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Genetic Testing in Epilepsy

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Outline

- Introduction to genomic era
- Type of genetic testing
- Pre-genetic testing counseling
- Case-based discussion
- How to handle the genetic result

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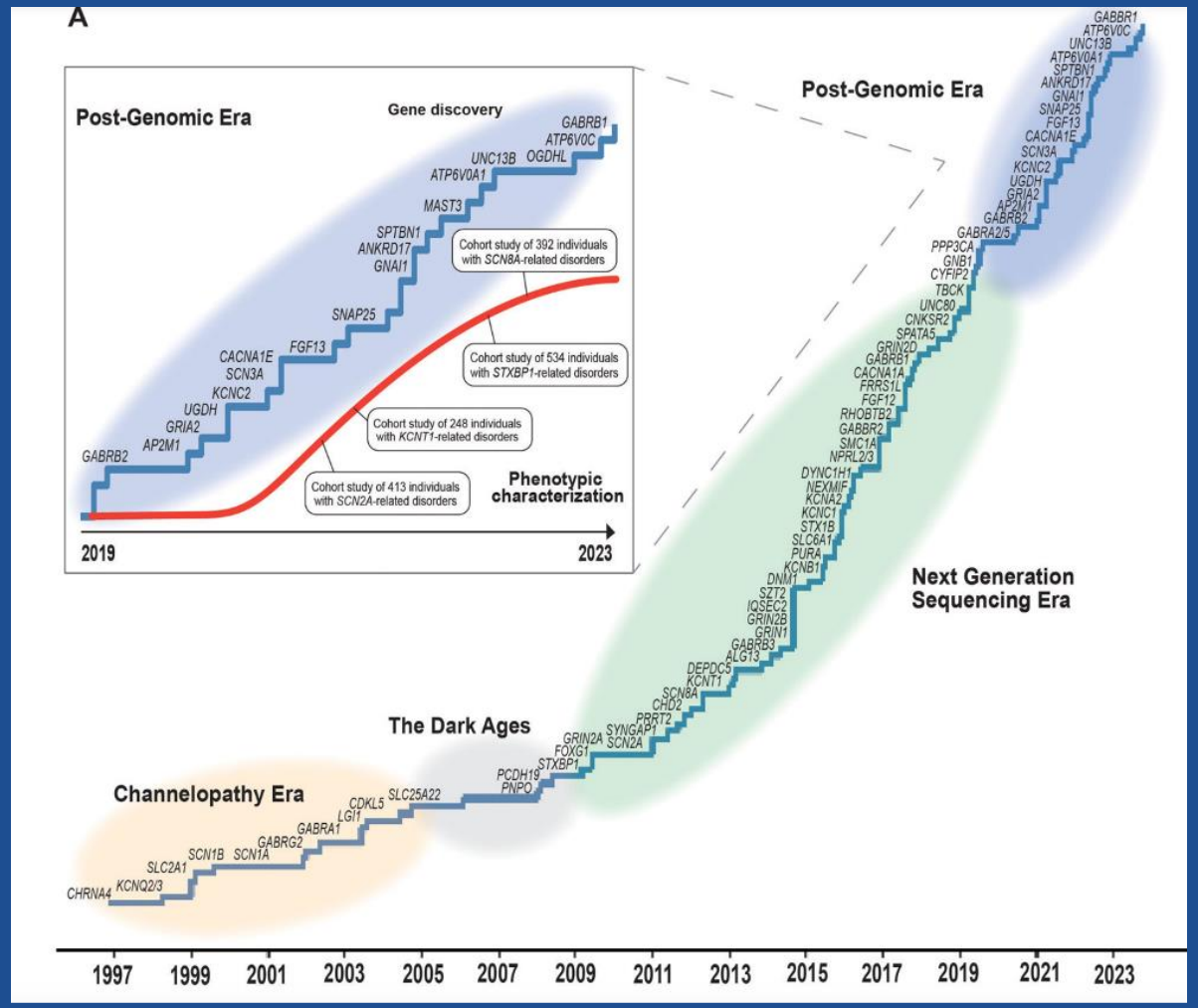
Innovations in Genetic testing

Over 1,000 genes
have been identified
that have been
associated with
monogenic epilepsy



Next-Generation Sequencing (NGS)

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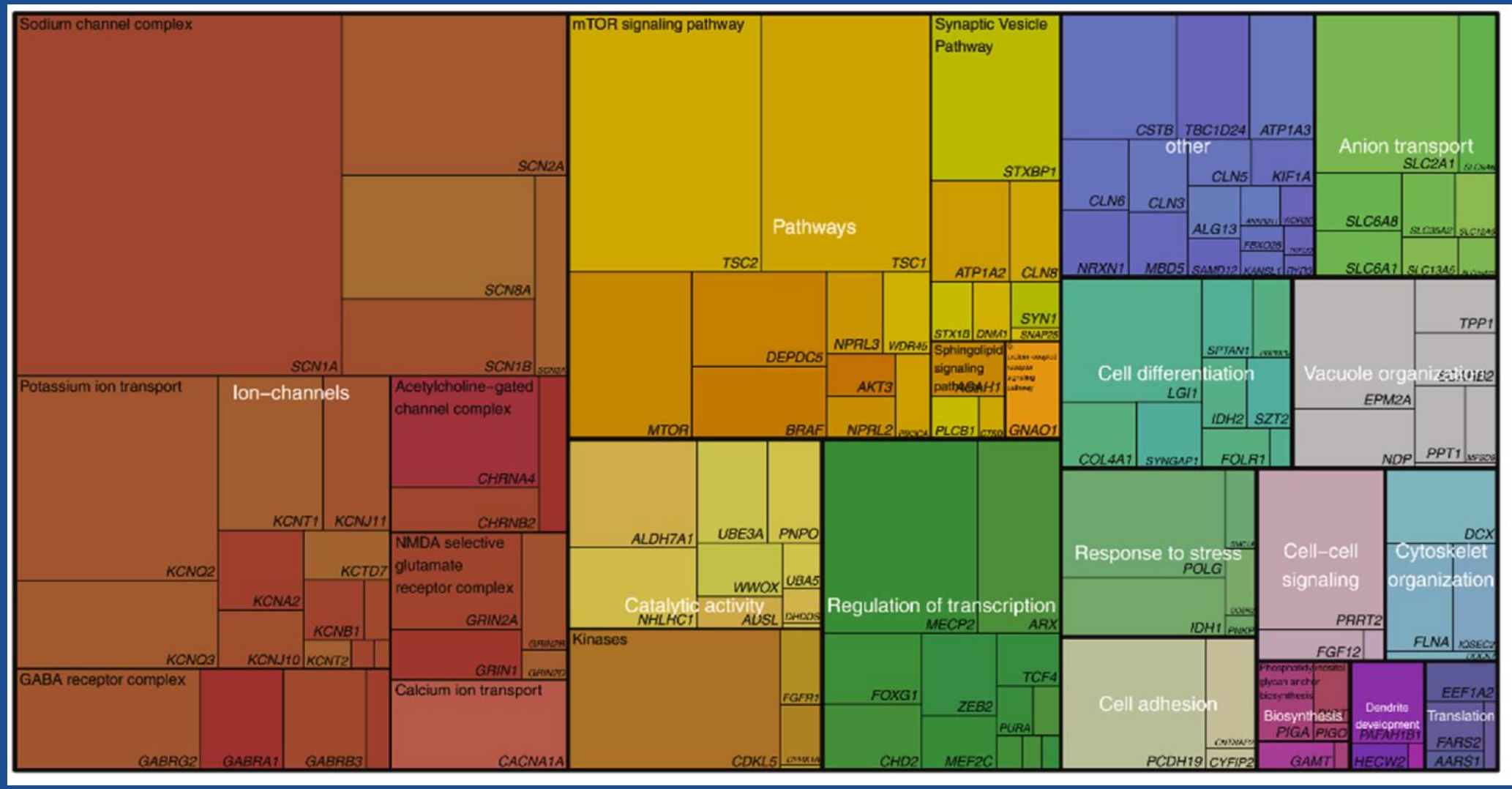
Treemap of Tier 1 epilepsy genes characterized in Gene Ontology term (GO) groups



