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Past, Present, and Future: The Ever-Changing Genetic Landscape of DEE

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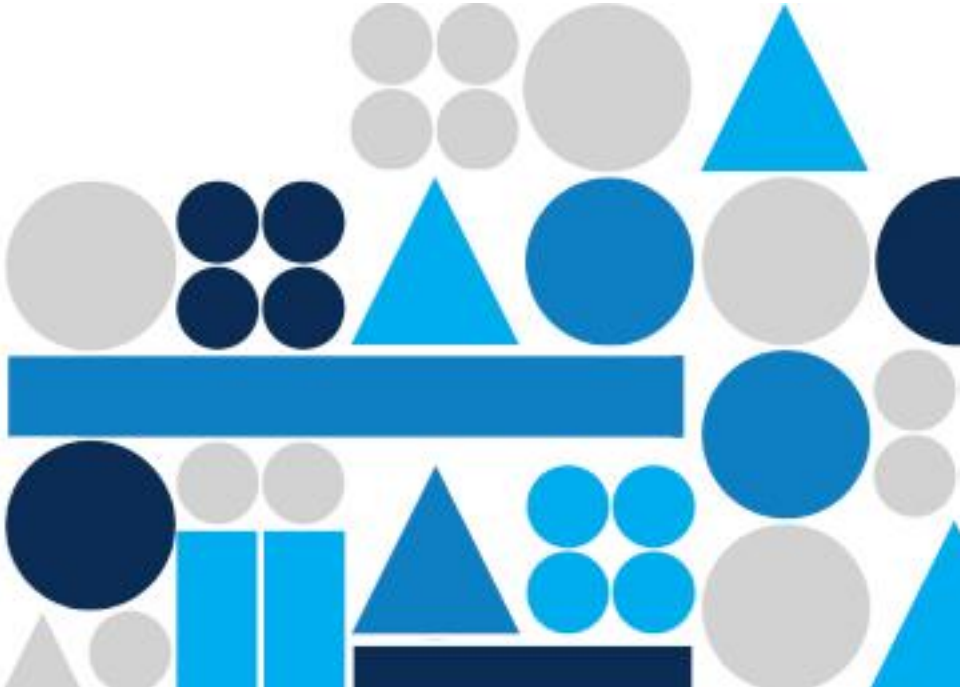

Disclosures:

Katie Angione, MS, CGC

No financial interests or relationships to disclose

Dianalee McKnight, PhD, FACMG

I am an employee and shareholder of Invitae

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Objectives:

1. Review how our knowledge of genetics in DEE has evolved over time
2. Discuss why genetic testing is important
3. Review different types of genetic testing
4. Review how to read a genetic testing report
5. Understand the difference between benign, pathogenic, and uncertain variants, and learn how variants are classified and reclassified

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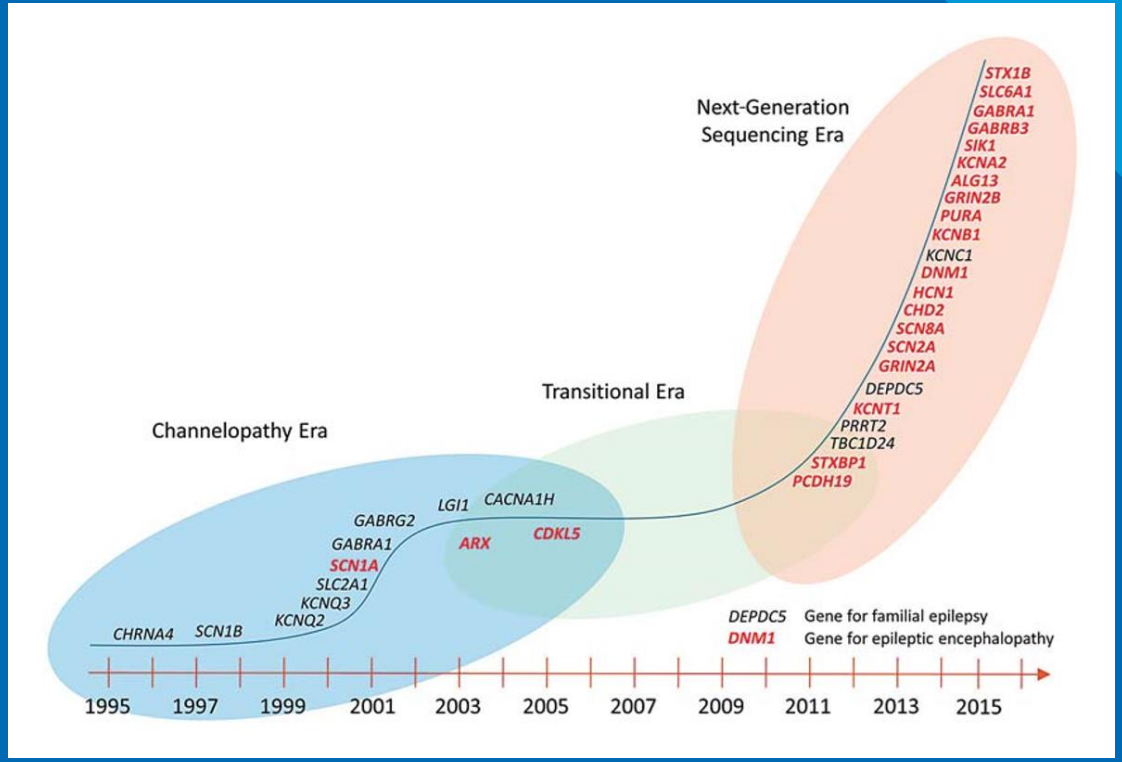
Genetics in DEE

