Genetic Testing in Epilepsy in practice: an exercise in selection, interpretation, and dealing with pitfalls.

Epilepsy Genetics Lecture
Michael J. Bresnan Child Neurology Course
September 2020

Christelle Moufawad El Achkar, MD
Epilepsy Genetics Attending
Boston Children's Hospital
Christelle.achkar@childrens.Harvard.edu
epilepsygenetics@childrens.Harvard.edu

Disclosures

- Nothing to disclose.

Give the following some thought

In your opinion, genetic testing is:

-Mostly academic, research driven, and rarely influences clinical decision

-Should be done on everyone with suspected genetic disorder

-Can have a lot of clinical benefits when used judiciously

-Has a high cost to benefit ratio, and thus is not worth it

-I am not sure, I will wait until the end of this lecture and make up my mind

The Clinical Value of Genetic Testing

- Choice or avoidance of certain treatments → **Precision Medicine**
- Better understanding of mechanisms→ **Drug Discovery**
- Knowledge of clinical spectrum → **Screening of other systems, range or possible outcomes**
- Ending a long, frequently invasive diagnostic journey → **More definitive answers**
- Family counseling → **Decrease risk of recurrence, family planning**
- Answers a very common question by parents: **Did I Do Something Wrong?**

Precision Medicine: relatively more studied examples

SCN1A	Avoid sodium channel blockers, indication for CBD and fenfluramine, several drug trials
SCN2A, SCN8A	Good response to phenobarb, phenytoin, sodium channel blockers
ALDH7A1, PNPO	Good response to P5P/pyridoxine
SLC2A1	Ketogenic Diet
PRRT2	Response to carbamazepine/oxcarbazepine
GRIN	Possible response to NMDA receptor antagonists -if gain of function (e.g.memantine)
KCNQ2	Targeted treatment with retigabine (discontinued, but other trials in preparation)
KCNT1	Trials of quinidine in EIFMS and ADNFLE (mixed response)
CLN2	Cerliponase alpha (Brineura) enzyme replacement intrathecal therapy, early therapy is key.

What is the optimal genetic test in 2020?

There still isn't one…. Several factors to consider

- Patient presentation
- Family history
- Clinical indication
- Benefit v/s disadvantage
- Cost effectiveness
- What does the test look for, and what does it miss
- Turn around time
- Availability of high quality counseling

Clinically Available Tests- 2020

- Karyotype
- Methylation studies
- FISH analysis
- Chromosomal microarray
- Single gene testing- sequencing, deletion and duplication
- Gene panels (syndrome or symptom specific), with or without deletion and duplication
- Large comprehensive panel (e.g. epilepsy panels, brain malformations, etc)
- Deep sequencing panels (focused on brain malformations or when mosaicism suspected)
- Mitochondrial genome sequencing
- Whole exome sequencing
- Whole genome sequencing (mostly research but clinical to some extent)
- RNA sequencing

Where to send the test: what does the lab offer?

- -Is the technology accurate? Sensitivity, specificity?
- -Does the lab also offer deletion/duplication testing
- -Does the lab have the appropriate panel for the patient's symptoms
- -Is Trio testing offered
- -Turnaround Time (especially in neonates/infants/acute situations)
- -Cost and insurance coverage
- -Hospital preferences/policies (which are typically assessed based on above factors)

Who to test: some suggestions- constantly changing

Well controlled, "benign" childhood epilepsy, e.g. JME, CAE, JAE, BRE, etc	No clinical indication for genetic testing but changing with time. Consider research enrolment.
Intractable epilepsy, generalized or multifocal, no other associated features	Panel, WES, CMA, in any order,
Focal epilepsy with negative MRI or FCD on MRI	Specific epilepsy panels for focal epilepsy- to include DEPDC5, NPRL2/3, KCNT1, etc. LOW YIELD, or WES. Might need tissue.
Epilepsy with other brain malformation (PMG, lissencephaly, micro/macrocephaly, etc)	Malformation specific panels, might need deep sequencing (such as overgrowth panels). Might need tissue.
Epilepsy + developmental delay or ASD	Panel, WES, CMA, in any order Fragile X- continued debate
Epilepsy + other neurological features (movement disorder, ataxia, hemiplegic migraine, myopathy)	WES and comprehensive epilepsy panel, in any order
Epilepsy + prominent dysmorphic features	Start with CMA, or CMA+WES

Do I start with WES or an epilepsy panel?