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Why is genetic testing important?

End of Diagnostic odyssey

Reduce unnecessary investigation

Screening for comorbidities

Connecting with with a support group

Personalized treatment

Opportunity for future research

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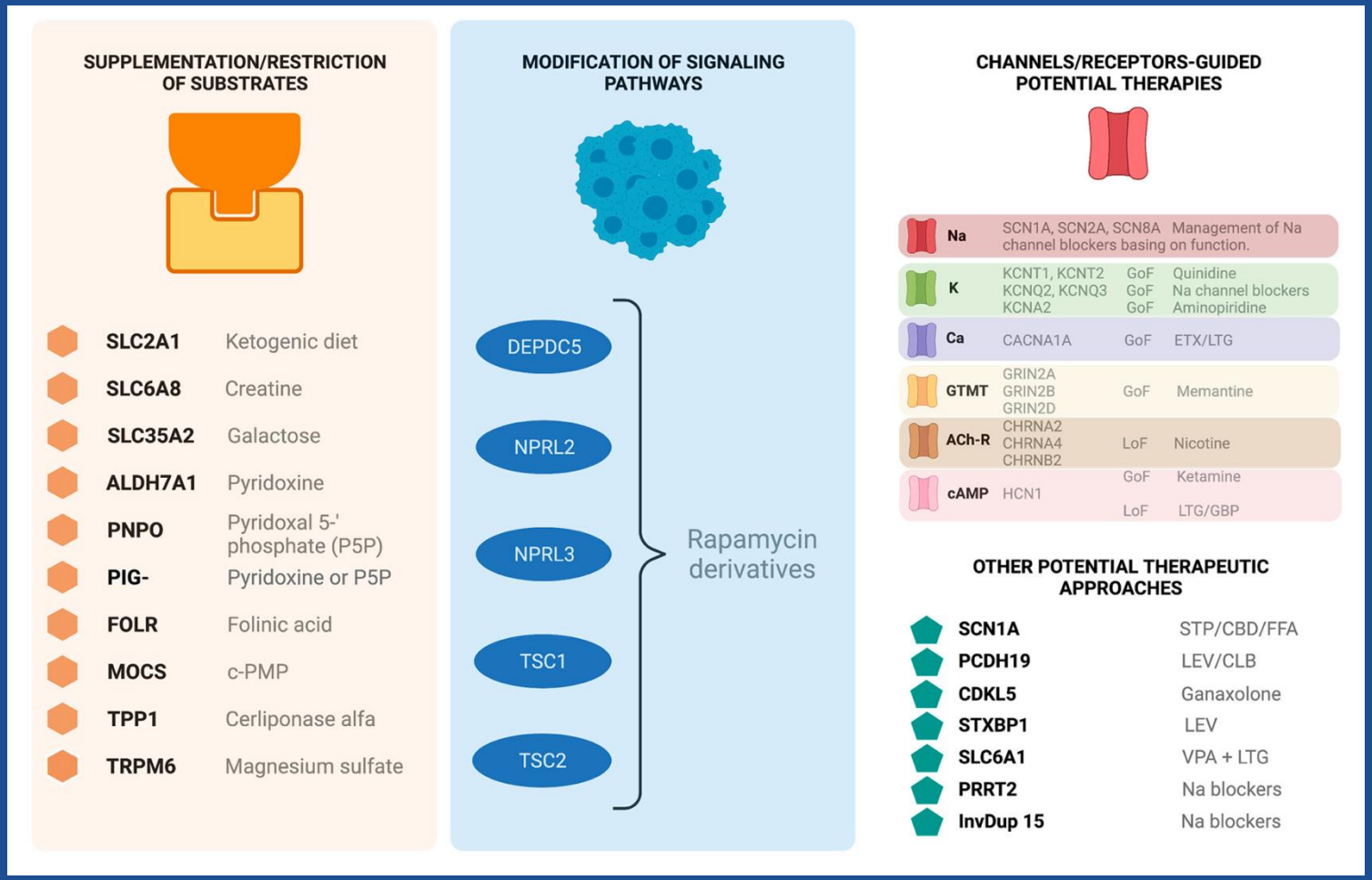
Epilepsy-related genetic conditions displaying potential specific therapeutic approaches



How to choose genetic testing

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Matching Genetic Disorder with Appropriate Testing

- Monogenic disorder
- Chromosomal disorder
- CNV disorder
- Imprinting disorder
- Repeat expansion disorder
- Mosaicism

- Chromosome study
- FISH
- Methylation test
- Chromosome microarray (SNP/CGH)
- Gene panel
- Whole exome sequencing
- Whole genome sequencing(long read/short read)

