

Baby Badger Network

DEC 8, 2025 12:30PM – 1:30PM



BBN ECHO: Welcome & Announcements

- **Introductions in the chat (name, discipline, institution)**
- **Attendance Code | 606-260-7097 | BUZSER**
- **2 Evaluations**
- **Building our Community (in chat)**
- **Agenda**
 - Knowledge Burst – An Uncertain Result: What Next? – April Hall
 - Problem of Practice – Jess Scott Schwoerer
- **Wrap Up**



Knowledge Burst | An Uncertain Result: What's Next?

April Hall PhD, CGC

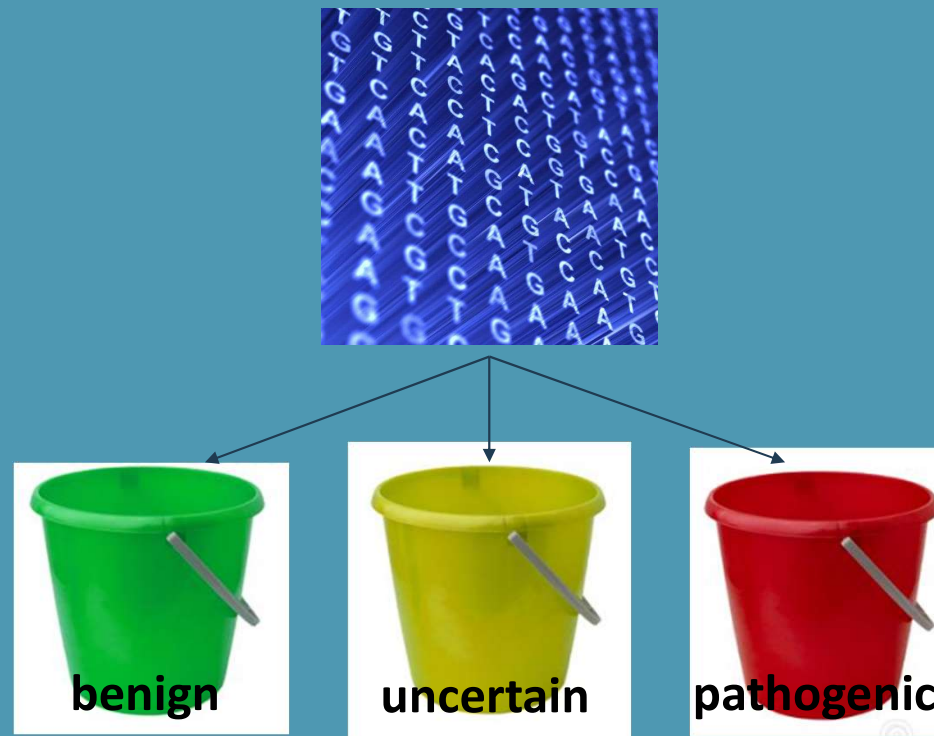
Learning Objectives

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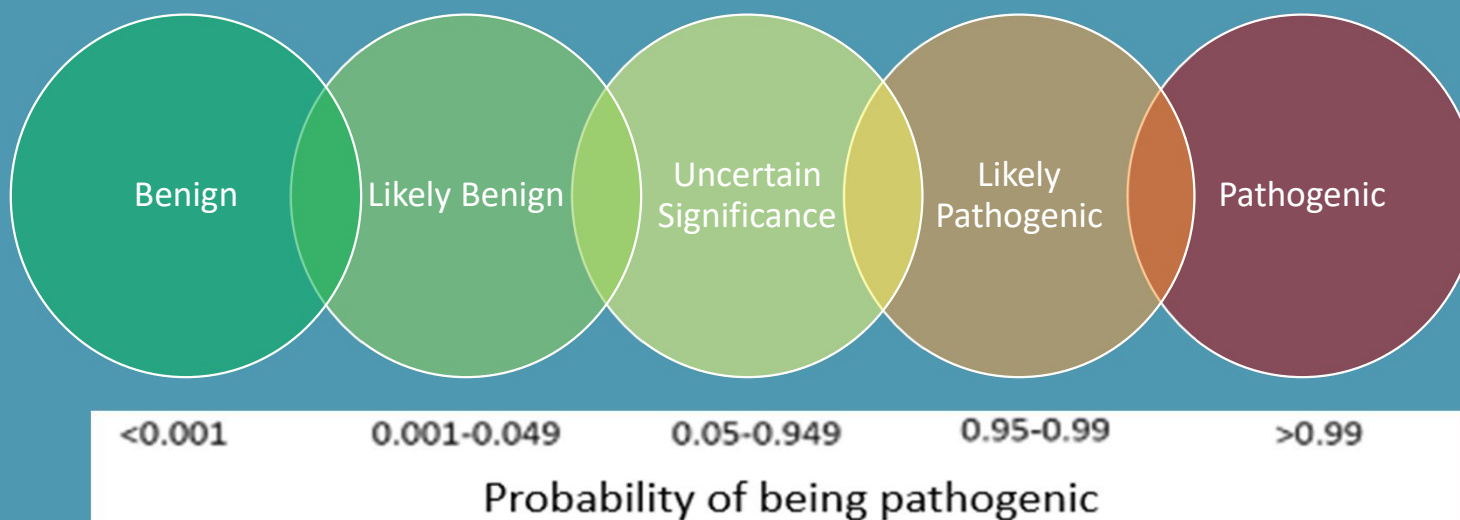
1. Summarize the ACMG variant classification scoring framework as it relates to an uncertain result
2. State the importance of clinical correlation when interpreting an uncertain genetic finding
3. Identify next steps such as communicating with the family, parental testing, referral to the outpatient setting and reanalysis
