistics

OMIM Morbid Map Scorecard (Updated August 26th, 2019) :

Total number of phenotypes* for which the molecular basis is known	6,492
Total number of genes with phenotype-causing mutation	4,143

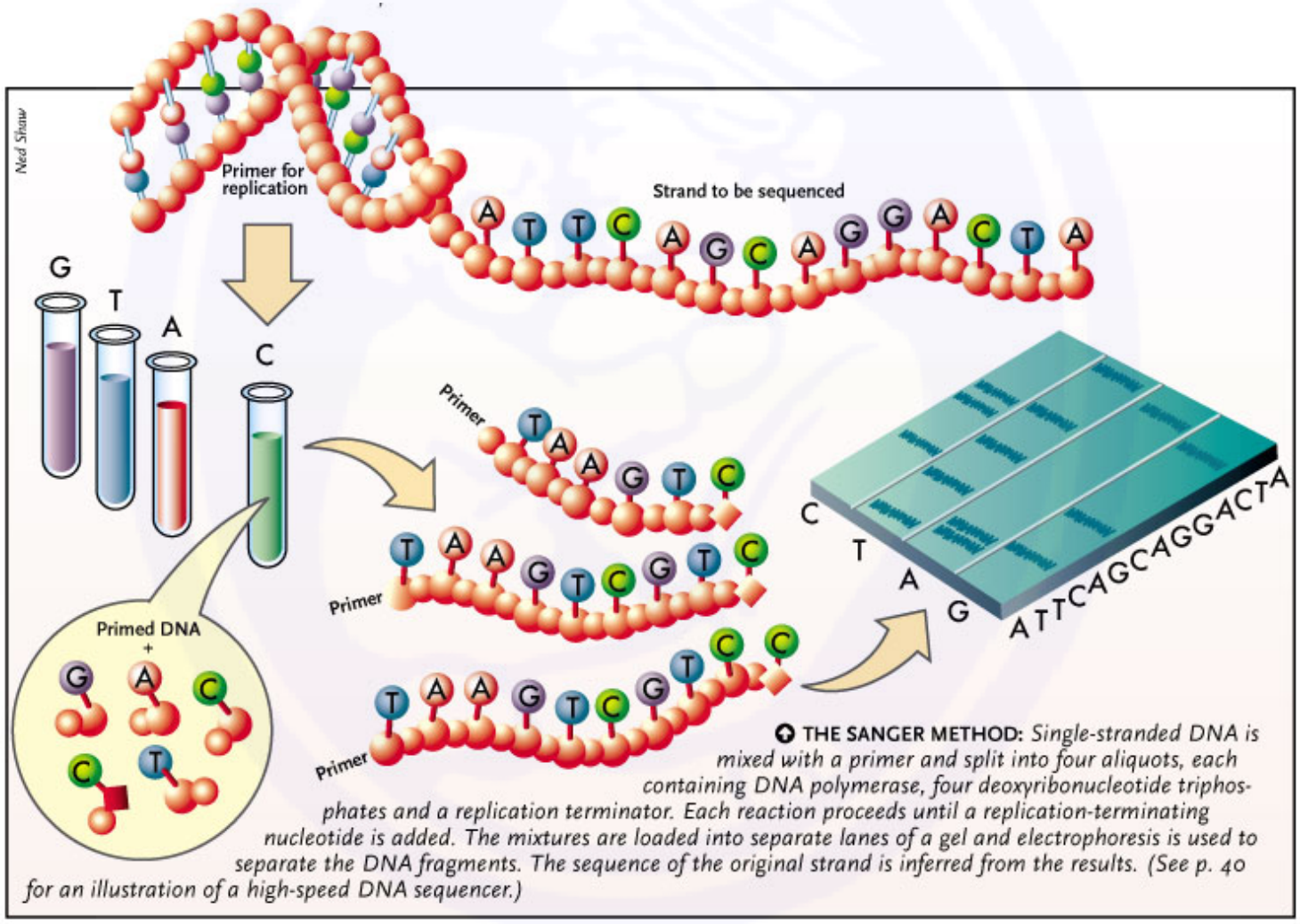
* Phenotypes include (1) single-gene mendelian disorders and traits; (2) susceptibilities to cancer and complex disease (e.g., BRCA1 and familial breast-ovarian cancer susceptibility, 113705.0001, and CFH and macular degeneration, 134370.0008); (3) variations that lead to abnormal but benign laboratory test values ("nondiseases") and blood groups (e.g., lactate dehydrogenase B deficiency, 150100.0001 and ABO blood group system, 110300.0001); and (4) select somatic cell genetic disease (e.g., GNAS and McCune-Albright syndrome, 139320.0008 and IDH1 and glioblastoma multiforme, 147700.0001.)