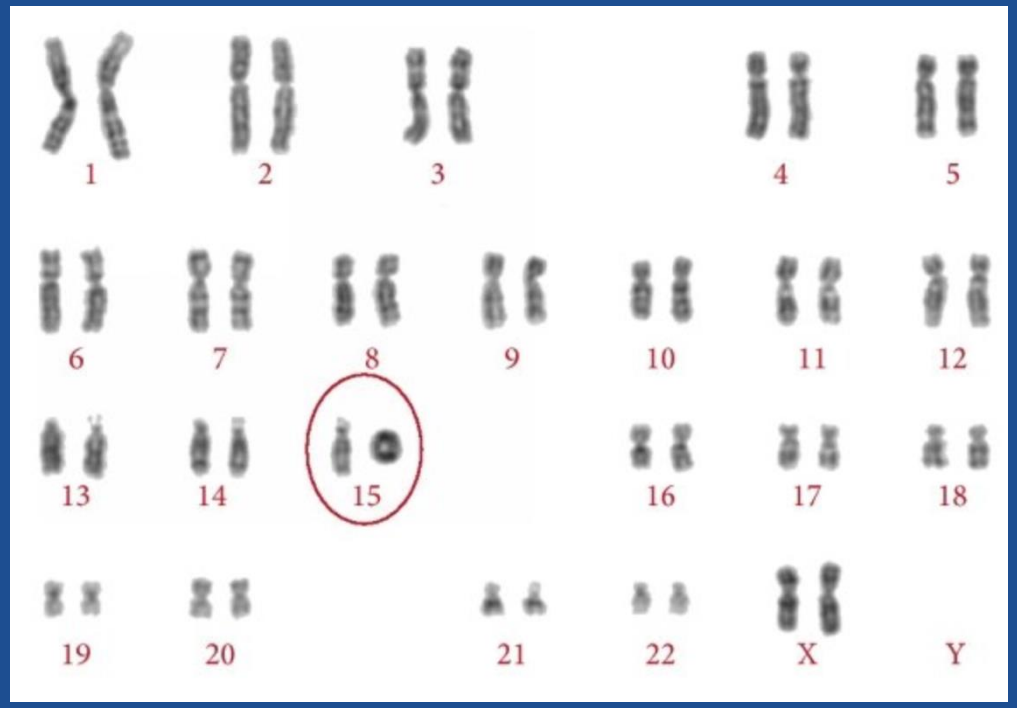
Type of Genetic Testing

Cytogenetic	Biochemical	Molecular
• Karyotype • FISH • CMA	• Plasma amino acids • Urine organic acids • Comprehensive metabolic test • Enzyme assays	• Sanger sequencing • MLPA • NGS - Gene panel - ES - GS

CMA: Chromosome microarray
ES: Exome sequencing
GS: Genome sequencing
MLPA: Multiplex Ligation-Dependent Probe Amplification
NGS: Next generation sequencing

Conventional karyotyping

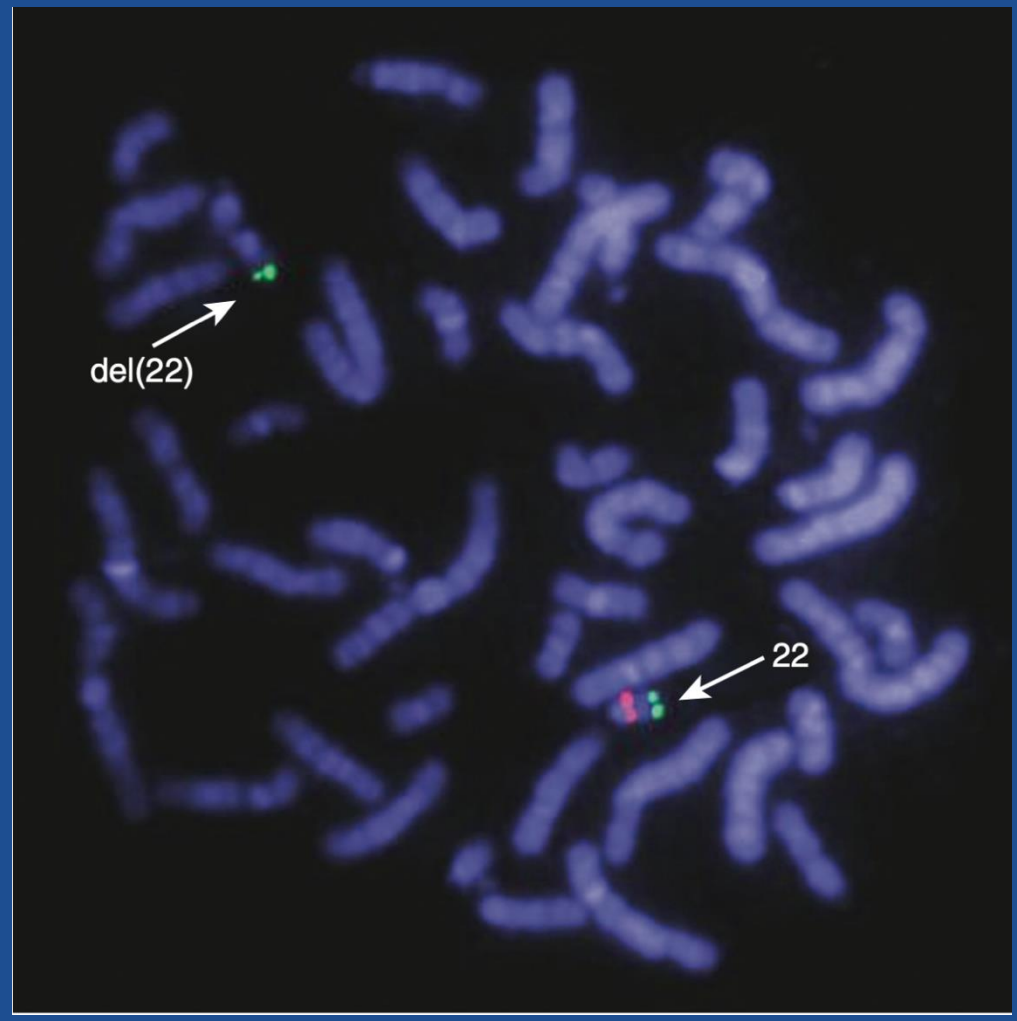
- Detect
  - Chromosomal abnormalities of 5 Mb and larger
  - 2–3 Mb (high-resolution banding)
- Analyze chromosomes for chromosomal rearrangements
    - Balanced or unbalanced translocations
    - Inversions
    - Ring chromosome