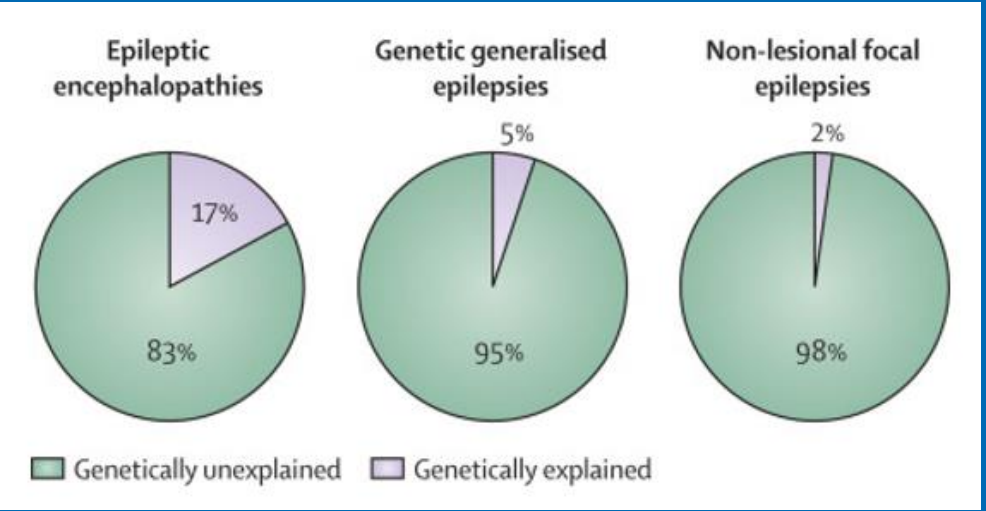
- Improvement in genetic testing methodology and technology has allowed us to identify more causative genes
- More widespread access to testing has expanded the known spectrum of presentation in many genetic disorders



Helbig & Abou Tayoun 2016

Genetics in DEE

- Diagnostic yield is higher for patients with DEE compared with other types of epilepsy (and highest in patients with infantile onset), but we are still unable to identify a genetic cause in the majority of patients



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EpiPM Consortium 2015

Genetics in DEE

- Testing is always improving - a genetic test done today can give you more information than the “same” test a few years ago would have
- Researchers, genetic testing laboratories, and family advocacy groups are continuing to push gene discovery forward and to expand our understanding of known genes

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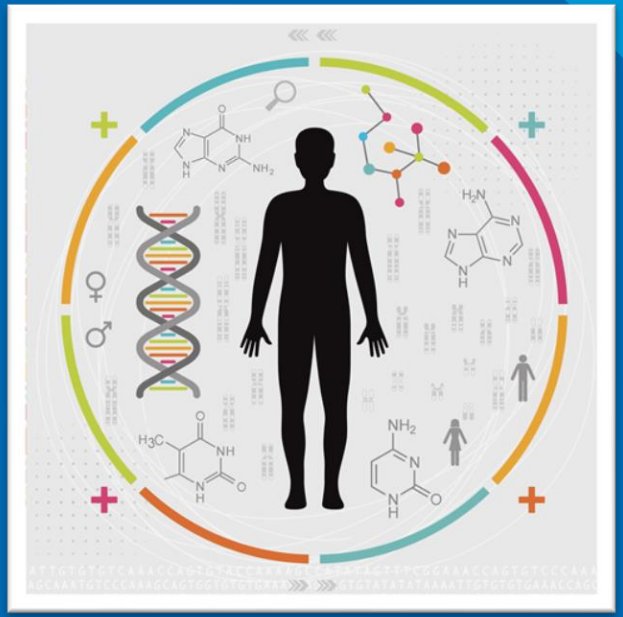
Why is genetic testing important?

- Recurrence
  - Can this happen again?
  - Are siblings at risk?
- Treatment
  - Is there a medication that will work better?
  - Are there any medications we should avoid?
  - Are there targeted therapies (gene therapy)?
  - Are there ongoing clinical trials or natural history studies?



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Why is genetic testing important?

- Prognosis
  - Will the seizures improve? Will they get worse?
  - What milestones can we hope for?
  - Do we need to be looking out for anything else?
  - Genotype/phenotype correlation
- Diagnostic Odyssey
  - Why did this happen?
  - Ease of anxiety, guilt, uncertainty
  - Connections

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Comparison of different sequencing methods

Single Gene	Panel	Exome/Genome
Clinical diagnosis is highly specific to a specific gene/variant	Clinically-indicated panel matches phenotype of the patient Test many genes at one time	Patient who has undergone a diagnostic odyssey with no answer Patients with large regions of homozygosity for which the differential is broad Patients with complex clinical presentations or multiple diagnoses

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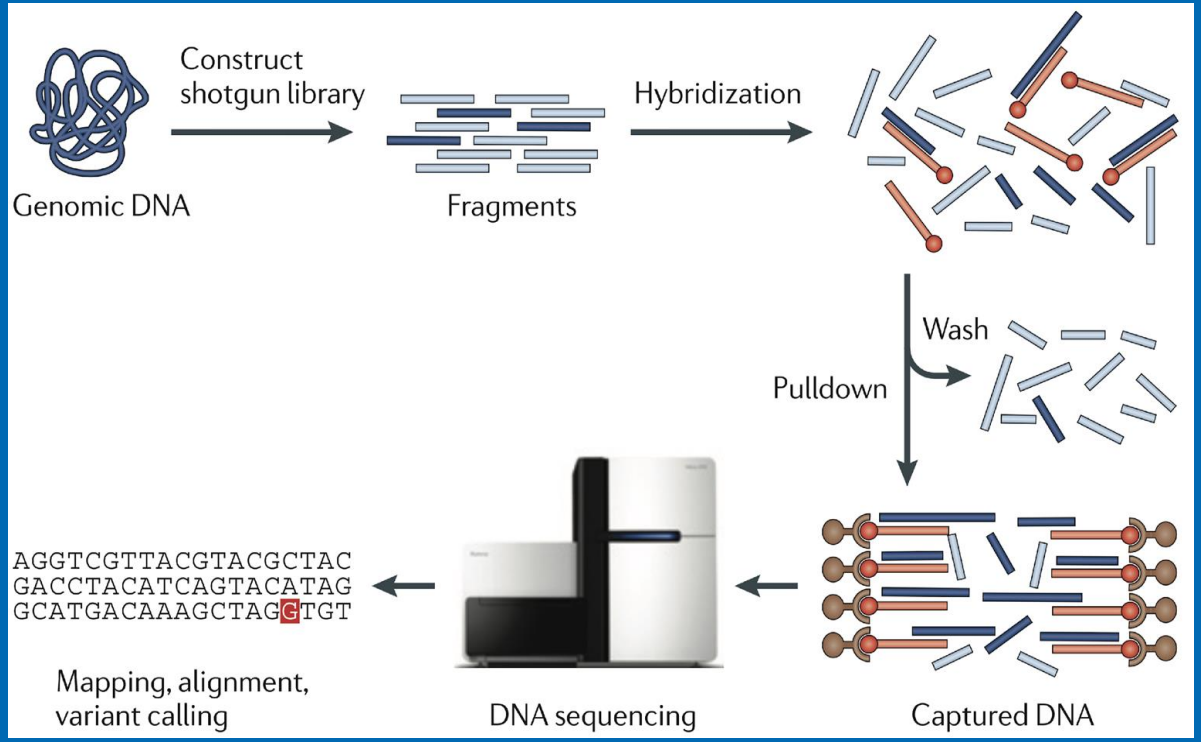
Next-Generation Sequencing