- WES is more comprehensive (looking at ~20-25K genes), while panels contain 100 to a few 100 genes
- WES takes 8-12 weeks, panels take 6-8 weeks
- WES involves trio testing from the get go, with panels it's a 2 step process
- Panels cost less than WES, but some insurance companies prefer one over the other
- WES will miss deletion/duplication, while most panels include del/dup
- WES is more likely to result in incidental findings than panels

Do I start with WES/Panel, or CMA?

- CMA tests for larger deletions/duplications (rarely picked up on WES and panels, depending on size and technology)
- Reasonable choice if prominent dysmorphic features
- TAT 4-6 weeks
- Currently favoring CMA testing sensitive enough to detect exon-level deletion/duplication
- Can do CMA and WES/Panel simultaneously

Clinical Illustration 1: Reasons for testing

16 y.o young man, presenting for the first time in our clinic, with intractable epilepsy, intellectual disability, abnormal gait.

His parents bring him for a second opinion regarding management and to consider the possibility of surgery.

1st seizure: 6 months of age, with left arm twitching and post-ictal paresis

Seizure types progressed over time

Seizure types at 16y: staring spells, GTCs, eyelid fluttering, drop attacks, reports of myoclonic seizures on EEG

Extremely refractory

Previous therapies	Current therapies	Potential options
-carbamazepine	-topiramate	Other medications:
-phenobarbital	-lamotrigine	perapampanel
-oxcarbazepine	-clobazam	vigabatrin
-valproic acid	-rufinamide	Felbamate (not willing)
-zonisamide		
-levetiracetam		Procedures:
-phenytoin		Corpus callosotomy
-Ketogenic diet		
-VNS x2		
-CBD		
-CBD+THC		

Meanwhile, sent for genetic testing

- CMA: Negative
- Infantile epilepsy panel

Gene	Coding DNA	Variant	Zygosity	Classification
SCN1A	c.5299delG	p.Val1767SerfsX12 (V1767SfsX12)	Heterozygous	Likely Pathogenic Variant

UNCERTAIN CLINICAL SIGNIFICANCE

Gene	Coding DNA	Variant	Zygosity	Classification
PNKP	c.416 G>A	p.Arg139His (R139H)	Heterozygous	Variant of Uncertain Significance

- *SCN1A* Variant confirmed *de novo*
- Note it is a premature stop codon, resulting in a truncated protein
- *PNKP* is associated with an autosomal recessive disorder

Implications

- 16 years of epilepsy without a diagnosis or explanation
- SCN1A-related epilepsy and Dravet are relatively well studied
- There are guidelines on medications to avoid and medications to use
- Potential candidate for drug trial or novel drugs when/if FDA approved (Epidiolex, *Ataluren*, Fenfluramine)
- Counseling: increased risk of SUDEP
- Of course- corpus callosotomy is not off the table

Clinical illustration 2: Test Selection

- 10 month old girl, coming to the US for treatment
- Healthy until seizure onset at 4 months of age
- Semiology: right or left clonic
- Triggers: fever, illness, warm baths
- Duration: up to 30 minutes at times
- Medications upon presentation: levetiracetam, phenobarbital, carbamazepine. Medications had not helped
- Development: no regression, slight motor delays
- Family history: negative for neurological disorders
- Work up: normal MRI, EEG with normal background with generalized and multifocal spikes

Results of epilepsy panel with del/dup

Test(s) Requested: Infantile Epilepsy Panel / Sequencing and Deletion/Duplication Analysis of 75 Genes

Test Indications: Not provided.

Result: **UNCERTAIN CLINICAL SIGNIFICANCE**

Gene	Coding DNA	Variant	Zygosity	Classification
POLG	c.156 G>C	p.Gln52His (Q52H)	Heterozygous	Variant of Uncertain Significance

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 complete list of genes included on the panel.

More expanded gene panel with del dup

Sequence Variant Results

This section of the report contains clinically-relevant, dual-platform confirmed sequence variants in the provider-selected gene lists in the *Pediatric Neurology Region of Interest*.