4. Discuss challenges and solutions to implementing genomic medicine in a NICU setting



Types of Genetic Test Results



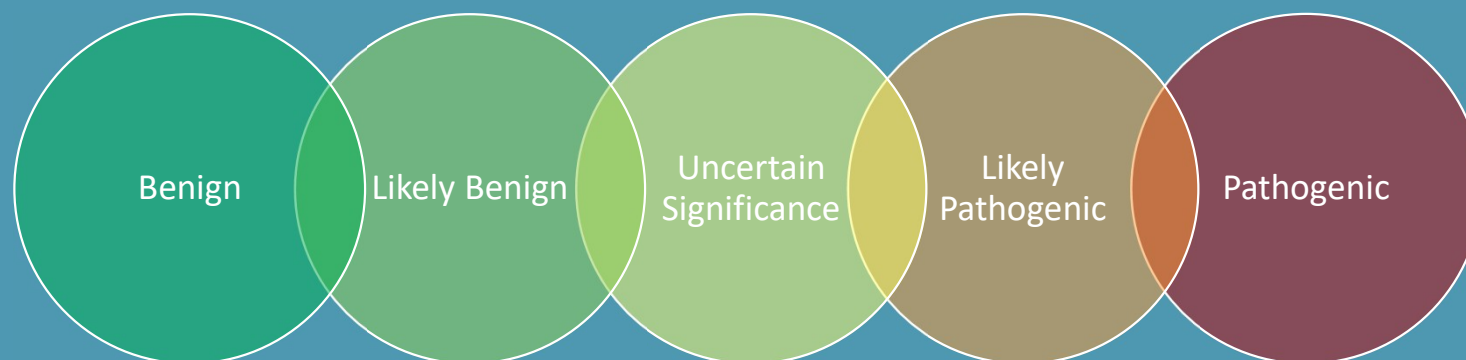
Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology



	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 OR observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in trans with a dominant variant BP2 Observed in cis with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			



Factors Influencing Variant Interpretation



- Population Data
- Computational/Predictive Data
- Functional Data
- Familial Data
- Allelic Data



Population Data

Is the variant seen in cases or controls?

gnomAD

genome aggregation database

SUMMARY OF RESULTS: *PNKP*: Homozygous for a Likely Pathogenic Variant

INDICATION FOR TESTING: Distal weakness, numbness, ophthalmoplegia, cerebellar ataxia, easily fatigued, normal cognition, cerebellar atrophy, ataxia, muscle weakness, hypotonia, hyporeflexia, contractions. Mother, father and brother unaffected; sister affected.