Exons and the Exome



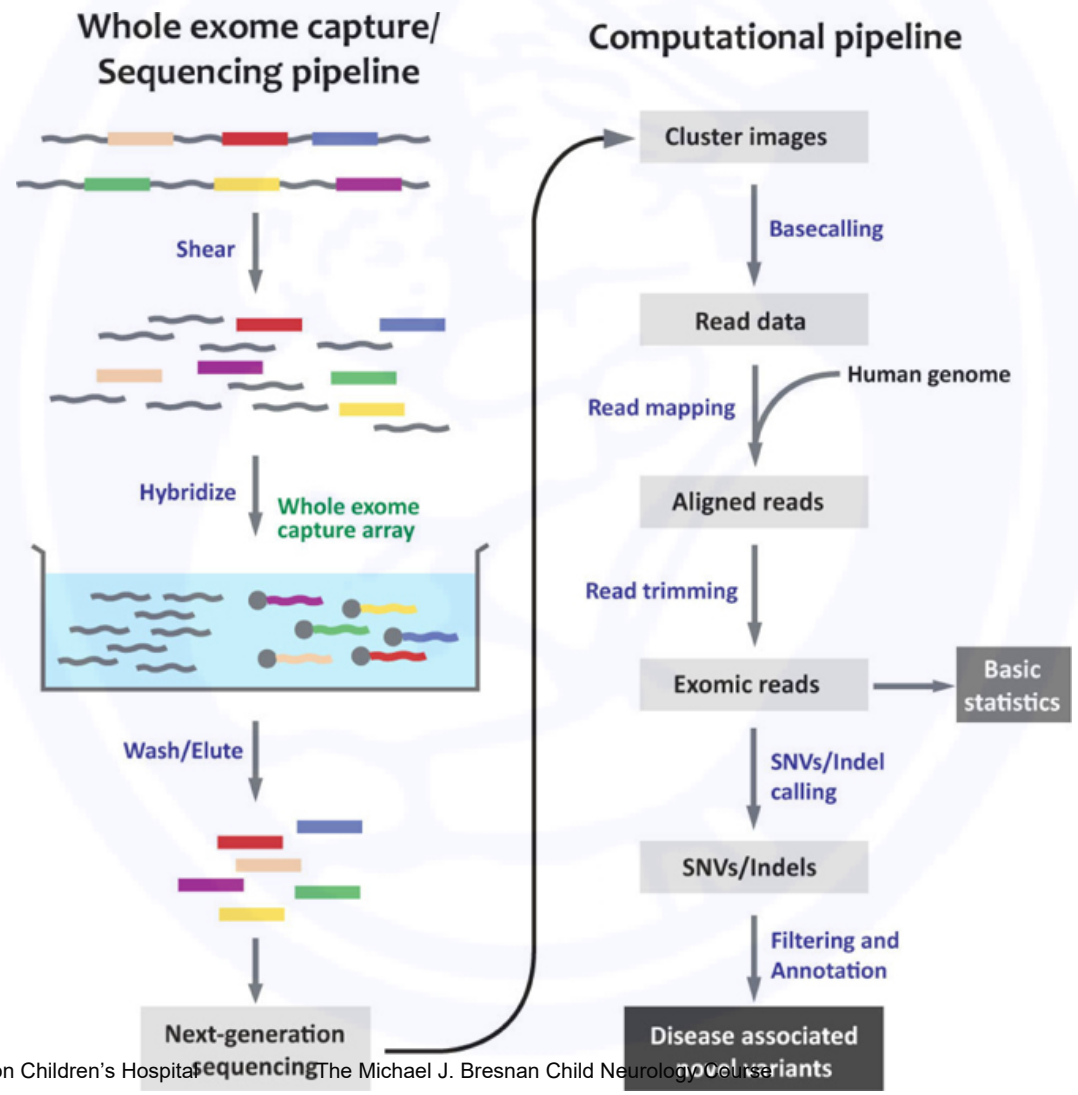
https://www.my46.org/intro/whole-genome-and-exome-sequencing

Traditional Sequencing



https://www.the-scientist.com/biobusiness/dna-sequencing-industry-sets-its-sights-on-the-future-49558

Whole Exome Sequencing (WES)



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3543920/

Advantages of WES

- It is efficient and cost-effective ($5,800-$9,000) compared to sequential gene testing
- It offers a genotype-first approach, for patients with:
  - Atypical presentations
  - Early disease courses
  - Multiple genetic disorders

Limitations of WES

- It does not really sequence the “whole” exome…
  - Not all exons are captured, and some not covered well
- Some mutation types not detectable:
  - Trinucleotide repeat expansion disorders (e.g. Fragile X)
  - Exon-level deletion/duplications
  - Chromosomal copy number variations
  - Balanced rearrangements
  - Introns and non-coding regions
- WES is limited by our knowledge: gene function, disease association, variants of unknown significance

Interpretation of WES Results

Possible Results

- Pathogenic
- Likely pathogenic
- Variant of unknown significance
- Likely benign variant
- Benign variant

Genetic counseling is a **MUST**

NEUROLOGY GRAND ROUNDS

Clinical Whole Exome Sequencing in Child Neurology Practice

Siddharth Srivastava, MD,[1] Julie S. Cohen, ScM,[1] Hilary Vernon, MD, PhD,[2] Kristin Barañano, MD, PhD,[1,3] Rebecca McClellan, MGC,[1] Leila Jamal, ScM,[1] SakkuBai Naidu, MD,[1,3,4] and Ali Fatemi, MD[1,3,4]

2014

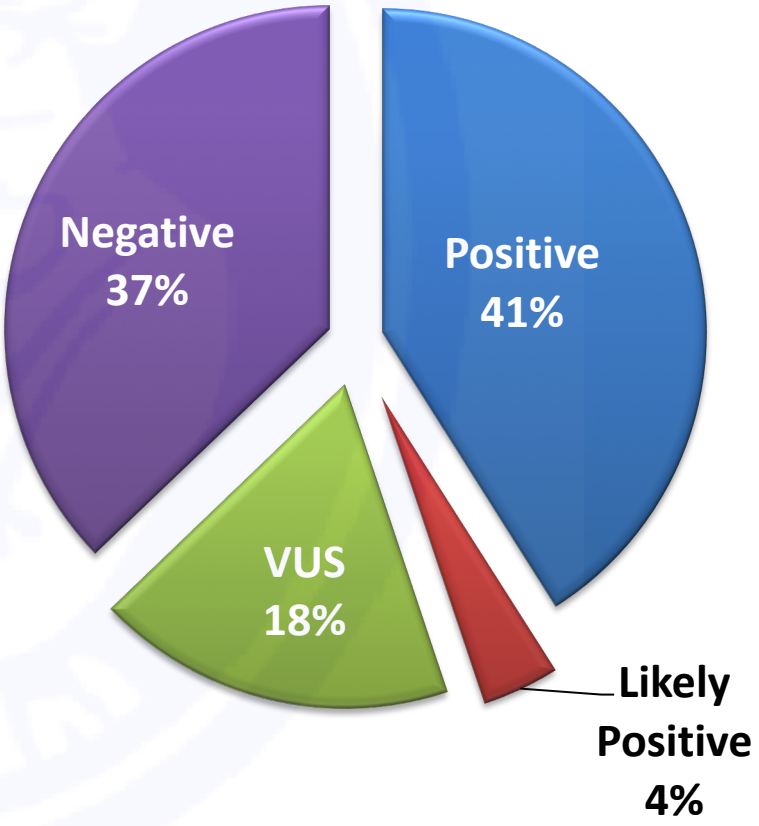
[25131622]

Patients

- 78 patients were included in the study
- Patients presented for etiological evaluation of previously unexplained NDD
- These disorders included ID, ASD, CP
- Already undergone extensive genetic/metabolic workup