- Highly automated and quality-controlled next-generation sequencing coupled tightly with custom bioinformatics algorithms



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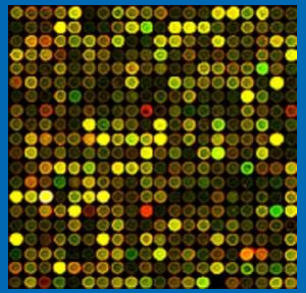
Evolution of deletion/duplication methods

- Classical cytogenetics
  - Microscopes and banding dyes
- Molecular cytogenetics
  - Fluorescence microscopy (FISH)
- Automatable multiplex targeted assays
  - qPCR, MIP and MLPA
- Microarrays
  - Array comparative genomic hybridization (aCGH)
  - High-resolution SNP arrays
- Next-generation sequencing (NGS)

1980s

Today



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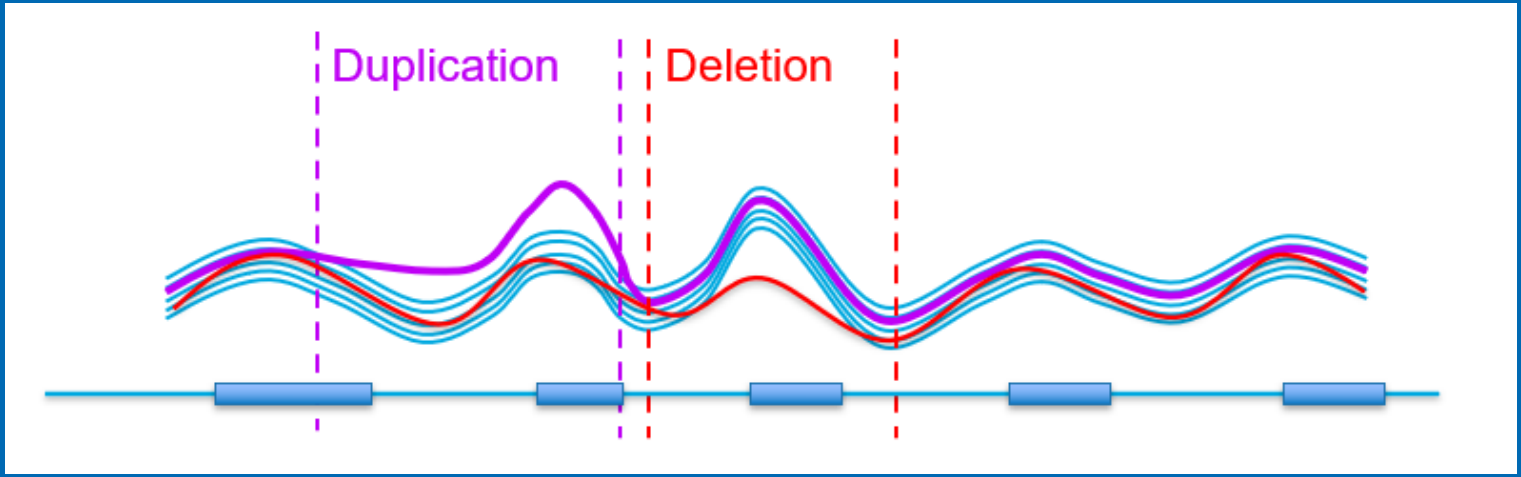
Copy number detection by NGS

Since depth profile is non-uniform but reproducible



Look for deviations with respect to *baseline samples.*

Perform this evaluation at the assay level to be able to detect del/dups down to single exon resolution across the panel.

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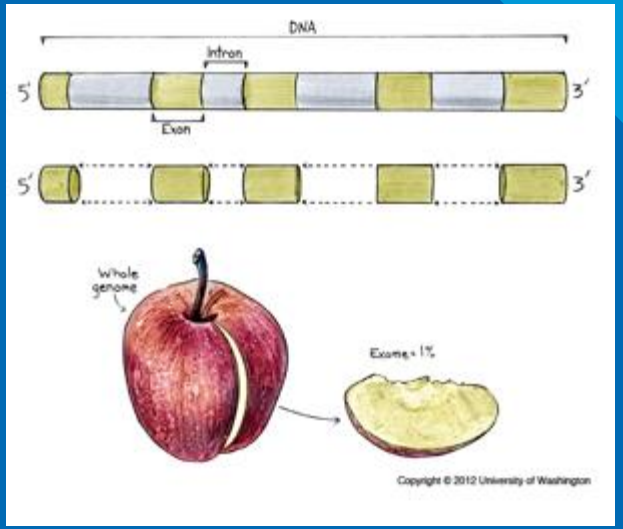
Exome (WES) vs. Genome (WGS)

- WES interrogates ~1.5% of the genome which contains protein coding sequences (exons)
  - Interrogates most exons in most coding genes in our genome (~250,000 exons and ~ 20,000 genes)
  - Capture-based NGS
  - Lower coverage than a panel
- WGS interrogates most of the genome
  - 6 billion base pairs
  - Lower coverage than an exome
  - Mainly done in a research setting but there is growing clinical use (i.e. Rady's NICU WGS)