1) Variants in genes known to be associated with phenotype:

Gene	Disorder(s)	Mode of Inheritance	Transcript	DNA Variation/ Predicted Effect	Reference	Classification
<i>PNKP</i>	Ataxia-oculomotor apraxia-4; Microcephaly, seizures, and developmental delay	Autosomal Recessive (Father and Mother)	NM_007254.3	c.1189-15_1191del18 (p.?) Homozygous	Undocumented	Likely Pathogenic

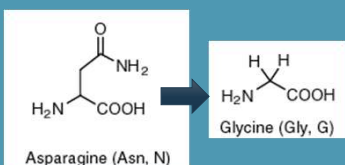
RESULTS AND INTERPRETATIONS: This patient is homozygous in the *PNKP* gene for a sequence variant designated c.1189-15_1191del18, which is predicted to alter splicing based on available splicing prediction programs (Alamut v2.8). To our knowledge, this variant has not been previously reported in literature or public allele frequency databases. Homozygous variants in *PNKP* have been described in patients with ataxia-



Computational/Predictive Data

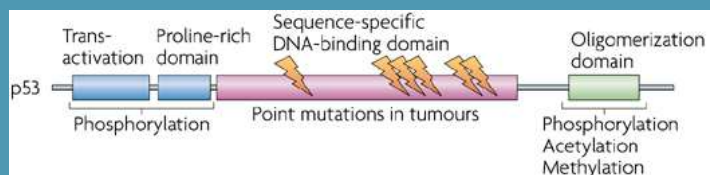
• What is the predicted affect of the variant on the protein?

• Nonsense or missense variant



p.R140W

Homo sapiens	IFQEKFRCPSTLS
Pan troglodytes	IFQEKFRCPSTLS
Macaca mulatta	IFQEKFRCPSTLS
Canis familiaris	IFQEEIRCPSTLS
Equus caballus	IFQEKLRCPSTLS
Bos taurus	IFQEEVQCPSTLS
Mus musculus	IFQEDIPCPASLS
Rattus norvegicus	IFQEDVPCPASLS
Gallus gallus	IYQEETECPSLS
Danio rerio	IYQGDNEVPAVAG



MOLECULAR GENETICS REPORT: Glycogen Storage Disease NextGen Sequencing Panel

SUMMARY OF RESULTS: **NEGATIVE**

RESULTS AND INTERPRETATIONS: In this patient, for the *AGL*, *G6PC*, *GAA*, *GBE1*, *GYS2*, *PFKM*, *PHKA1*, *PHKA2*, *PHKB*, *PHKG2*, *PYGL*, *PYGM*, *SLC2A2* and *SLC37A4* genes, we found no sequence variants that are likely to be a primary cause of disease.

This patient is heterozygous in the *PFKM* gene for a likely benign missense which bears further mention. This variant is defined as c.2087G>A, and is predicted to result in the amino acid substitution p.Arg696His. This variant was reported in a patient with GSD Type VII (Raben et al. 1995. *Am. J. Hum. Genet.* 56:131-141). However, a second clearly causative variant was not found in this patient, and *in vitro* expression of the Arg696His protein in yeast showed no reduction in enzyme activity. Furthermore, the programs PolyPhen-2 and SIFT (Adzhubei et al. *Nat Methods* 7:248-249, 2010; Ng and Henikoff *Genome Res* 11:863-874, 2001) predict the p.Arg696His change to be "benign" and "tolerated", respectively. It should be noted that this variant has a minor allele frequency greater than 1.0% in several different populations (<http://www.1000genomes.org/>) and has been observed in several presumably unaffected individuals in the homozygous state (<http://www.ncbi.nlm.nih.gov/SNP/>; evs.gs.washington.edu/EVS/; <http://exac.broadinstitute.org/>). In addition, we have observed this variant in several patients at PreventionGenetics in the absence of a second causative variant in the *PFKM* gene. In summary, we classify this variant as likely benign.