Diagnostic Rate

- The overall diagnostic rate was **41%** (n = 32 out of 78 patients)
- The diagnostic yield of WES in this heterogeneous pediatric neurology cohort represents an improvement over other forms of genetic testing



Management Changes

Management Change	Number Patients
Reproductive planning	27 (84%)
Alteration of presumed disease inheritance pattern	7 (22%)
Changing of prognosis	10 (31%)
Initiation of disease monitoring	4 (13%)
Investigation of systemic involvement	6 (19%)
Discontinuation of medication	5 (16%)
Initiation of medication	2 (6%)
Education about clinical trials	3 (9%)

SYSTEMATIC REVIEW | Genetics inMedicine

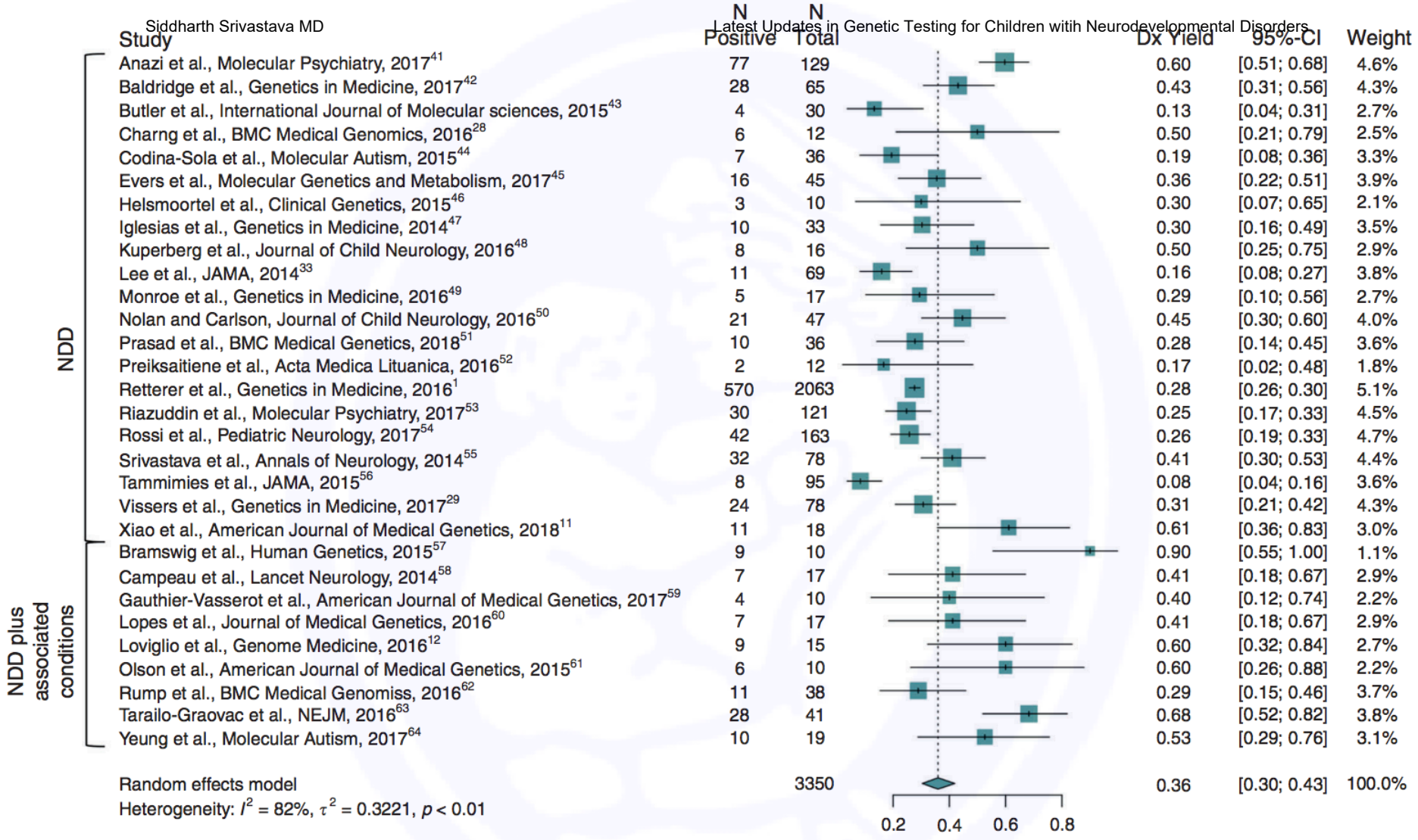
Open

Meta-analysis and multidisciplinary consensus statement: exome sequencing is a first-tier clinical diagnostic test for individuals with neurodevelopmental disorders

Siddharth Srivastava, MD[1], Jamie A. Love-Nichols, MS, MPH[1], Kira A. Dies, ScM[1], David H. Ledbetter, PhD[2], Christa L. Martin, PhD[2], Wendy K. Chung, MD, PhD[3,4], Helen V. Firth, DM, FRCP[5,6], Thomas Frazier, PhD[7], Robin L. Hansen, MD[8], Lisa Prock, MD, MPH[1,9], Han Brunner, MD[10,11,12], Ny Hoang, MS[13,14,15], Stephen W. Scherer, PhD[14,15,16,17], Mustafa Sahin, MD PhD[1], David T. Miller, MD PhD[18] and the NDD Exome Scoping Review Work Group