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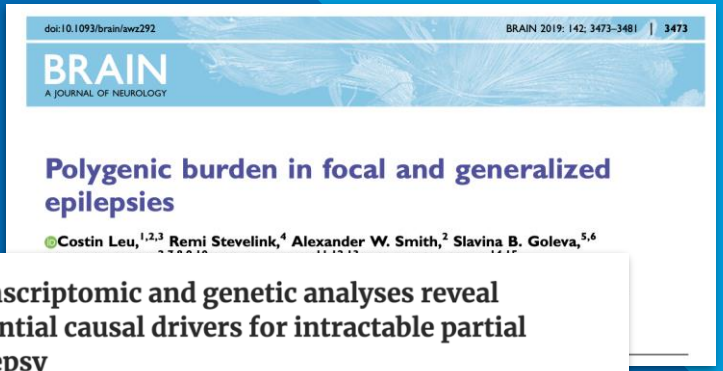
Future Diagnostics…

→ Polygenic Risk Scores and/or Modifier Genes

→ Omics

- Transcriptome
  - Sequence all messenger RNA
- Proteome
  - Study all proteins
- Metabolome
  - Study all metabolites



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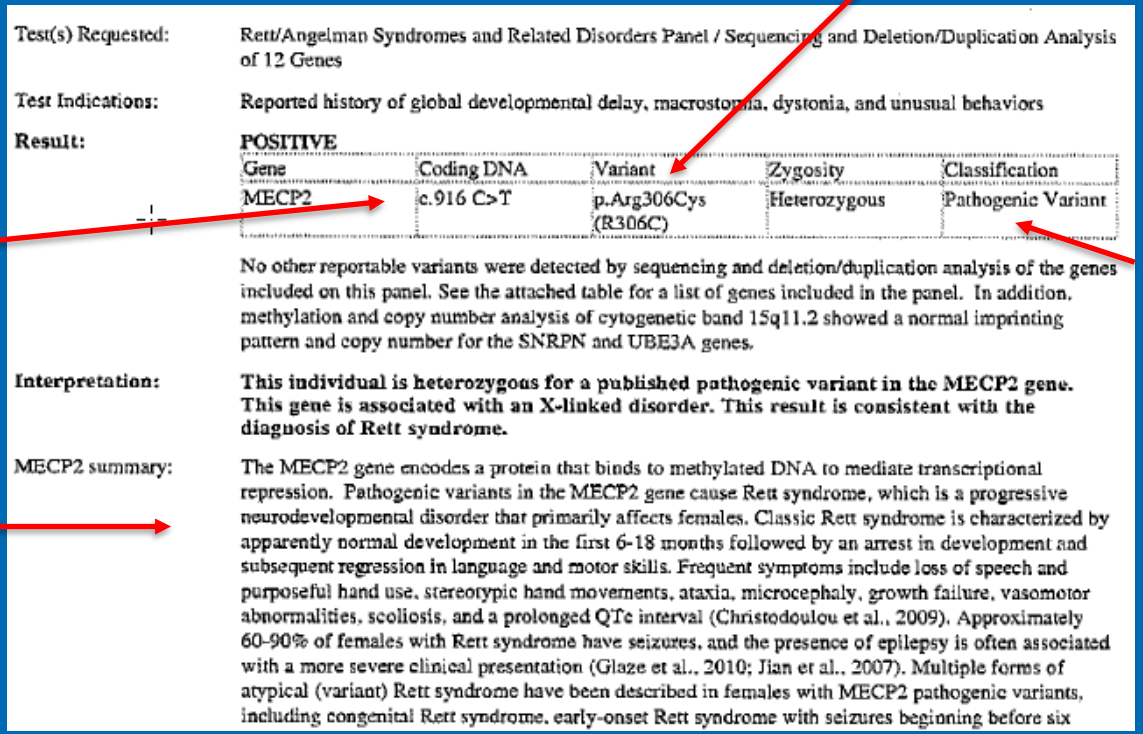
Example Test Report

change in the amino acid (p= protein)

change in the DNA (c = coding)

disease-causing

information about the disorder



Test(s) Requested: Rett/Angelman Syndromes and Related Disorders Panel / Sequencing and Deletion/Duplication Analysis of 12 Genes

Test Indications: Reported history of global developmental delay, macrostomia, dystonia, and unusual behaviors

Result: **POSITIVE**

Gene	Coding DNA	Variant	Zygosity	Classification
MECP2	c.916 C>T	p.Arg306Cys (R306C)	Heterozygous	Pathogenic Variant

No other reportable variants were detected by sequencing and deletion/duplication analysis of the genes included on this panel. See the attached table for a list of genes included in the panel. In addition, methylation and copy number analysis of cytogenetic band 15q11.2 showed a normal imprinting pattern and copy number for the SNRPN and UBE3A genes.

Interpretation: **This individual is heterozygous for a published pathogenic variant in the MECP2 gene. This gene is associated with an X-linked disorder. This result is consistent with the diagnosis of Rett syndrome.**

MECP2 summary: The MECP2 gene encodes a protein that binds to methylated DNA to mediate transcriptional repression. Pathogenic variants in the MECP2 gene cause Rett syndrome, which is a progressive neurodevelopmental disorder that primarily affects females. Classic Rett syndrome is characterized by apparently normal development in the first 6-18 months followed by an arrest in development and subsequent regression in language and motor skills. Frequent symptoms include loss of speech and purposeful hand use, stereotypic hand movements, ataxia, microcephaly, growth failure, vasomotor abnormalities, scoliosis, and a prolonged QTc interval (Christodoulou et al., 2009). Approximately 60-90% of females with Rett syndrome have seizures, and the presence of epilepsy is often associated with a more severe clinical presentation (Glaze et al., 2010; Jian et al., 2007). Multiple forms of atypical (variant) Rett syndrome have been described in females with MECP2 pathogenic variants, including congenital Rett syndrome, early-onset Rett syndrome with seizures beginning before six