These results should be interpreted in context of clinical findings, family history and other laboratory data. All genetic tests have limitations. Please see limitations and other information for this test on pages 3 - 5.



Functional Data

Does the variant affect the function of the protein?

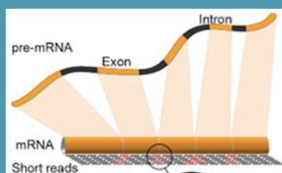
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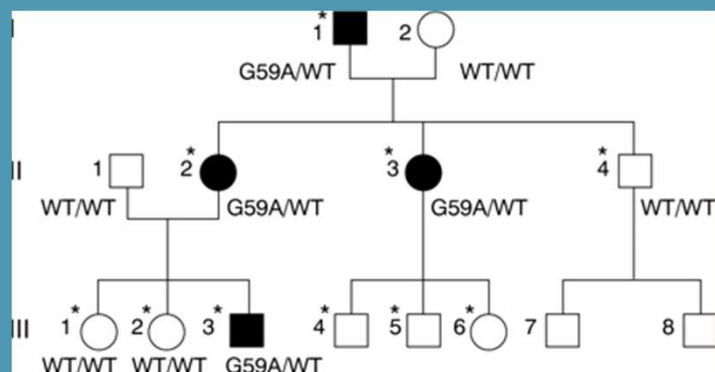
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Familial Studies Data

Is the variant seen in other members of the family?



MOLECULAR GENETICS REPORT:
Malignant Hyperthermia via *RYR1*, *CACNA1S* and *STAC3* Gene NextGen Sequencing

SUMMARY OF RESULTS: POSITIVE

RESULTS AND INTERPRETATIONS: This patient is heterozygous in the *RYR1* gene for a sequence variant designated c.6487C>T, which is predicted to result in the amino acid substitution p.Arg2163Cys. This variant has been repeatedly documented to be causative for Malignant Hyperthermia (MH) (see for example Manning et al. Am J Hum Genet 62:599-609, 1998; Robinson et al. Hum Mutat 27:977-989, 2006; www.emhg.org). It is found much more frequently in cases than controls ($p < 10^{-4}$) (Robinson et al. Hum Mutat 27:977-989, 2006; <http://exac.broadinstitute.org/>). The p.Arg2163Cys variant has been shown to segregate with MH susceptibility in two large families (Manning et al. Am J Hum Genet 62:599-609, 1998). Exon 39 is a hotspot for MH pathogenic variants in *RYR1*. A different substitution at the same amino acid (p.Arg2163His) has also been reported to be pathogenic for MH. Two studies have shown that the p.Arg2163Cys variant affects the function of the *RYR1* Ca⁺⁺ channel in model systems (Tong et al. J Biol Chem 272:26332-26339, 1997; Dirksen and Avila Biophys J 87:3193-3204, 2004). We conclude that the c.6487C>T (p.Arg2163Cys) variant is clearly pathogenic.