#	Classification	Gene	Nucleotide	Amino Acid	Zygosity	Transcript	Gene Coverage*	Variant Inheritance
1	Pathogenic	*SCN1A*	c.2556+2T>C	n/a	Heterozygous	NM_006920.5	100%	*De novo*
2	Uncertain	*FLNA*	c.7769G>A	p.Arg2590Lys	Heterozygous	NM_001456.3	100%	Maternal
3	Uncertain	*GLDC*	c.190G>A	p.Ala64Thr	Heterozygous	NM_000170.2	95.6%	*De novo*
4	Uncertain	*POLG*	c.156G>C	p.Gln52His	Heterozygous	NM_002693.2	100%	Maternal

*Percentage of gene with >20x coverage per base pair

- Identify the pitfalls
- Clinical significance of this result
- How do you counsel the family?

- Identify the pitfalls- variant missed by first lab.

- Clinical significance of this result- Dravet spectrum/SCN1A related epilepsy: medication choice- avoid Na Channel blockers, consider VPA, CLB, Ketogenic diet, CBD, clinical expectations, early developmental support

- How do you counsel the family?

Risk of recurrence is mainly related to mosaicism (1-10%)

POLG variant and valproic acid- this particular variant not associated with hepatotoxicity

SUDEP risk

Clinical illustration 3: result interpretation

- 4 y.o girl with early developmental delays then clearer regression at 8 months of age
- First seizure at 2 months of age
- Semiology: wakes up scared, goes limp and unresponsive
- Never captured on EEG- but per primary neurologist who saw videos, events very stereotyped and concerning for seizures
- Family history negative
- Exam; HC 46.7cm (3-5%) alert, babbling, but not interactive. no dysmorphic features or abnormal movements, hypotonia
- Work up: negative CMA, WES as follows

WES results

Clinical Indication: Female with global developmental delay, developmental regression, hemiparesis, hypotonia, brain atrophy, failure to thrive, and dysphagia.

A sample from this individual's mother (GeneDx #1680209) and father (GeneDx #1680213) were also submitted for variant segregation analysis by whole exome sequencing.

1. Causative Variants in Disease Genes Associated with Reported Phenotype:

None identified. Whole exome sequencing did not identify any variants that can be interpreted at this time to definitively explain this patient's reported phenotype.

2. Variants in Genes Possibly Associated with Reported Phenotype:

None identified.

3. Candidate Genes with a Potential Relationship to a Disease Phenotype:

Gene	Disease	Mode of Inheritance	Variant	Coding DNA	Zygosity	Inherited From	Classification
RYR3	None Currently Described	Unknown	p.A2284T	c.6850 G>A	Heterozygous	Mother	Variant of Uncertain Significance
RYR3	None Currently Described	Unknown	p.I3104N	c.9311 T>A	Heterozygous	Father	Variant of Uncertain Significance

ACMG Secondary Findings: None identified.

mtDNA Test Results: Negative, no pathogenic variants were identified. See separate report for details.

- What do you make of those results?
- How do you proceed with it?

Premise:

You are not supposed to know anything about this gene. So start from scratch.

Steps

- Literature search
- Normal databases search (e.g. gnomAD, no homozygotes found with these variants, and given parents are carriers- this is not a heterozygous condition in this case)
- Pathogenic variant databases search (e.g. ClinVar: one variant present p.A2284T, VUS, heterozygous, no second variant reported, phenotype epileptic encephalopathy)➔ inconclusive
- Gene Matcher

Literature search

- For now candidate gene
- Encodes part of a ryanodine/calcium release channel
- Highly expressed in brain
- *De novo* variant recently associated with epileptic encephalopathy in literature (2 unrelated individuals)
- Compound heterozygous individuals found by the lab, but not reported in literature yet, with a range of neurodevelopmental disabilities, hypotonia, microcephaly, epileptic encephalopathy.

Response from Gene Matcher

- Several individuals with compound heterozygous variants with history of developmental delays, with or without regression, with or without epilepsy, some epileptic encephalopathies
- Patient's variants not found in that small database

- Discussion with family: this is probably the diagnosis but cannot confirm yet until more information about this gene/functional analysis/larger series

- Plan to re-analyze in a year, enrolled in research

But this was not the end of the story

- Months after the visit, we receive message from family wanting to follow up for “new diagnosis of Rett Syndrome”
- Whole genome had been sent locally under research and showed abnormality in the MEPC2 gene
- Clinical confirmation as follows
- Did we really need whole genome? What could we have done?