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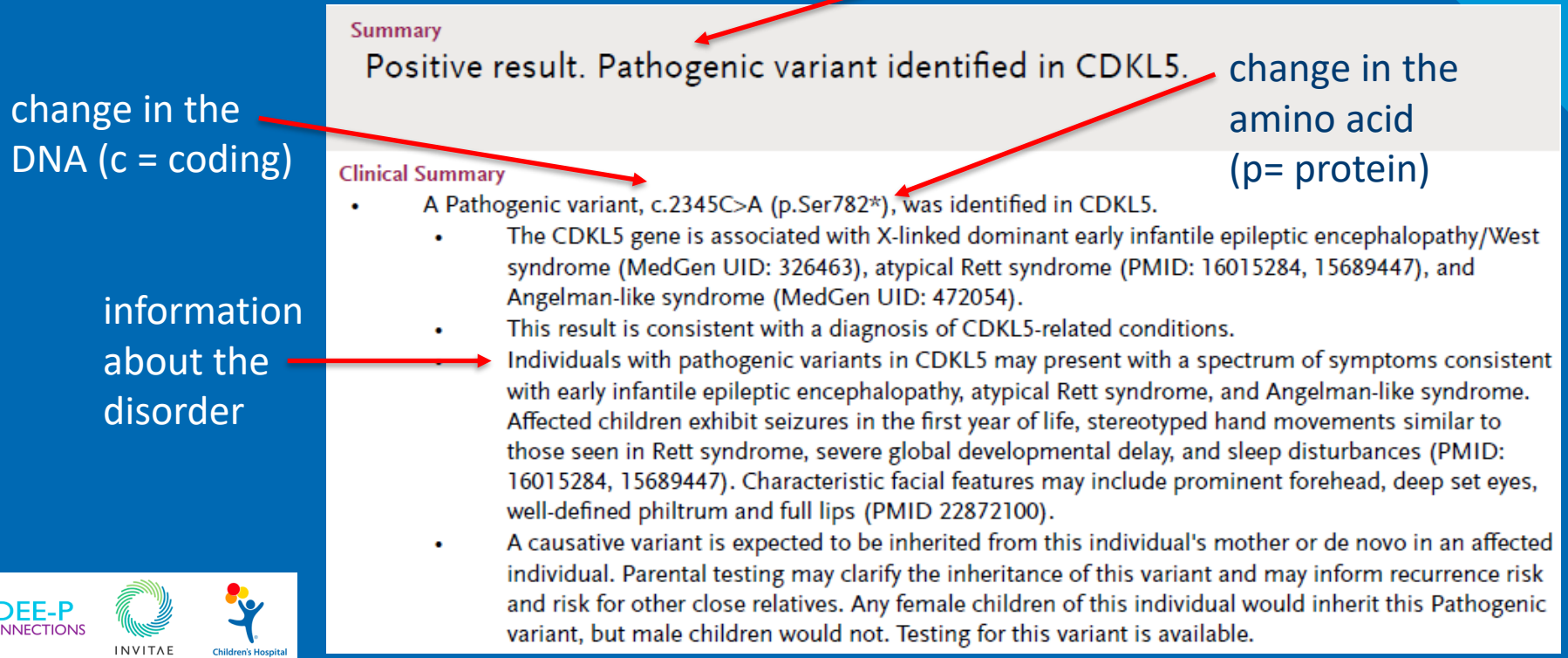
Example Test Report



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Not every test report is straightforward…

Summary

Variants of Uncertain Significance identified in ALG13, CACNA2D2, DOCK7, FLNA, MBD5, PIGQ, PNKD, RYR3, SLC13A5 and SPTAN1.

Clinical Summary

- A Variant of Uncertain Significance, c.2798_2799insACCTCC (p.Pro944_Pro945dup), was identified in ALG13.
  - The ALG13 gene is associated with the X-linked congenital disorder of glycosylation ALG13-CDG (CDG-Is) (MedGen UID 763818) and early infantile epileptic encephalopathy (EIEE) (MedGen UID: 763818).
  - The clinical significance of this result is uncertain. Until this uncertainty can be resolved, caution should be exercised before using this result to inform clinical management decisions.
  - Familial VUS testing is not offered. Testing family members for this variant will not contribute evidence to allow variant reclassification. Details on our VUS Resolution and Family Variant Testing Programs can be found at https://www.invitae.com/en/family/.
- A Variant of Uncertain Significance, c.525T>G (p.Ser175Arg), was identified in CACNA2D2.
  - The CACNA2D2 gene is associated with autosomal recessive early infantile epileptic encephalopathy (PMID: 24358150, 23339110).
  - The clinical significance of this result is uncertain. Until this uncertainty can be resolved, caution should be exercised before using this result to inform clinical management decisions.
  - Familial VUS testing is not offered. Testing family members for this variant will not contribute evidence to allow variant reclassification. Details on our VUS Resolution and Family Variant Testing Programs can be found at https://www.invitae.com/en/family/.
- A Variant of Uncertain Significance, c.2203C>G (p.Pro735Ala), was identified in DOCK7.
  - The DOCK7 gene is associated with autosomal recessive early infantile epileptic encephalopathy (EIEE) 23 (MedGen UID: 862929).
  - The clinical significance of this result is uncertain. Until this uncertainty can be resolved, caution should be exercised before using this result to inform clinical management decisions.
  - Familial VUS testing is not offered. Testing family members for this variant will not contribute evidence to allow variant reclassification. Details on our VUS Resolution and Family Variant Testing Programs can be found at https://www.invitae.com/en/family/.

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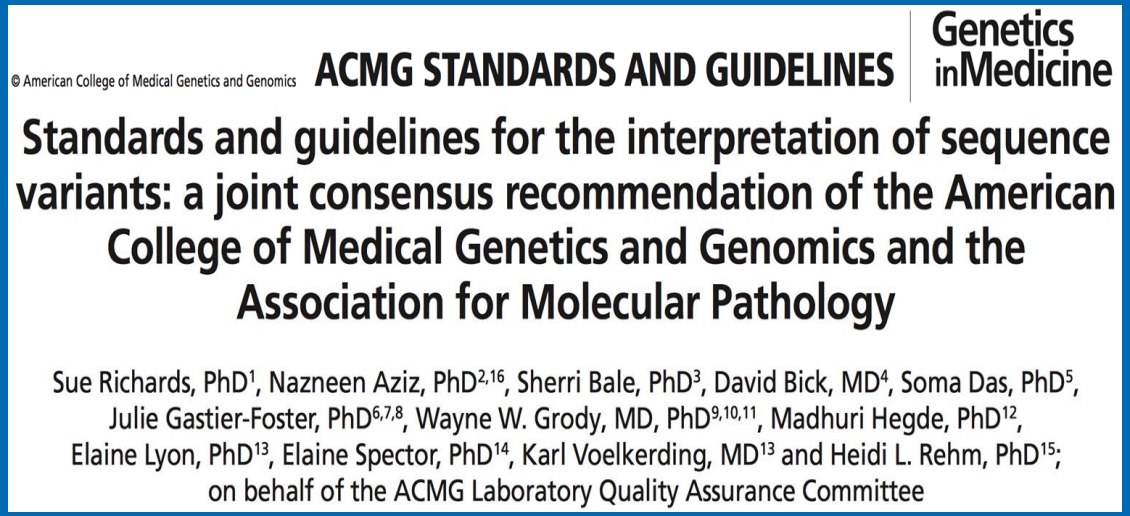
Challenges in germline genetic variant interpretation