May 2019

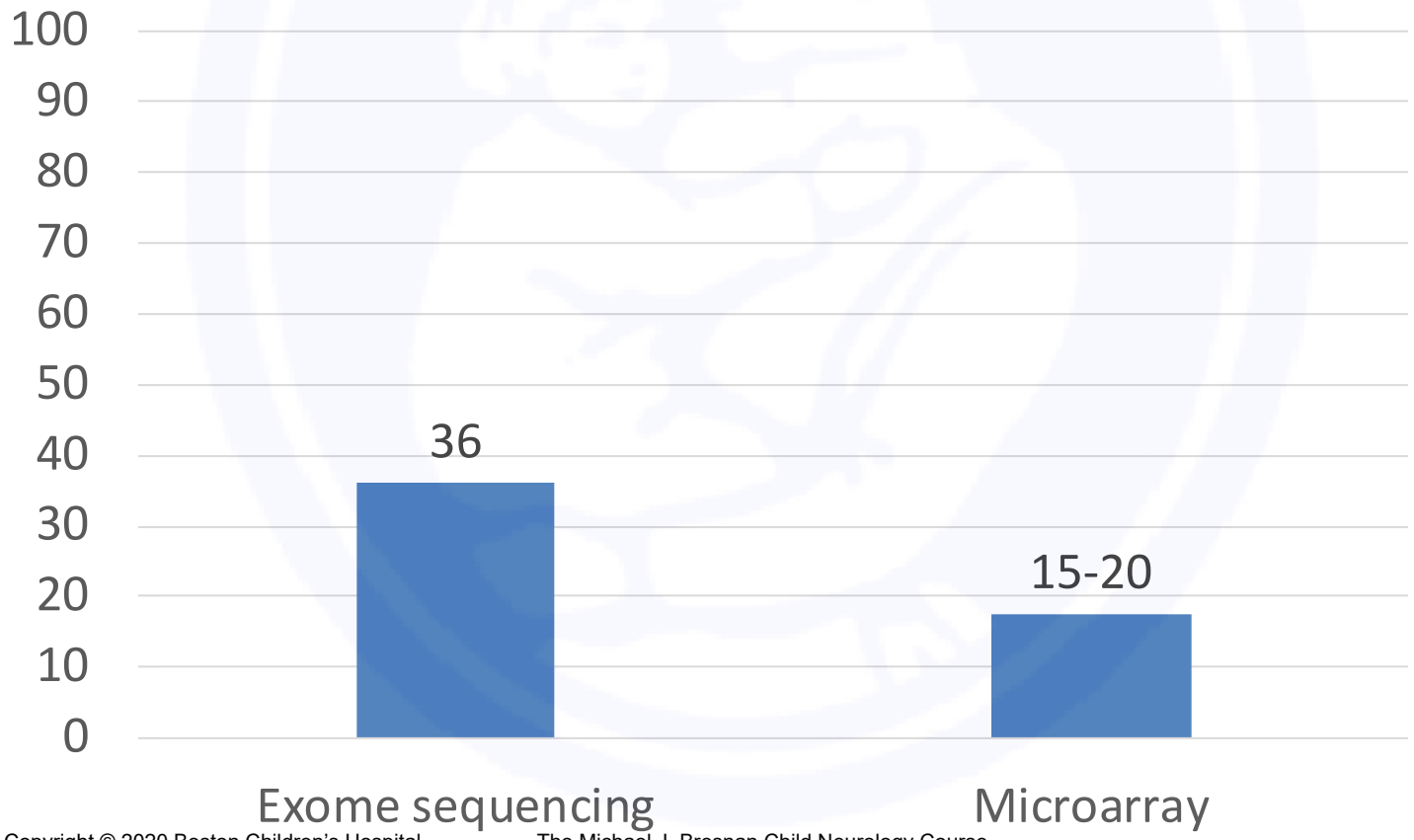
[31182824]



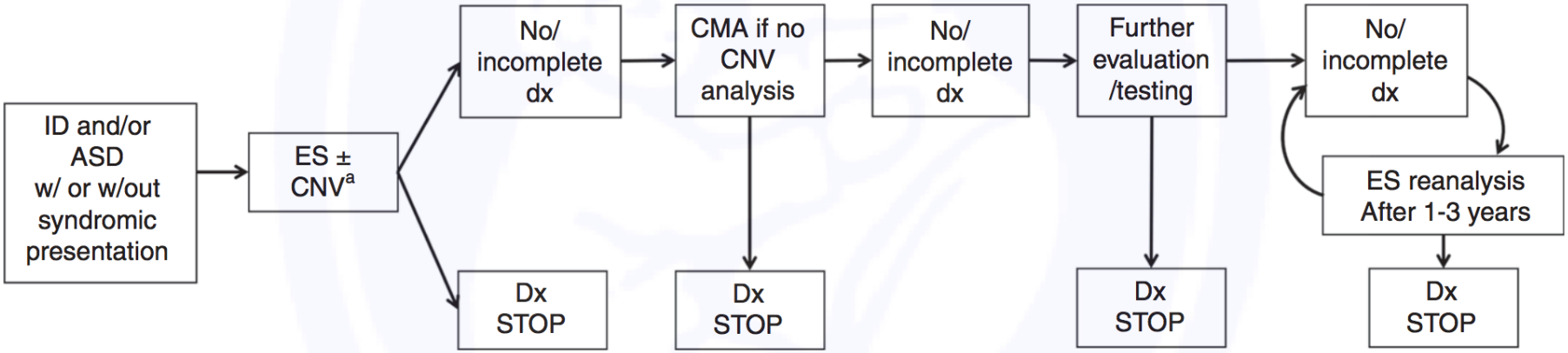
Diagnostic yield of WES for NDD (ASD and/or GDD/ID) is ~36%

Rationale for WES

High Diagnostic Yield (Even Higher than CMA)