[redacted]

[redacted] / Male Progressive Neurodevelopmental Syndrome

Result: **POSITIVE: Heterozygous for a Partial Deletion of the MECP2 Gene.**

Targeted array CGH analysis with exon-level resolution (ExonArrayDx) indicates that this individual is heterozygous for a deletion including at least exons 3-4 of the MECP2 gene, based on the reference transcript and genomic sequence coordinates specified below.* A normal copy number was observed for the remaining exons that were evaluated.

Interpretation: This individual is heterozygous for a deletion of the 3' end of the MECP2 gene that includes at least exons 3-4. Partial deletions of the MECP2 gene including exons 3-4 have been reported numerous times in association with Rett syndrome (for examples see Archer et al., 2006; Scala et al., 2007; Erlandson et al., 2003; Fukuda et al., 2005). The presence of the deletion is consistent with the diagnosis of Rettt syndrome in this individual. As the 3' boundary of this deletion cannot be determined by this assay alone, the deleted interval may encompass additional gene(s).

Recommendation: 1. This result permits targeted testing for the deletion and prenatal diagnosis for at-risk family members, if desired. Genetic counseling is recommended.

Pitfalls: think about what the test misses

- Del/Dup is not done as part of the WES
- It was picked up on the WGS- although the usual epilepsy panel would have picked up the exon deletion
- When WES is normal or results uncertain, consider del/dup testing for epilepsy genes

Stepwise approach to interpretation of results/reports

1-does the gene make sense? (keep in mind, new genes are being discovered constantly)

2-inheritance type: is it an autosomal, recessive, or X-linked gene

3-inherited v/s *de novo* **[importance of parental testing in broad panels and WES]**

4-Is the variant established as either pathogenic or benign (literature, gene databases, normal population databases)

5-How does the variant affect the protein (conserved location, amino acid change, truncating, prediction in-silico)

6-Just because the lab lists a variant as a VUS- does not mean it is NOT the explanation, and just because the lab lists a variant as pathogenic does not mean it IS the explanation

7-Take the date of the test into account- anything 1-2 years old is worth re-analyzing (newly discovered genes, re-classifications, new literature, etc)

Clinical illustration 4: Example of result interpretation

5 year old girl

- Normal development
- Staring spells, dozens of times a day, starting a little after 2nd birthday
- EEG with 3Hz spike and wave during seizures
- Partial response to ethosuximide, better but incomplete response to lamotrigine (still with 10-15 seizures/day)
- Continues with normal development

An epilepsy panel was done at the time

Test(s) Requested: Childhood Epilepsy Panel / Sequencing and Deletion/Duplication Analysis of 50 Genes

Test Indications: Reported history of generalized absence seizures.

Result: **SEE INTERPRETATION**

Gene	cDNA	Variant	Zygosity	Classification
POLG	c.2982-9 T>G	IVS18-9 T>G	Heterozygous	Variant, likely disease-causing

No other reportable variants were detected by sequencing and deletion/duplication analysis of the 50 genes included on this panel.

Interpretation: **This individual is heterozygous for a single variant, likely mutation, in the POLG gene. No second mutation was identified by sequencing and deletion/duplication analysis of POLG. Mutations in the POLG gene causing infantile or childhood-onset symptoms typically have an autosomal recessive inheritance pattern; therefore, the clinical significance of a single POLG variant is unknown.**

Importance of counseling

- Normal development, no issues in school
- Mom convinced she will never outgrow seizures based on the POLG variant
- It was explained to mom that this is “inherited” from her grandfather because there is a cousin with JME
- Mom also asked the cousin with JME to stop taking VPA given the POLG variant

Report re-analyzed in 2017:

Last 100 Documents : 49 out of 49 documents are accessible. (Document Count) In Error Documents Filtered

Test(s) Requested: Childhood Epilepsy Panel / Sequencing and Deletion/Duplication Analysis of 50 Genes