- Diagnostic clinical genetic testing typically adheres to variant interpretation guidelines from the American College of Medical Genetics and Genomics (ACMG).
- 2015 guidelines provide more guidance but are still not specific for many types of evidence.



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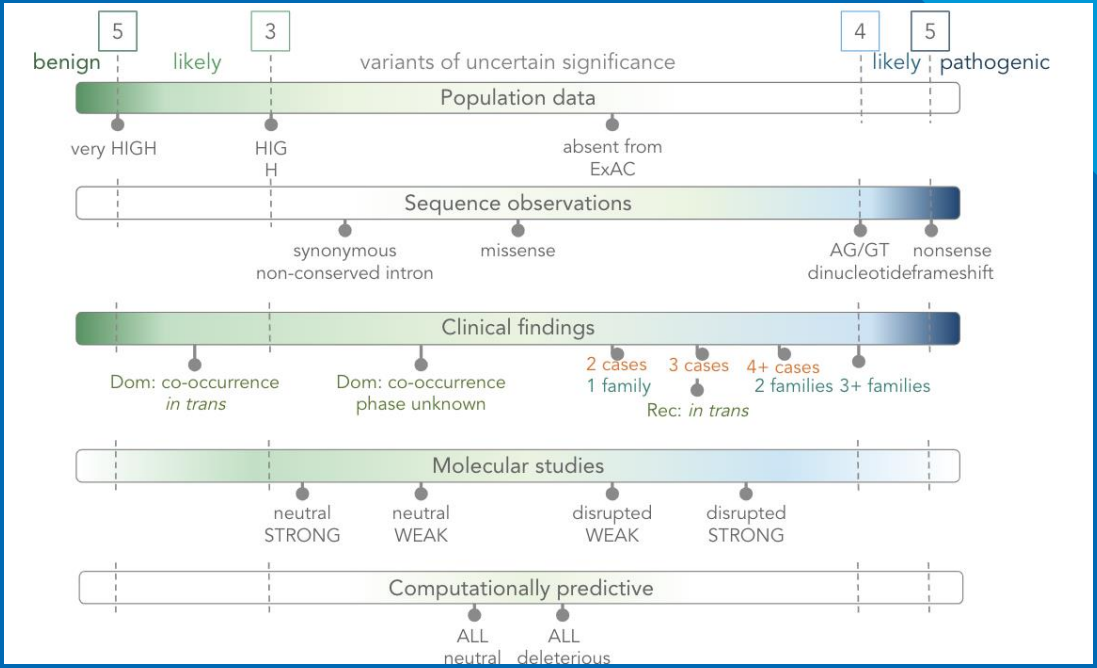
Categories of evidence in variant interpretation

Pathogenic (Path)
Likely Pathogenic (LPath)

Variant of Uncertain Significance (VUS)

Likely Benign (LBen)
Benign (Ben)



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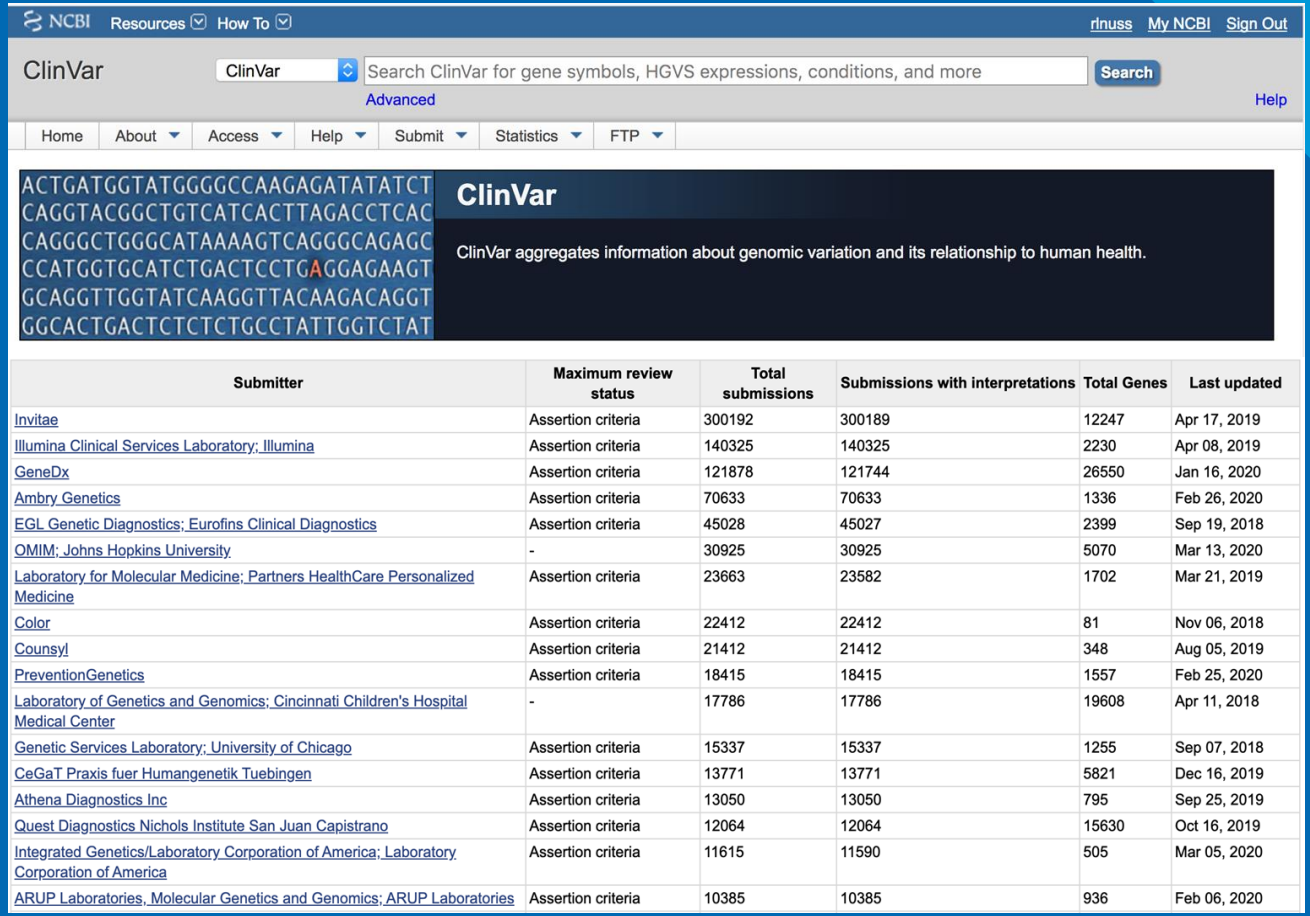
Data sharing among laboratories