Ring chromosome 15

FISH

- Specific probe
- Resolution: 150-200 kb
- Can not detect uniparental disomy



Next generation sequencing

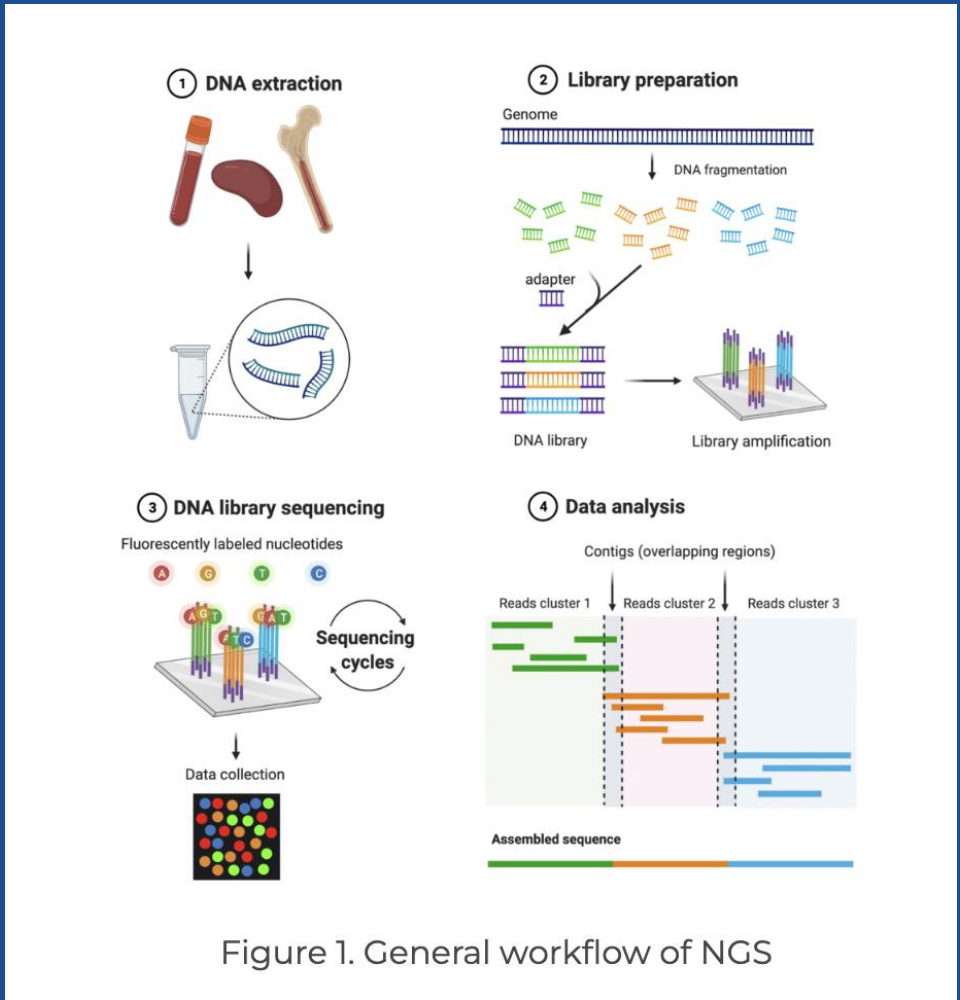
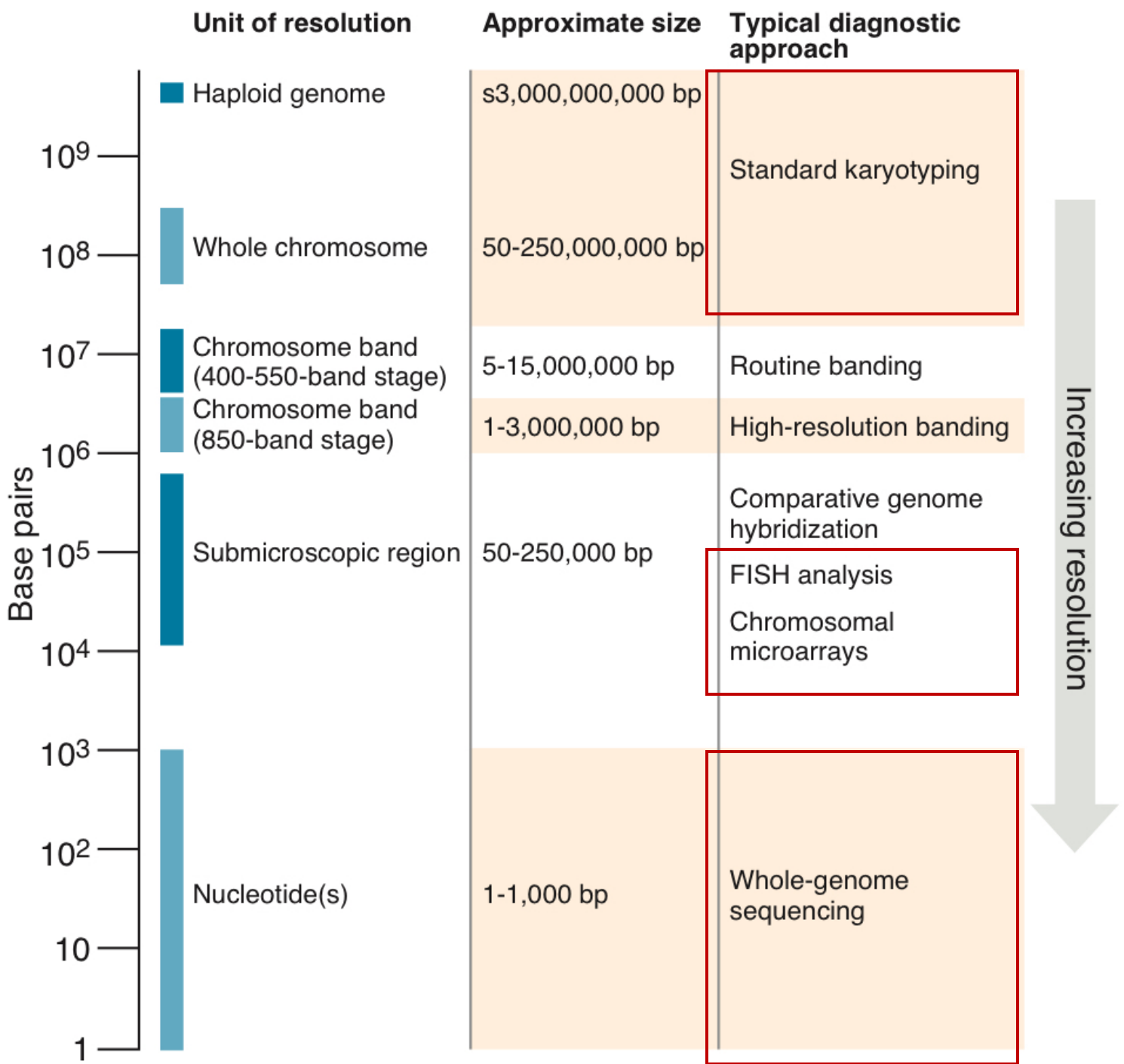