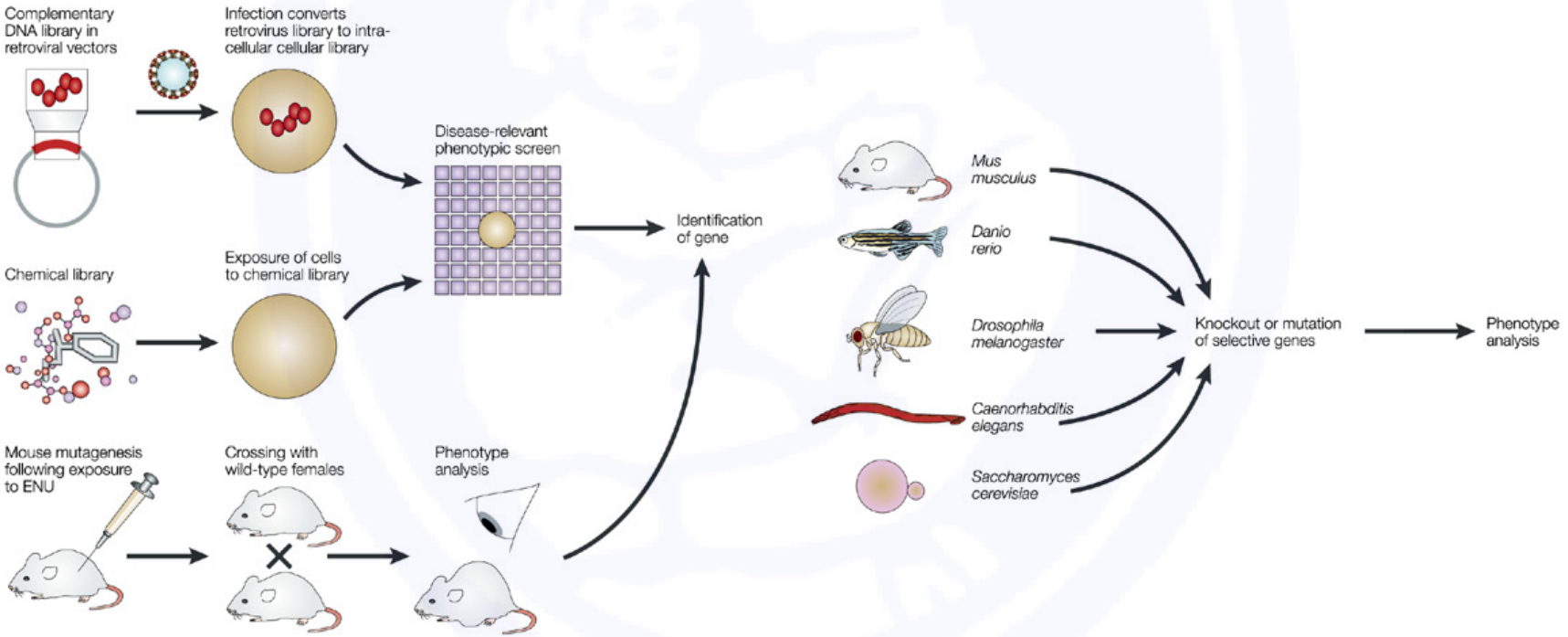
Workup of NDD in Post-Exome Sequencing Era


ID and/or ASD w/ or w/out syndromic presentation
ES ± CNV[a]
No/ incomplete dx
Dx STOP
CMA if no CNV analysis
Dx STOP
No/ incomplete dx
Further evaluation /testing
Dx STOP
No/ incomplete dx
ES reanalysis After 1-3 years
Dx STOP

Evaluation Framework

Forward Genetics

Reverse Genetics



https://www.nature.com/articles/nrd1202

Cases

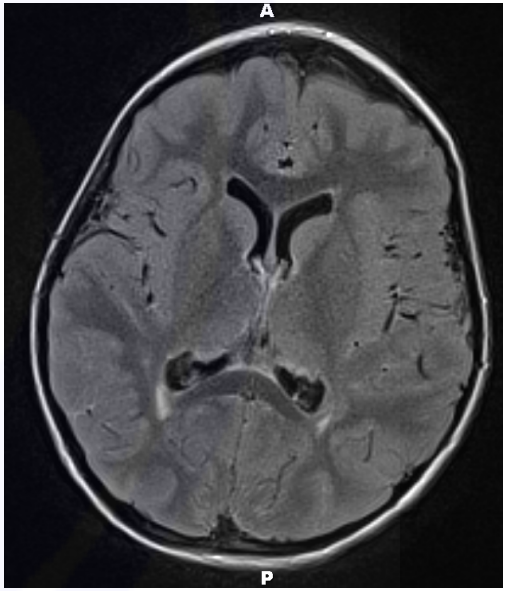
Case #1

- **12 year-old boy with:**
  - Motor delay and spasticity since infancy
  - Later onset of dysarthria
  - Progressive weakness
  - Normal cognitive abilities
- Parents are 1st cousins once-removed
- He was labeled as having cerebral palsy due to "hypoxic-ischemic encephalopathy" in spite of normal pregnancy and birth

Extensive Work-Up

- Amino acids, organic acids
- Lactate, pyruvate
- Lysosomal enzyme panel
- Very long chain fatty acids
- Cholestanol
- Urine sialic acid, MPS spot
- Vitamin E, B12, folate
- CDG

Brain MRIs:
"Periventricular Leukomalacia"

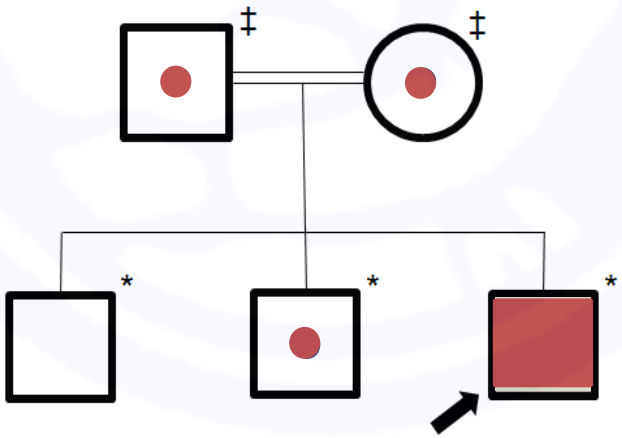


- **SNP array = 8% homozygosity**
  - 3,486 genes, many candidates… where to start?

WES Result

Homozygous nonsense mutation in *ALS2*
c.4897C>T (p.Q1633X)

Infantile-onset Ascending Hereditary Spastic Paralysis

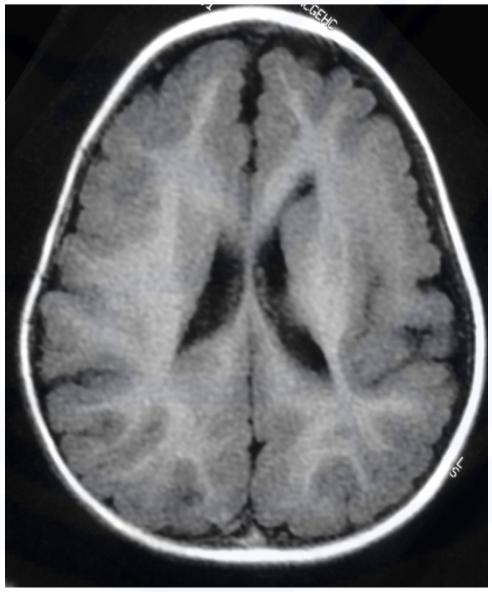


Case #1 Conclusions

- **Impact of diagnosis**
  - Etiology changed (genetic, not acquired)
  - Prognosis changed (motor deterioration, preserved cognition)
  - Recurrence risk and genetic counseling became important for siblings
- **Lessons from case**
  - There are genetic masqueraders of cerebral palsy
  - WES may be useful when there are many candidate genes