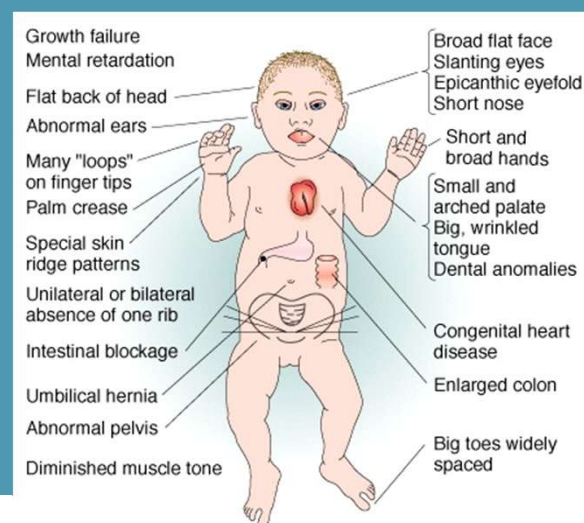


Clinical Correlation

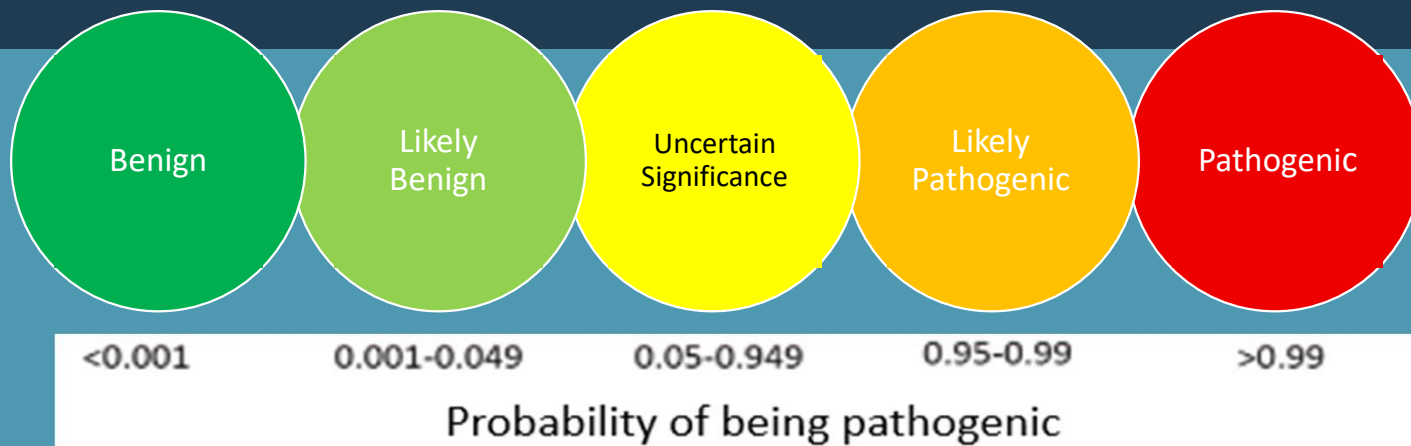
Does the patient's phenotype match that of which is described?

Variable Expressivity

Symptoms occurring outside of newborn period



Considerations for Variant of Uncertain Significance



- Communicating results to family
- Next Steps
 - Referral to outpatient genetics
 - Parental testing
 - Reanalysis



Case Example

- Patient is a 2 week old female with seizures. She is eumorphic. You are suspicious of syndrome and order a seizure panel.
- Result: TCF4 c.839T>C (p.Met280Thr) VUS
- What information do you want to know in communicating this result to the family?



Case Example

- Eumorphic 2 week old female with seizures.
- Result: TCF4 c.839T>C (p.Met280Thr) VUS
- **OMIM**
 - TCF4 is associated with autosomal dominant Pitt-Hopkins Syndrome characterized by moderate to severe intellectual disability, developmental delays, intermittent hyperventilation, seizures, distinctive facial features
 - Missense mutations have been reported with Pitt-Hopkins syndrome
- **Control databases**
 - Not seen in control populations
- **Conservation**
 - Methionine - Non-polar; Hydrophobic; Threonine - No charge; Polar; Hydrophilic
 - Conserved through squirrels
- **Family studies**
 - Only Mom was available for parental testing and she did NOT have the variant