Figure 1. General workflow of NGS

https://www.rapidnovor.com/de-novo-protein-sequencing-vs-dna-sequencing/

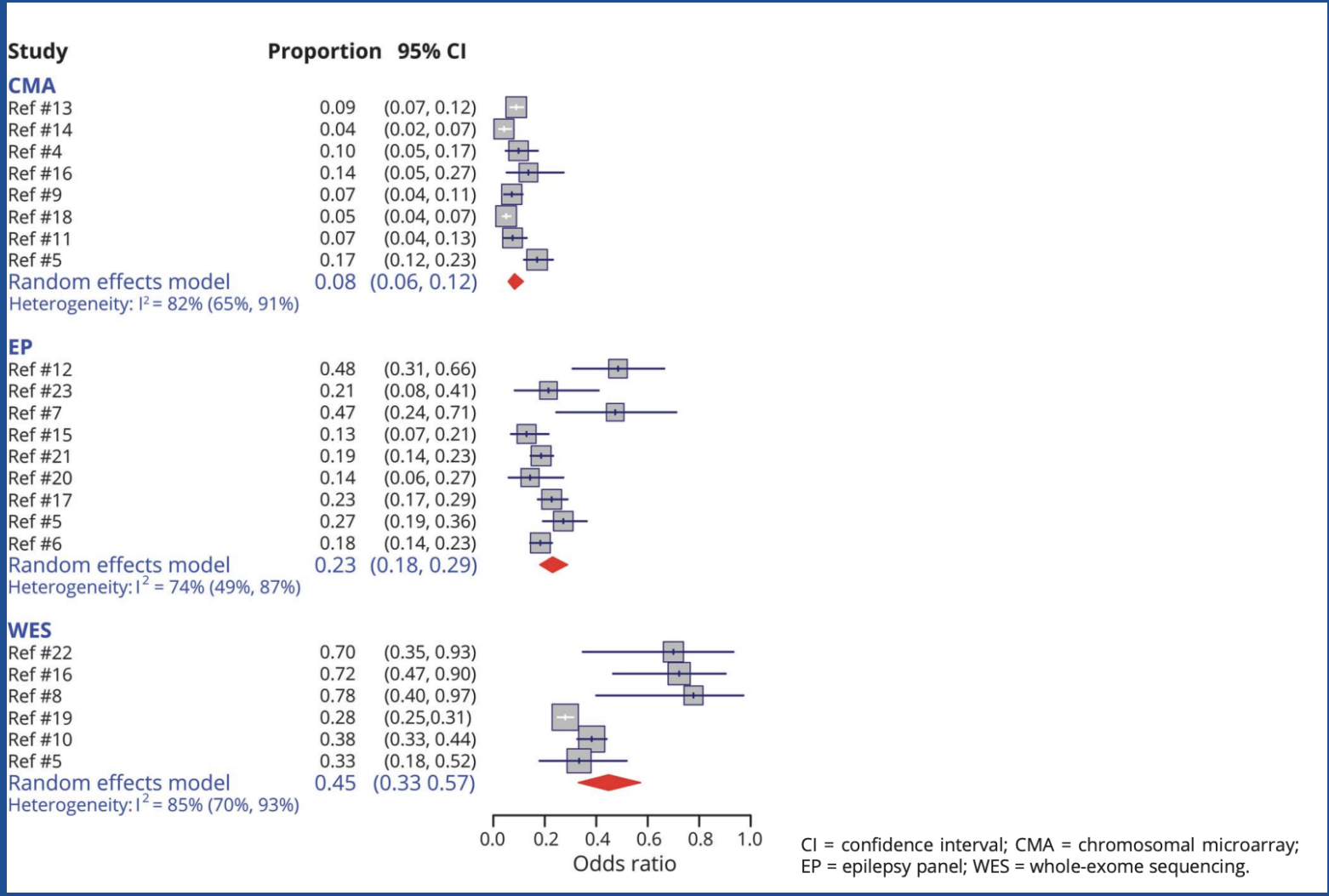
Resolution



Thompson & Thompson genetics in medicine, 8th Ed.

	Resolution	Applications	Limitations
Karyotyping	2-3 Mb / 5 Mb	Scanning for aneuploidy, large deletion or duplication and structural variants.	Cannot identify microdeletion/duplication
CMA: SNP array	Few kb or longer	Scanning the entire genome for any CNV, Can identify ROH	Cannot identify balanced translocation and inversion
CMA: Array-CGH	Few kb or longer	Scanning the entire genome for any CNV	Same as SNP-array. Cannot identify ROH
Gene panel	One to several bp	Scanning for genes of interest. Can be supplemented with other technologies to solve low-coverage regions and detect small CNV. "True" panel testing with deep coverage can detect low-level mosaicism.	Cannot detect deep-intronic changes. Different labs have different set of genes for targeted phenotype.
ES	One to several bp	Scanning for all genes of interest excluding non-coding and deep intronic regions.	Might miss variants in low-coverage regions and deep intronic changes, low-level mosaicism. Less reliable for copy number calling.
GS	One to several bp	Scanning for entire genome including non-coding and deep intronic regions.	Costly and mostly research-driven. Most variants in non-coding regions are uninterpretable

Diagnostic yield in genetic testing



CI = confidence interval; CMA = chromosomal microarray; EP = epilepsy panel; WES = whole-exome sequencing.

Genetic Counseling

- Types of Genetic Testing Performed on Patients
- Sample Collection
- Advantages of Genetic Testing
  - Personalized treatment
  - Prognostic information and guidance
  - Recurrence risk assessment
- Possible Results
  - Positive findings
  - Negative findings
  - Variants of uncertain significance (VUS)
  - Secondary findings (ES/GS)

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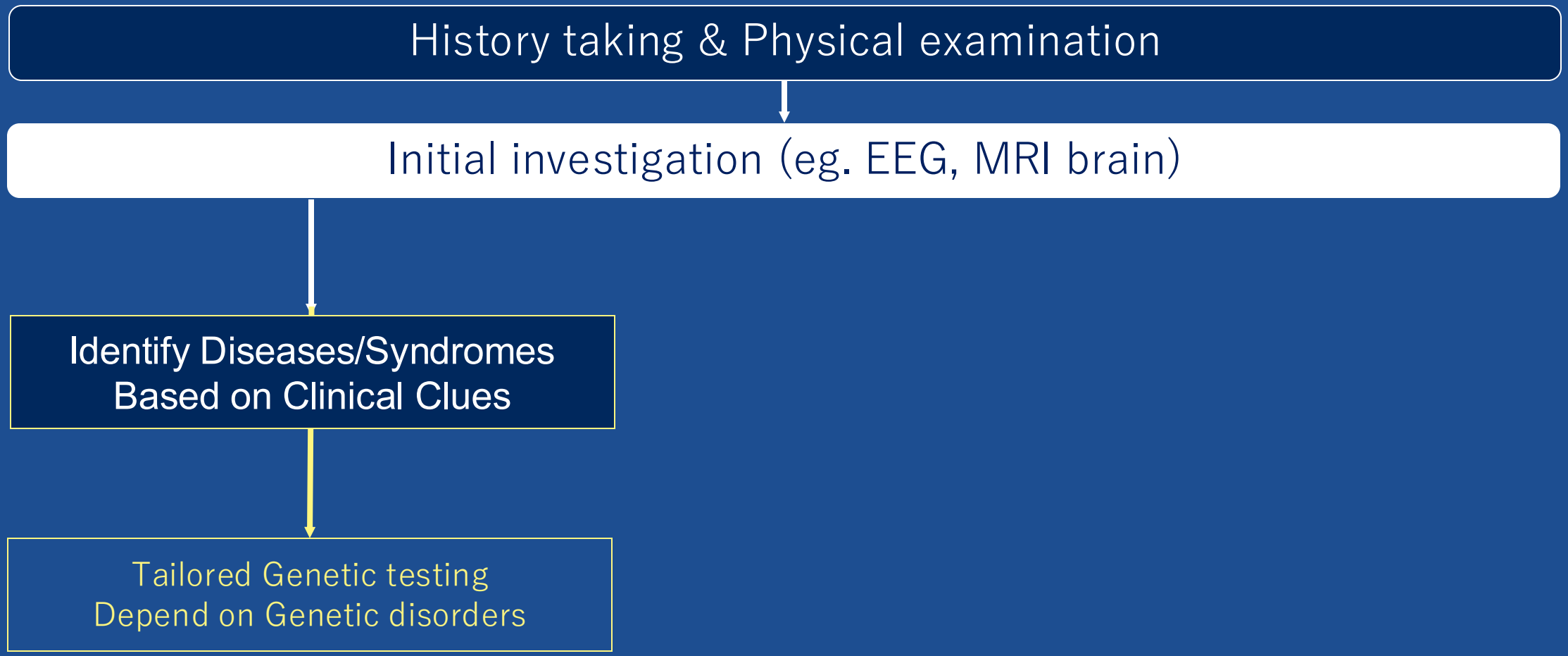
Approach