Submitter	Maximum review status	Total submissions	Submissions with interpretations	Total Genes	Last updated
Invitae	Assertion criteria	300192	300189	12247	Apr 17, 2019
Illumina Clinical Services Laboratory; Illumina	Assertion criteria	140325	140325	2230	Apr 08, 2019
GeneDx	Assertion criteria	121878	121744	26550	Jan 16, 2020
Ambry Genetics	Assertion criteria	70633	70633	1336	Feb 26, 2020
EGL Genetic Diagnostics; Eurofins Clinical Diagnostics	Assertion criteria	45028	45027	2399	Sep 19, 2018
OMIM; Johns Hopkins University	-	30925	30925	5070	Mar 13, 2020
Laboratory for Molecular Medicine; Partners HealthCare Personalized Medicine	Assertion criteria	23663	23582	1702	Mar 21, 2019
Color	Assertion criteria	22412	22412	81	Nov 06, 2018
Counsyl	Assertion criteria	21412	21412	348	Aug 05, 2019
PreventionGenetics	Assertion criteria	18415	18415	1557	Feb 25, 2020
Laboratory of Genetics and Genomics; Cincinnati Children's Hospital Medical Center	-	17786	17786	19608	Apr 11, 2018
Genetic Services Laboratory; University of Chicago	Assertion criteria	15337	15337	1255	Sep 07, 2018
CeGaT Praxis fuer Humangenetik Tuebingen	Assertion criteria	13771	13771	5821	Dec 16, 2019
Athena Diagnostics Inc	Assertion criteria	13050	13050	795	Sep 25, 2019
Quest Diagnostics Nichols Institute San Juan Capistrano	Assertion criteria	12064	12064	15630	Oct 16, 2019
Integrated Genetics/Laboratory Corporation of America; Laboratory Corporation of America	Assertion criteria	11615	11590	505	Mar 05, 2020
ARUP Laboratories, Molecular Genetics and Genomics; ARUP Laboratories	Assertion criteria	10385	10385	936	Feb 06, 2020

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Next steps when a VUS is identified

- Test parents for phase
  - De novo, compound heterozygous
- Family testing
  - Does the variants segregate with other affected family members?
- Assess clinical correlation
  - Does the gene/variant type match with the patient's phenotype?
  - Is further phenotyping of the patient needed?
- Additional laboratory testing
  - Metabolic analysis
  - MRI

→ **Collaborative process between the provider and the lab to help with the interpretation of variants and unexpected results**

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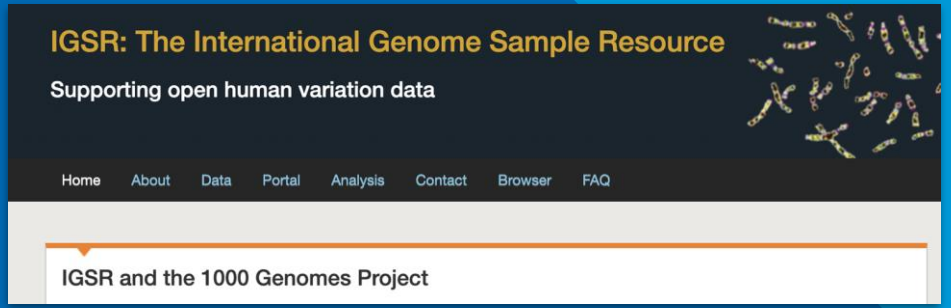
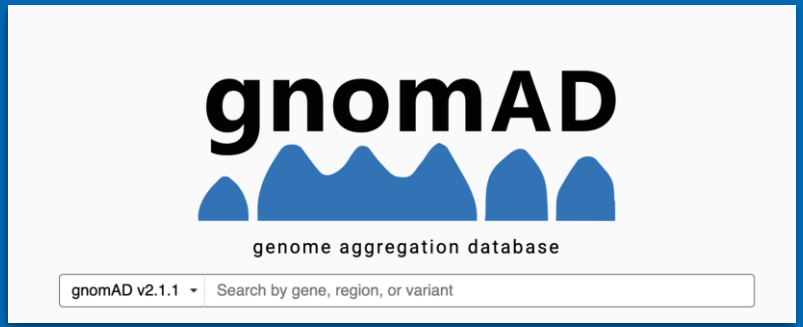
Evolution of large public databases

- **1000 Genomes (2010)**
  - First large public database of genome data from “control” individual
- **ExAC (2014)**
  - Second large public database
  - 60,000 exomes
- **gnomAD (2017)**
  - Current largest public database
  - v2: 125,748 exomes and 15,708 whole genomes
  - v3: 71,702 whole genomes