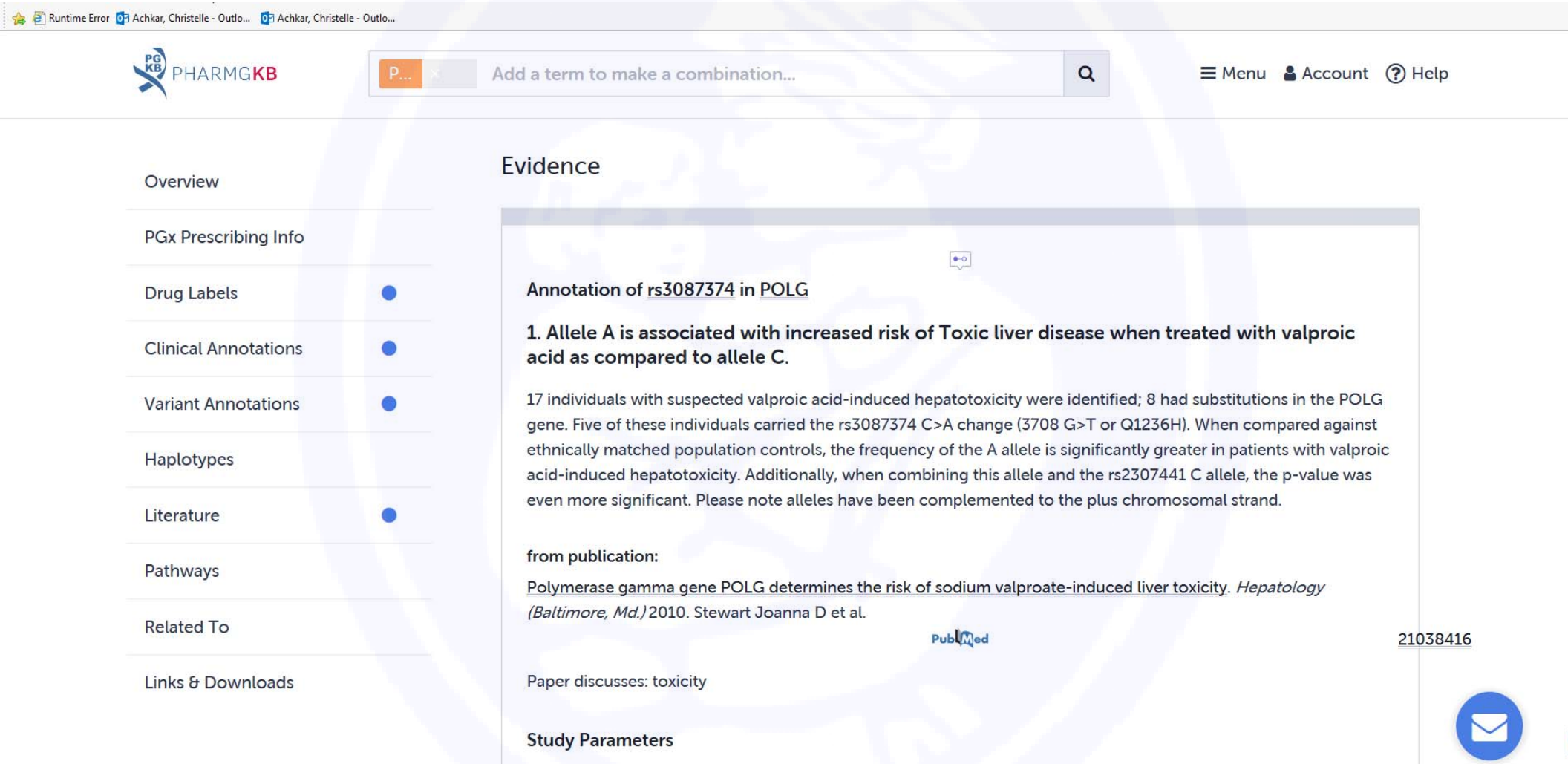
Reason for Update: **This report supersedes the report dated 9/18/2015. The c.2982-9 T>G variant in the POLG gene, which was previously reported as a variant, likely disease-causing, has been classified as a variant of uncertain significance based on review of the data in the context of the 2015 ACMG Standards and Guidelines for the interpretation of sequence variants (Richards et al., 2015). This classification now applies to all individuals, including relatives, who were found by GeneDx to harbor this variant. Updates have been made to the following sections: Result, Interpretation, POLG gene paragraph, POLG c.2982-9 T>G Variant Interpretation, and References. The remainder of the report is unchanged.**

Test Indications: Reported history of generalized absence seizures.