History taking & Physical examination
Initial investigation (eg. EEG, MRI brain)
Identify Diseases/Syndromes
Based on Clinical Clues
Tailored Genetic testing
Depend on Genetic disorders

Wolf–Hirschhorn syndrome

- 4p Deletion
- Onset: 6 to 12 months of age
- Seizure type: GTC, tonic spasm, focal seizure
- Facial dysmorphism
- Triggered by fever
- Developmental delay
- Heart defect, Hearing defect
- Recommended ASMs: CLB, LEV
- ASMs to Avoid: PHT, CBZ, OXZ

Justyna P, et al. European Journal of Epilepsy 116 (2024) 14–23

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- Four-year-old girl presented with developmental regression, hand wringing movement and progressive microcephaly

MECP2 sequencing: negative

Suspected disease	Molecular pathology	Genetic testing
Rett syndrome	Monogenic disorder	Single testing vs gene panel

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What causes a negative result?

1. Limitations of single-gene sequencing:

Unable to detect copy number variation (CNV) disorders

Genetic abnormalities in *MECP2*

Gene	Method	Proportion of Pathogenic Variants
MECP2	Sequence analysis	90%-95%
	Gene-targeted deletion/duplication analysis	5%-10%

https://www.ncbi.nlm.nih.gov/books/NBK1497/

MECP2, Deletion (Exon 4), heterozygous (gene panel)

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What causes a negative result?

2. Alternative diagnosis (Rett-like syndrome)

- Angelman syndrome

- Other genetic disorders

such as FOXG1, CDKL5, GABBR2, PPT1

Case 3

- 12-year-old boy
- Presented with GT seizure at 4 months related with fever.
- He had recurrent/prolong febrile seizure.
- Other seizure type: focal seizure, myoclonic seizure
- U/D: Developmental delay
- MRI brain: normal
- Drug resistance

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Dravet syndrome

- Loss of function in *SCN1A* gene (65-80% of patients)
- Onset: 4-7 months (mean 5 months)
- Seizure
  - Febrile seizures, prolong or cluster
  - Multiple type seizures (GT, hemiclonic Sz, myoclonic seizure)
- Triggering Factors: Fever, Hot bath, Vaccine, Sodium channel blocker
- Developmental delay
- Drug resistance

S Viravan, et al. JHSMR. July 2021

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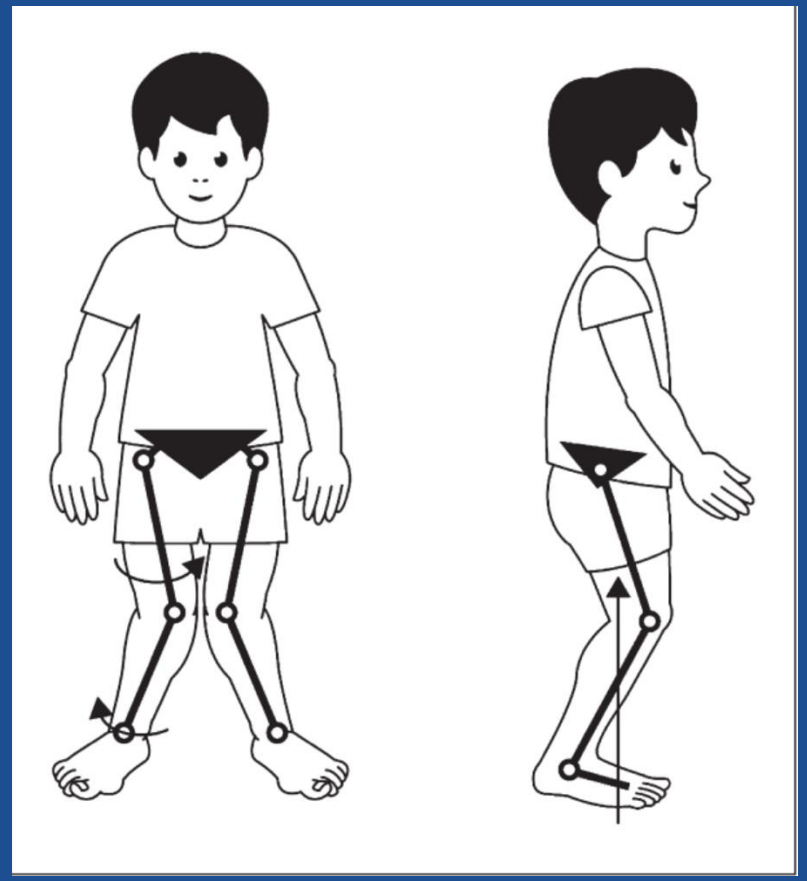
Crouch gait



ARCH NEUROL/VOL 69 (NO. 7), JULY 2012

Approach

History taking & Physical examination

Key Findings

Pattern recognition

Presumptive diagnosis

Tailored Genetic testing
Depend on Genetic disorders

Stepwise recognition

Differential diagnosis
Initial Investigation

Non-genetic disease
Genetic disease
- Common in rare diseases
- Treatable diseases