Case #2

- **3 year-old girl with:**
  - Global developmental delay
  - Hypotonia
  - Microcephaly
  - Polymicrogyria



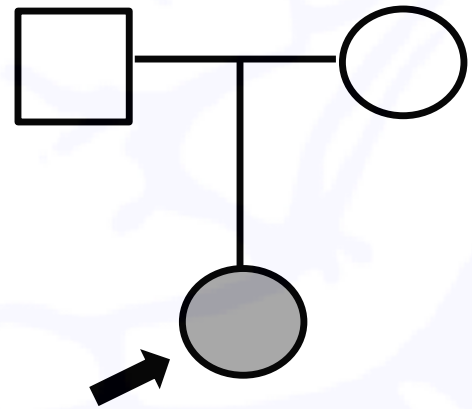
Extensive Work-Up

- Karyotype
- SNP chromosome microarray
- *GPR56, WDR62*, and *TUBB2B* gene sequencing
- *MECP2* gene sequencing and del/dup analysis
- 7-dehydrocholesterol
- Very long chain fatty acids
- Plasma amino acids
- Urine organic acids
- Lactate
- CMV

All Results NORMAL

Exome Sequencing Result

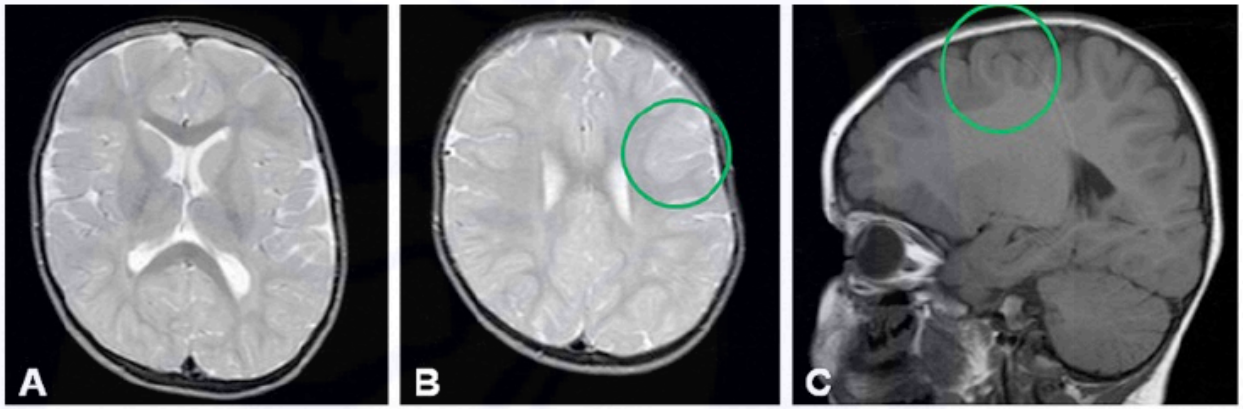
Heterozygous *de novo* mutation in *DYNC1H1:*
c.4700G>A (p.R1567Q)



Mutations in *DYNC1H1* cause severe intellectual disability with neuronal migration defects

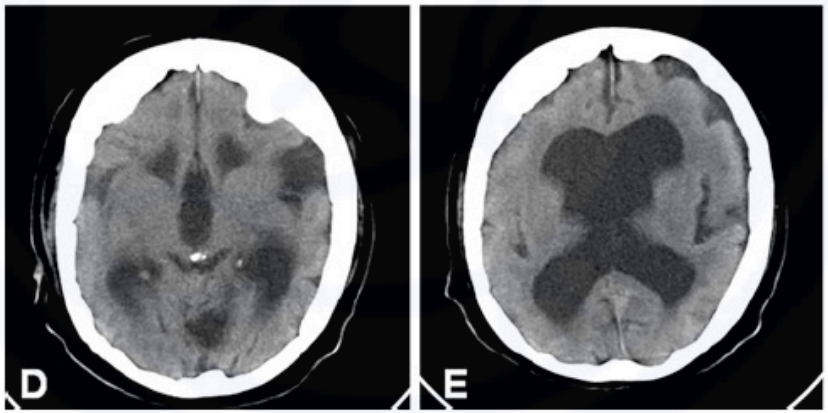
Patient 1

- 5 year old male
- p.H3822P



Patient 2

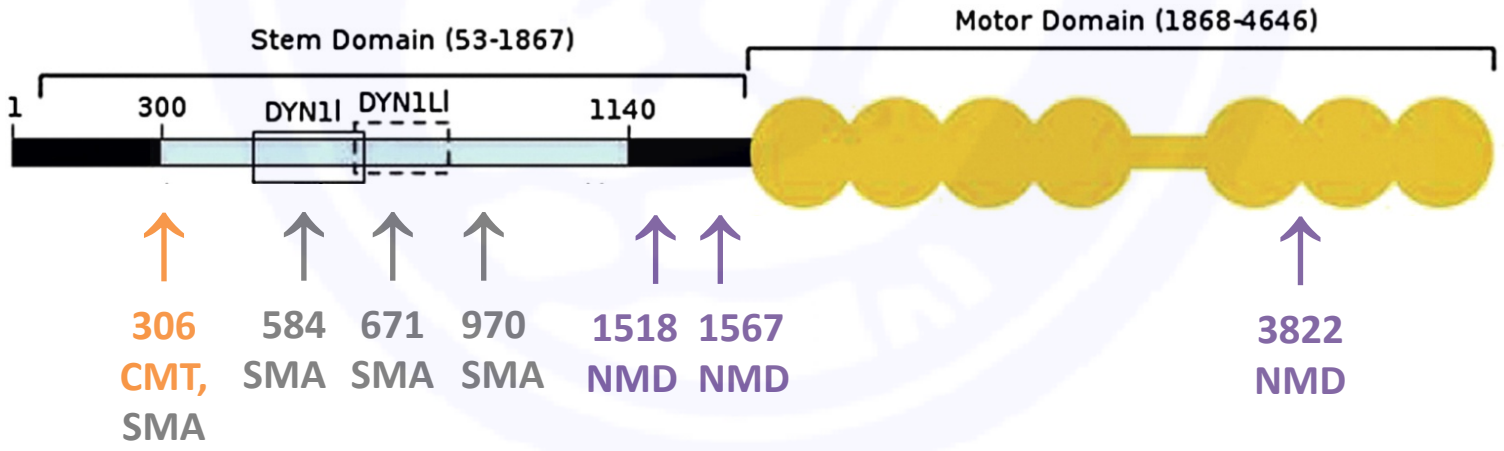
- 51 year old female
- p.Q1518K



[21076407]; [22368300]