Result: **SEE INTERPRETATION**

Gene	cDNA	Variant	Zygosity	Classification
POLG	c.2982-9 T>G	IVS18-9 T>G	Heterozygous	Variant of Uncertain Significance

No other reportable variants were detected by sequencing and deletion/duplication analysis of the 50 genes included on this panel.


Runtime Error
Achkar, Christelle - Outlo...
Achkar, Christelle - Outlo...
PHARMGKB
P...
Add a term to make a combination...
Menu
Account
Help
Overview
PGx Prescribing Info
Drug Labels
Clinical Annotations
Variant Annotations
Haplotypes
Literature
Pathways
Related To
Links & Downloads
Evidence
Annotation of rs3087374 in POLG
1. Allele A is associated with increased risk of Toxic liver disease when treated with valproic acid as compared to allele C.
17 individuals with suspected valproic acid-induced hepatotoxicity were identified; 8 had substitutions in the POLG gene. Five of these individuals carried the rs3087374 C>A change (3708 G>T or Q1236H). When compared against ethnically matched population controls, the frequency of the A allele is significantly greater in patients with valproic acid-induced hepatotoxicity. Additionally, when combining this allele and the rs2307441 C allele, the p-value was even more significant. Please note alleles have been complemented to the plus chromosomal strand.
from publication:
Polymerase gamma gene POLG determines the risk of sodium valproate-induced liver toxicity. Hepatology (Baltimore, Md.) 2010. Stewart Joanna D et al.
PubMed
21038416
Paper discusses: toxicity
Study Parameters

Clinical illustration 5: more interpretation, and importance of counseling

6y.o boy

- Normal development
- Intractable left frontal lobe epilepsy
- Negative MRI
- Would you pursue genetic testing?

Extensive epilepsy panel ~500 genes

Sequence Variant Results

This section of the report contains clinically-relevant, dual-platform confirmed sequence variants in the provider-selected gene lists in the *Pediatric Neurology Region of Interest*.

#	Classification	Gene	Nucleotide	Amino Acid	Zygosity	Transcript	Gene Coverage*	Variant Inheritance
1	Uncertain	*GRIN2A*	c.2663C>T	p.Thr888Met	Heterozygous	NM_000833.4	100%	Paternal
2	Uncertain	*HGSNAT*	c.409C>T	p.Leu137Phe	Heterozygous	NM_152419.2	93.9%	Maternal
3	Uncertain	*MFSD8*	c.1006G>C	p.Glu336Gln	Heterozygous	NM_152778.2	100%	Maternal
4	Uncertain	*PPT1*	c.904A>G	p.Ile302Val	Heterozygous	NM_000310.3	100%	Maternal
5	Uncertain	*UBR5*	c.2851A>G	p.Thr951Ala	Heterozygous	NM_015902.5	100%	Maternal
6	Uncertain	*UBR5*	c.6031T>C	p.Ser2011Pro	Heterozygous	NM_015902.5	100%	Paternal

*Percentage of gene with >20x coverage per base pair

Copy Number Variant Results

No pathogenic, likely pathogenic, or uncertain copy number variants were identified in the genes or genomic regions targeted in the genes and genomic regions targeted in the provider selected gene lists in the *Pediatric Neurology Region of Interest*.
This section of the report contains the incidental finding that is included in this report based on the ACMG recommendations.

#	Classification	Copy Loss/Gain	Chromosome Region	Min/Max Coordinates*	Min/Max Size
4	Pathogenic	Loss	17q21.31	41199715-41276310 41199728-41277265	76.6kb 77.5kb

Copy Number Variant Interpretations

4 The 17q21.31 copy number deletion includes exons 1 through 20 of the *BRCA1* gene. Heterozygous pathogenic sequence variants and large deletions of the *BRCA1* gene have been associated with an increased risk to the development of cancer,[7] while certain homozygous or compound heterozygous pathogenic variants have been reported in association with Fanconi anemia.[8] Similar large deletions have been reported in association with a more severe clinical presentation such as the development of bilateral breast cancer, diagnosis <40 years, ovarian cancer, and male breast cancer.[9] Therefore, based on the predicted truncating and loss of function effect of this copy number variant on the protein, this variant has been classified as pathogenic.