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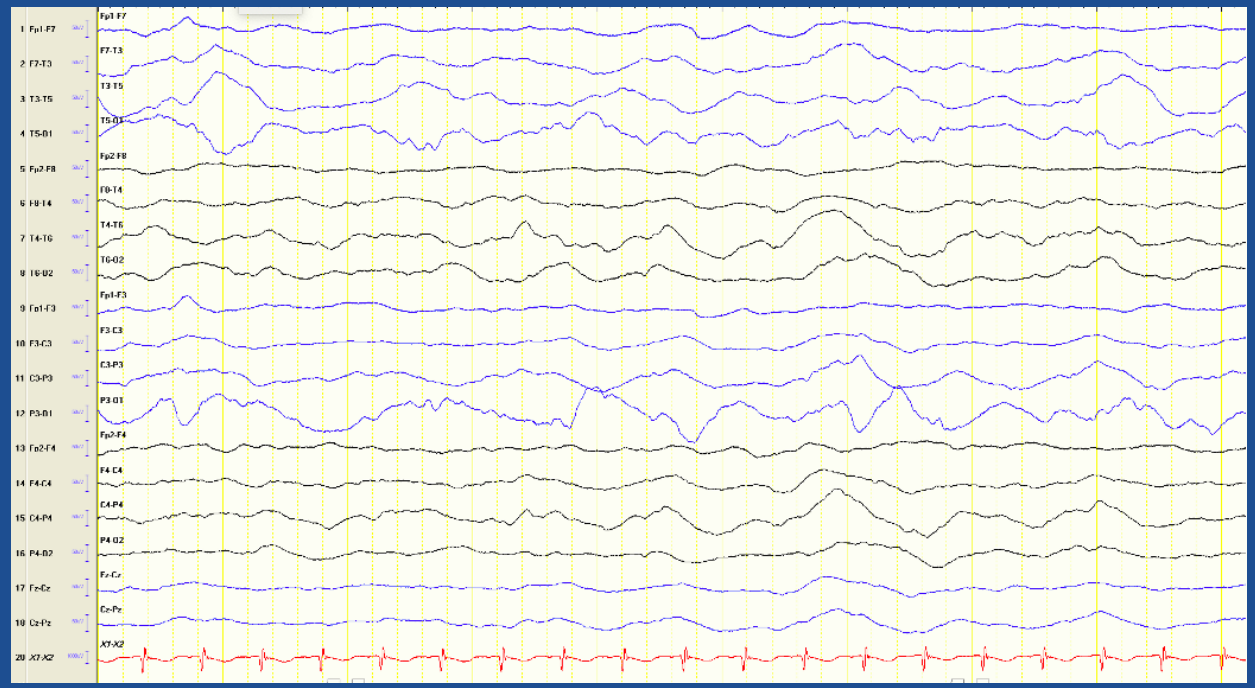
Case 4

- 3-month-old girl
- Presented with refractory status epilepticus 2 day ago
- Failed ASM: PHB, LEV, MDZ, Pentobarbital
- U/D: Developmental delay
- CT brain: Mild hydrocephalus

After pyridoxine treatment



Suspected disease

Pyridoxine dependent epilepsy

Molecular pathology

ALDH7A1 gene

Genetic testing

Gene panel

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Who should get genetic testing?

- Unexplained refractory epilepsies
- Neonatal with epileptic encephalopathies (up to 83%)
- Children with onset < 3 years (37%)
- Epilepsy with developmental delay or regression
- Suspicious genetic epileptic syndrome
  - EIEE, Dravet syndrome, familial self-limited epileptic syndrome, DEE
- Features suggest a genetic syndrome eg. dysmorphic facies

Epilepsia. 2021 Jan;62(1):143-151
Epilepsia. 2022 Feb;63(2):375-387
JAMA Netw Open. 2023;6(7)

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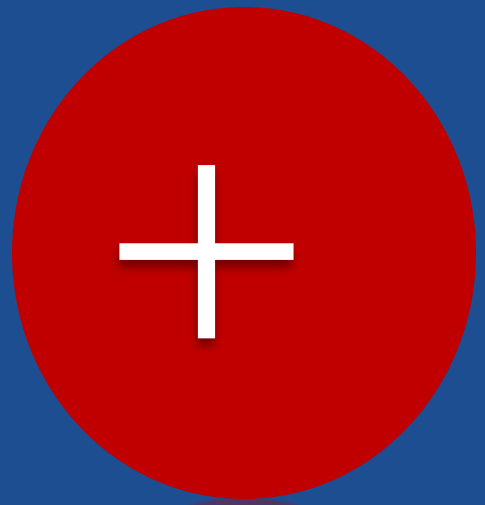
Managing Genetic Test Results

Negative

Positive



VUS

Variant of
uncertain significant

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How to Handle a Positive Result