DYNC1H1 gene

- Mutations have been identified in 7 patients/families with:
  - **Neuronal migration defects + intellectual disability**
  - **Charcot-Marie Tooth disease**
  - **Spinal muscular atrophy**



[21820100]; [22459677]; [22847149]

Case # 2 Conclusions

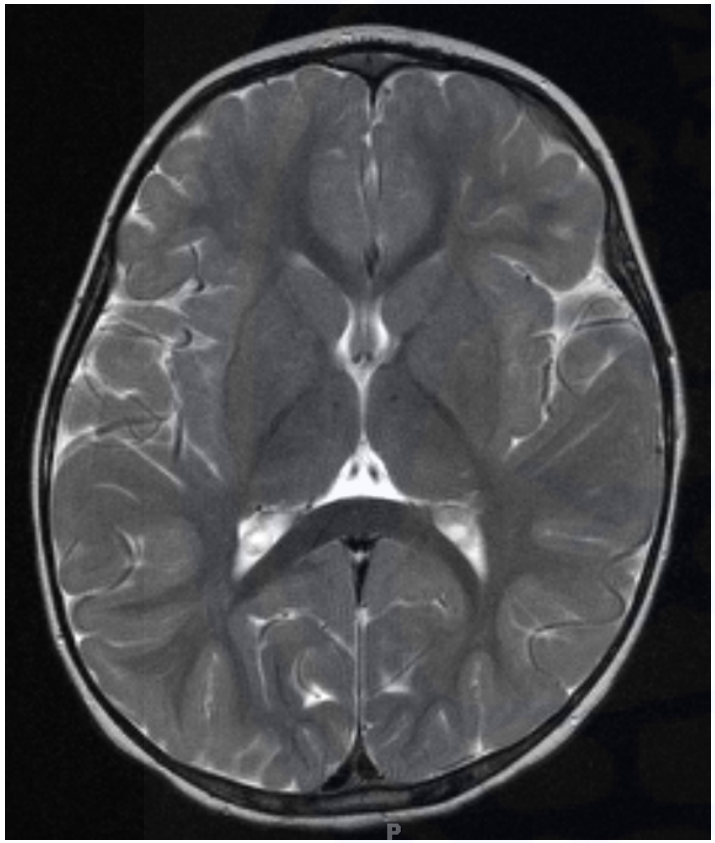
- **Impact of diagnosis**
  - Diagnosis prompted search for associated conditions
    - **EMG/NCS: chronic denervation consistent with mild SMA**
  - Prognosis changed (progressive motor difficulties)

- **Lessons from case**
  - WES can enable diagnosis of newly-described disease genes for which clinical testing not available
  - Unbiased diagnosis by WES can contribute to knowledge about expanding phenotypic spectrum

Case #3

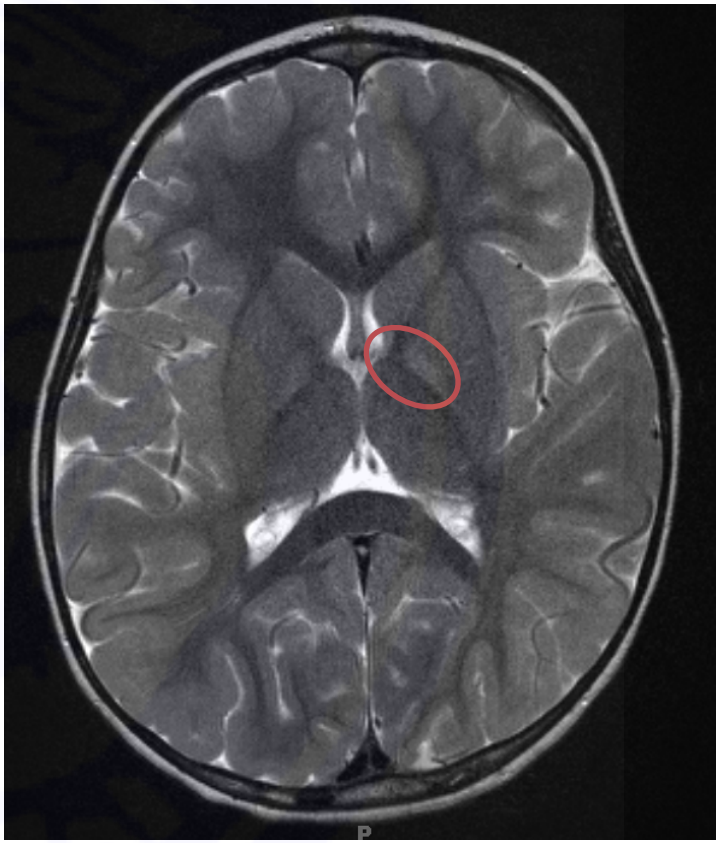
- **4 year-old girl with:**
  - Cognitive/language delay, failure to thrive
  - Early motor milestones normal, then “slowed” after 1y
  - Gradual motor deterioration from ~2.5y
  - Dystonia and opisthotonus
  - Swallowing difficulties
  - Staring spells