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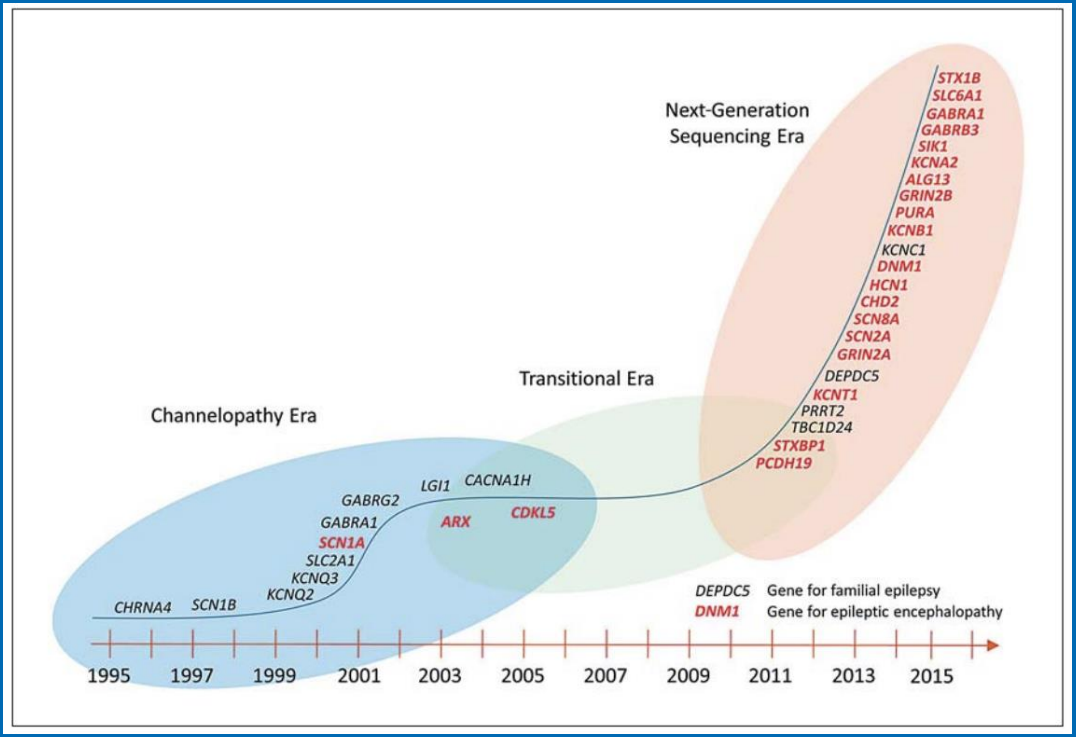
Gene Discovery



Molecular Syndromology

Review Article

Mol Syndromol 2016;7:172–181
DOI: 10.1159/000448530

Published online: August 20, 2016

Understanding Genotypes and Phenotypes in Epileptic Encephalopathies

Ingo Helbig[a, c] Ahmad N. Abou Tayoun[b]

Divisions of [a]Neurology and [b]Genomic Diagnostics, Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pa., USA; [c]Department of Neuropediatrics, Christian Albrechts University of Kiel and University Medical Center Schleswig-Holstein (UKSH), Kiel, Germany

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Impact of sponsored testing programs

CHALLENGE

Previously, it has not been common to look for the underlying genetic causes of epilepsy, resulting in delayed or incorrect diagnosis of affected individuals

APPROACH

The first-ever pediatric epilepsy sponsored testing program launched in 2016 with the goal of reducing time to diagnosis.[1]

- Invitae's comprehensive epilepsy panel is available at no charge to patients
- Carefully selected clinical criteria were used to determine eligibility for the program

IMPACT

Average age of disease diagnosis within the program has been reduced by 1-2 years and continues to improve.

EVOLUTION

Additional partners continue to join the program and patient eligibility continues to expand.

1.Miller N, Truty R, Bailey M, *et al.* Behind The Seizure™: A no-cost, 125-gene epilepsy panel for pediatric seizure onset between 2–4 years. Poster presented at the ACMG Annual Clinical Genetics Meeting; April 10–14, 2018; Charlotte, NC.

Summary:

- Genetic testing is continually evolving and improving
- A genetic diagnosis can help families to understand, prepare, learn, and connect
- Genetic testing is not one-size-fits-all! Work with your child's team to determine what testing makes sense for your family
- There is value in repeating and reanalyzing inconclusive results, and in revisiting a diagnosis over time

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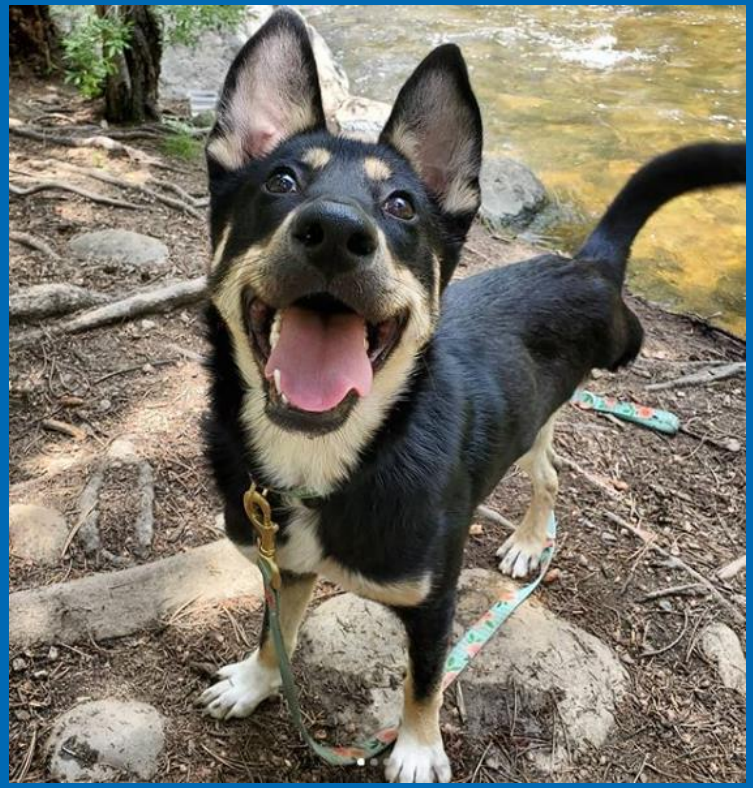
Questions?



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Thank you for joining us!