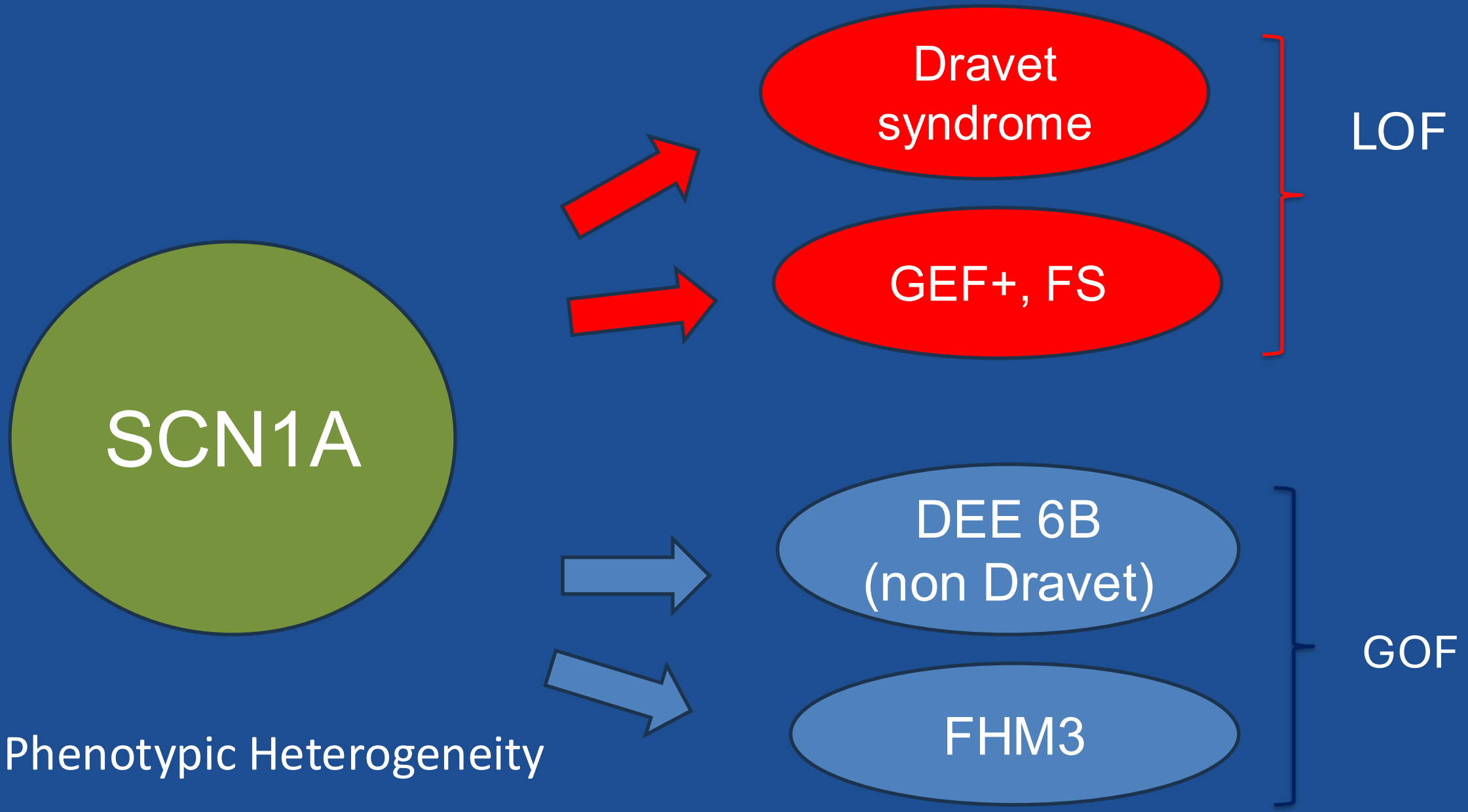
RESULT: POSITIVE

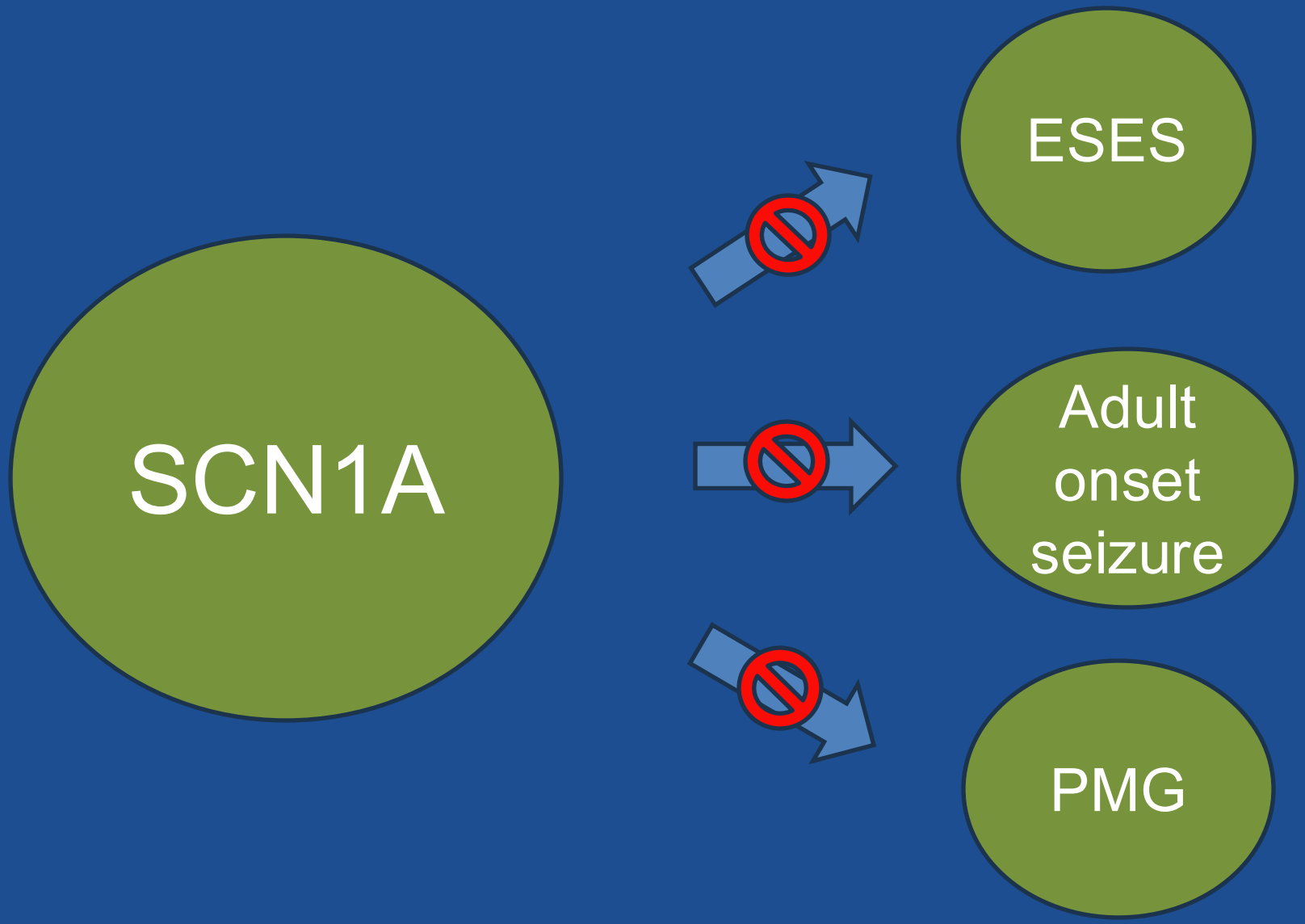
One Pathogenic variant identified in SCN1A. SCN1A is associated with a spectrum of autosomal dominant seizure and other neurological and musculoskeletal conditions.

Additional Variant(s) of Uncertain Significance identified.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
SCN1A	c.680T>G (p.Ile227Ser)	heterozygous	PATHOGENIC

SCN1A ≠ Dravet syndrome


Dravet syndrome
GEF+, FS
LOF
SCN1A
DEE 6B (non Dravet)
FHM3
GOF
Phenotypic Heterogeneity


SCN1A
ESES
Adult onset seizure
PMG

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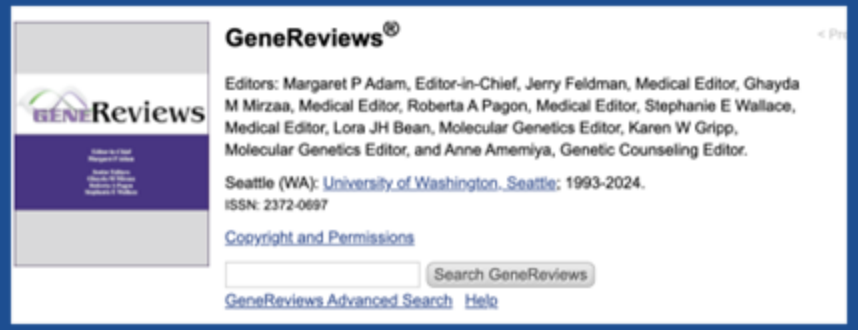
How to Handle a Positive Result

No need to memorize diseases; just know how to find the information



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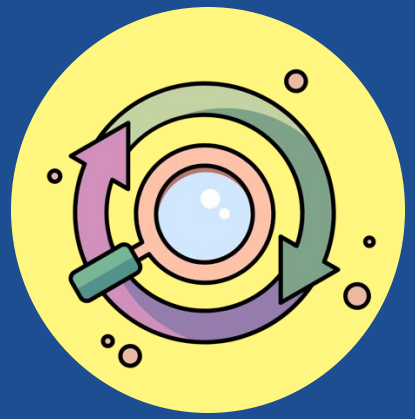
How to Handle a Positive Result

Personalized medicine

- Dravet syndrome:
  - avoid Na channel blocker, Antisense oligonucleotide
- SCN8A or SCN2A related DEE (GOF):
  - Sodium channel blocker, Relutrigine (PRAX-562)



Surveillance

- Rett syndrome
  - QT prolong (18-55%)

Genetic counseling and family planning

- Prenatal diagnosis
- Preimplantation diagnosis
- Carrier testing
- Advice prognosis

How to Handle a Negative Result

- Review test limitations
- Consider alternative diagnoses
- Reevaluate the patient's condition
  - Some phenotypes may emerge with age
  - Ongoing discovery of new genes and conditions
  - Updates in genetic variant data

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Acknowledgement







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