Copy Number Variant Interpretations

4 The 17q21.31 copy number deletion includes exons 1 through 20 of the *BRCA1* gene. Heterozygous pathogenic sequence variants and large deletions of the *BRCA1* gene have been associated with an increased risk to the development of cancer,[7] while certain homozygous or compound heterozygous pathogenic variants have been reported in association with Fanconi anemia.[8] Similar large deletions have been reported in association with a more severe clinical presentation such as the development of bilateral breast cancer, diagnosis <40 years, ovarian cancer, and male breast cancer.[9] Therefore, based on the predicted truncating and loss of function effect of this copy number variant on the protein, this variant has been classified as pathogenic.

Risks and Remedies

- Wrong diagnosis due to errors in interpretation → thorough evaluation by experts
- Missed diagnosis due to technical errors or errors in interpretation → check and recheck with lab, ask for re-evaluation of unclear variants on a yearly basis
- Incidental findings, patient and family anxiety → PRE AND POST- testing Genetic counseling
- Completely negative results → re-evaluation yearly, research avenues, affected tissue testing when available and applicable

Other Aspects

The following should be taken into account

- Genotype-phenotype correlation is far from perfect, e.g.: same *KCNT1* variant can cause EIMFS in one family member and ADNFLE in another (other examples are SCN1A, KCNQ2, SCN2A, etc)
- Variable penetrance (e.g. *DEPDC5*)
- Unusual modes of inheritance related expression (e.g. *PCDH19*)
- Consider using confirmatory tests (e.g. skin biopsies with NCL-related variants, metabolic markers, etc)
- Candidate genes, communication through labs, MatchMaker, Rare Epilepsy Network, Undiagnosed Disease Network

Overall yield of genetic testing- not systematically studied yet

- Factors affecting those numbers include:

-population tested- severe v/s less severe epilepsy, presence or absence of developmental delays, age of epilepsy onset

-sequence of testing performed- e.g. overall yield of WES after negative panels is lower than yield of WES done as a first test

-content of panels studied and availability of deletion/duplication studies

Reported Yields in literature

- CMA: *High* yield in epilepsy + developmental delay or ASD (>5%, Olson et. Al, Annals of Neurology, 2014)
- CMA: Epileptic encephalopathy (>3%, EPGP/EPI4K, Annals of Neurology, 2015)

Reported Yields in literature

Helbig K. et al, Genetics in Medicine, Sept 2016

- WES done on 1,131 patients (314 with epilepsy, 817 without seizures- these were family members)
- Positive or likely positive in 38.2% (compared to 28.7% in patients without epilepsy)
- 43.4% of epileptic encephalopathies had positive findings (higher in neontal onset encephalopathies- 58.3%)
- Likely positive novel etiology proposed in 17% (candidate genes/uncharacterized genes)

- 29.9% of the 1,131 patients had prior genetic testing that was negative (mainly NGS or X-linked ID, mitochondrial, Rett, Angelman panels, or negative CMA)

Other helpful tools (databases and programs)

- gnomAD (Genome Aggregation Database, >120,00 exomes and >15,000 whole genomes) gnomad.broadinstitute.org/
- ClinVar: processing of submitted variants and clinical assertion
- In-silico prediction models, but use with caution (e.g. PolyPhen-2, MutationTaster, SIFT, etc)
- GeneMatcher https://genematcher.org/
- SCN1A databases (e.g Molgen https://www.molgen.vib-ua.be/SCN1AMutations/)
- KCNQ2 database (RIKEE Project) https://www.rikee.org/about-the-database
- CLN database at UCL (collecting since 1998) https://www.ucl.ac.uk/ncl/mutation.shtml

Take home points

- Genetic testing has a growing and vital role in understanding and treating epilepsies
- More is not necessarily always best: Choosing a reasonable test for the right patient is key
- On the other hand, when the answer matters, keep looking!
- A result is as good as its interpretation
- Genotype-phenotype correlation is far from perfect
- A negative result does not mean there is no genetic etiology
- Genetic counseling is an indispensable part of this process