Brain MRIs



1y 10m

Normal



3y 2m

Subtle T2/FLAIR hyperintensity in globus pallidus bilaterally

Extensive Work-Up

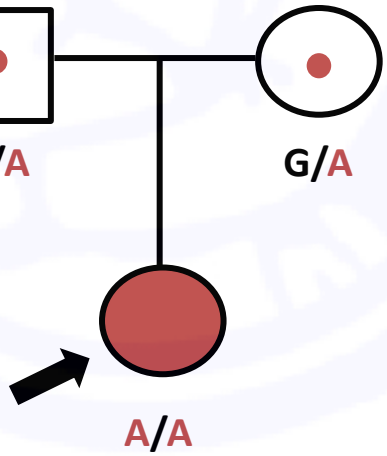
- SNP array
- Fragile X repeat analysis
- Amino acids, organic acids
- Lactate, CoQ10
- Lysosomal enzyme activity
- CLN1, CLN2 enzyme activity
- Very long chain fatty acids
- CDG
- *MECP2* sequencing
- mtDNA sequencing

All Results NORMAL

Exome Sequencing Result

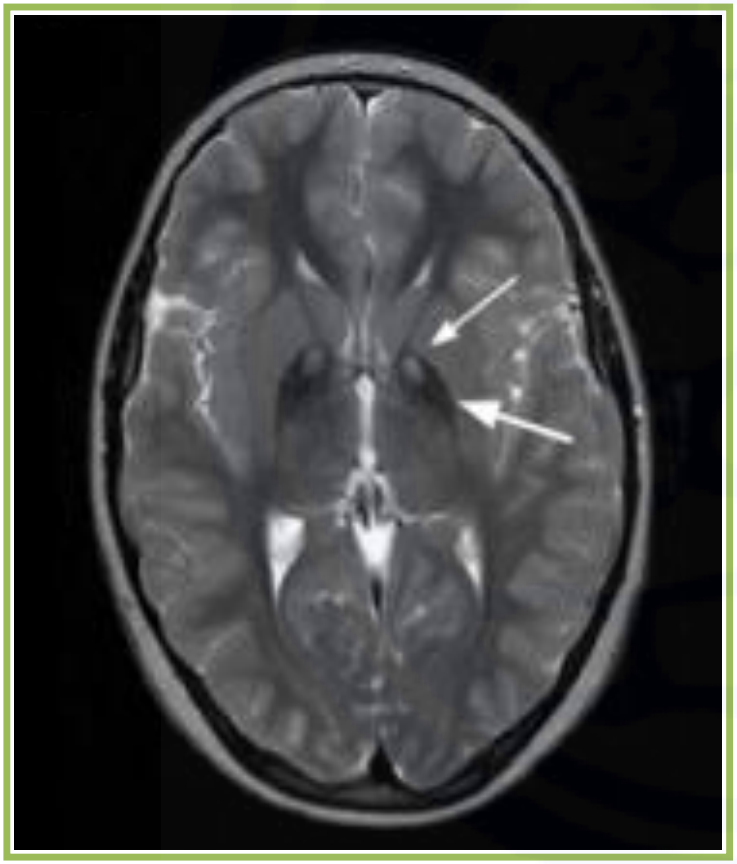
Homozygous mutation in *PANK2* gene:
c.1561G>A (p.G521R)

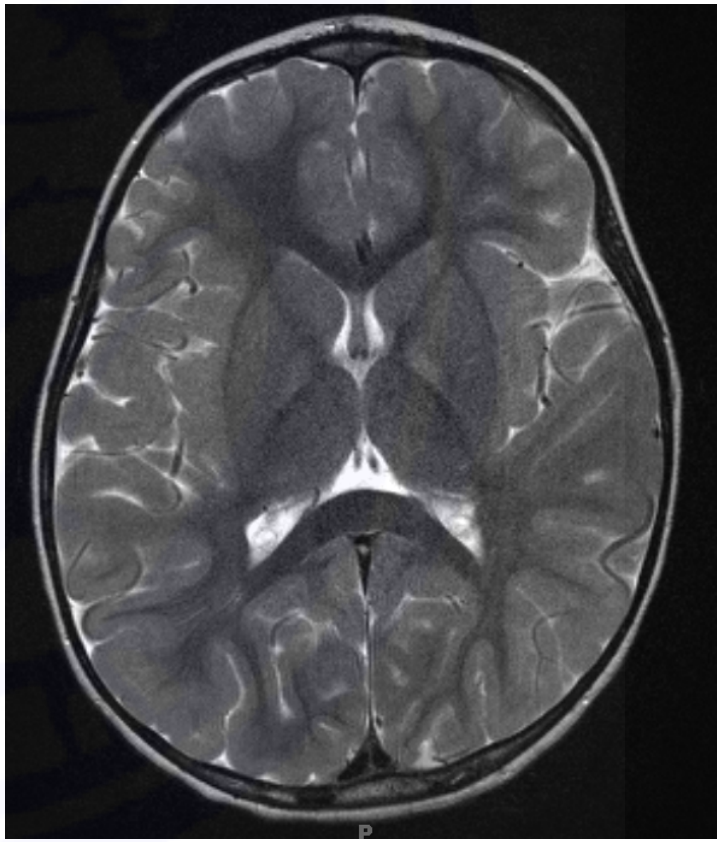
Pantothenate Kinase Associated Neurodegeneration (PKAN)



PKAN

Basal ganglia iron deposition → "Eye of the Tiger"



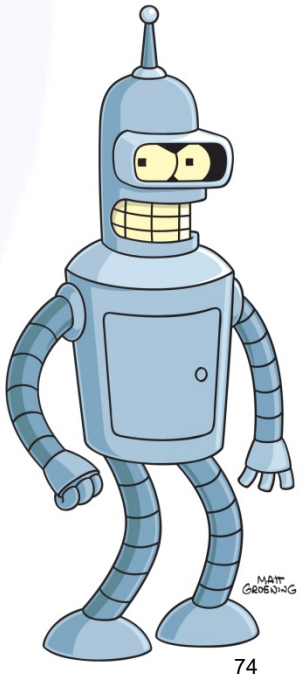


Patient

Case #3 Conclusions

- **Impact of diagnosis**
  - Diagnosis identified before pathognomonic MRI changes
  - She became eligible for a clinical trial
- **Lessons from case**
  - WES may facilitate diagnosis earlier in disease course
  - WES may shorten diagnostic odyssey

Conclusions and Future Directions



Summary

- All patients with unexplained NDD deserve work-up
- Etiologic diagnosis can improve management and have benefits for patient and family
- Rapid technological advances are improving ability to identify underlying cause for neurodevelopmental disabilities
- The high diagnostic yield by WES supports the use of WES in pediatric neurology practices
- Genetic counseling throughout the process is indispensable