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Better health through laboratory medicine.

PEARLS OF LABORATORY MEDICINE

Variants of Uncertain Significance

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CCTC Clinical Chemistry Trainee Council

Genetic Testing:
An expanding component of laboratory medicine

Compiled using genetests.org (accessed 1/2/2016)

Standardization efforts by the American College of Medical Genetics & Genomics (ACMG)

- Molecular genetics laboratories are encountering an increasing number of novel genetic variants
- Previous ACMG recommendations did not provide detailed guidance on classification of variants
- Lack of standardization of variant classification across testing laboratories limits the positive influence genetic testing can have on medical decisions

5-tier ACMG variant classification system

Pathogenic	• Strong and conclusive evidence present • Targeted testing of at-risk family members and changes in medical management for pathogenic mutation carriers may be recommended • A pathogenic variant should be included in results report
Likely Pathogenic	• Strong evidence that favors pathogenicity; more limited than for pathogenic variants • Similar implications for a patient as a pathogenic variant • These variants should be included in the results report
Likely Benign	• Variants with strong evidence against pathogenicity • Targeted testing of at-risk family members is not recommended • A likely benign variant is not routinely included in results reports (with some exceptions, i.e. pseudodeficiency alleles)
Benign	• Variants with very strong evidence against pathogenicity • Targeted testing of at-risk family members should not be recommended • Same criteria applies for reporting as for “Likely Benign” variants
Uncertain Significance	• Variants with limited or conflicting evidence regarding pathogenicity • Targeted testing of family members may be useful in some situations • A variant of “uncertain significance” (VUS) should not alter medical management • Included in results report

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Pathogenic evidence - ACMG

Evidence	Strength
Variant causes a nonsense change, a frameshift, occurs in the canonical splice site, alters the initiation codon, or leads to loss of one or more exons in **a gene where loss of function is a known mechanism of disease**	**Very Strong**
Variant causes the same amino acid change as a well-established pathogenic variant	**Strong**
De novo change (confirmed absent in both unaffected biological parents)	**Strong**
Functional studies supportive of a damaging effect on the gene or gene product	**Strong**
Strong enrichment of variant in the affected population	**Strong**

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Pathogenic evidence - ACMG

Evidence	Strength
Variant found in a mutational hotspot or on in a domain with well-established functional significance	**Moderate**
Absence (or extremely low frequency) in control populations	**Moderate**
In recessive disorders – it occurs in trans with an established pathogenic variant	**Moderate**
Missense change at an amino acid residue where a distinct amino acid change at the same residue has been established as pathogenic	**Moderate**
De novo (presumed) without parental confirmation	**Moderate**
Co-segregation with affected family members in a gene known to cause disease	**Supporting**
Missense variant in gene where missense changes are common mechanisms of disease	**Supporting**
Multiple lines of in silico support: conservation, splicing impact, structural changes	**Supporting**
Reputable source recently reported the variant as pathogenic but evidence is not available	**Supporting**

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Benign evidence - ACMG

Evidence	Strength
Allele frequency >5% in Exome Sequencing Project (ESP), 1000 Genomes Project, or Exome Aggregation Consortium (ExAC)	**Sufficient**
Allele frequency is greater than that of the expected disorder (Hardy-Weinberg)	**Strong**
Observed in a healthy adult in recessive conditions (homozygous), dominant (heterozygous), or X-linked disorders (hemizygous), where full-penetrance is anticipated	**Strong**
Functional studies show no damaging effect on the gene or gene product	**Strong**
Variant does not segregate with disease in affected families	**Strong**

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Benign evidence - ACMG

Evidence	Strength
Observed *in trans* with a pathogenic variant for a completely penetrant gene or disorder or observed in *cis* with an established pathogenic variant with any inheritance pattern	**Supporting**
In silico predictions suggest no impact	**Supporting**
In-frame deletion or insertion in a repetitive region without a known function	**Supporting**
De novo (presumed) without parental confirmation	**Supporting**
Variant found in combination with an alternate molecular cause of disease	**Supporting**
Missense variant in gene where missense changes are common mechanisms of disease	**Supporting**
Synonymous (silent) variant with no predicted impact on splicing AND a poorly conserved nucleotide	**Supporting**
Reputable source recently reported the variant as benign but evidence is not available	**Supporting**

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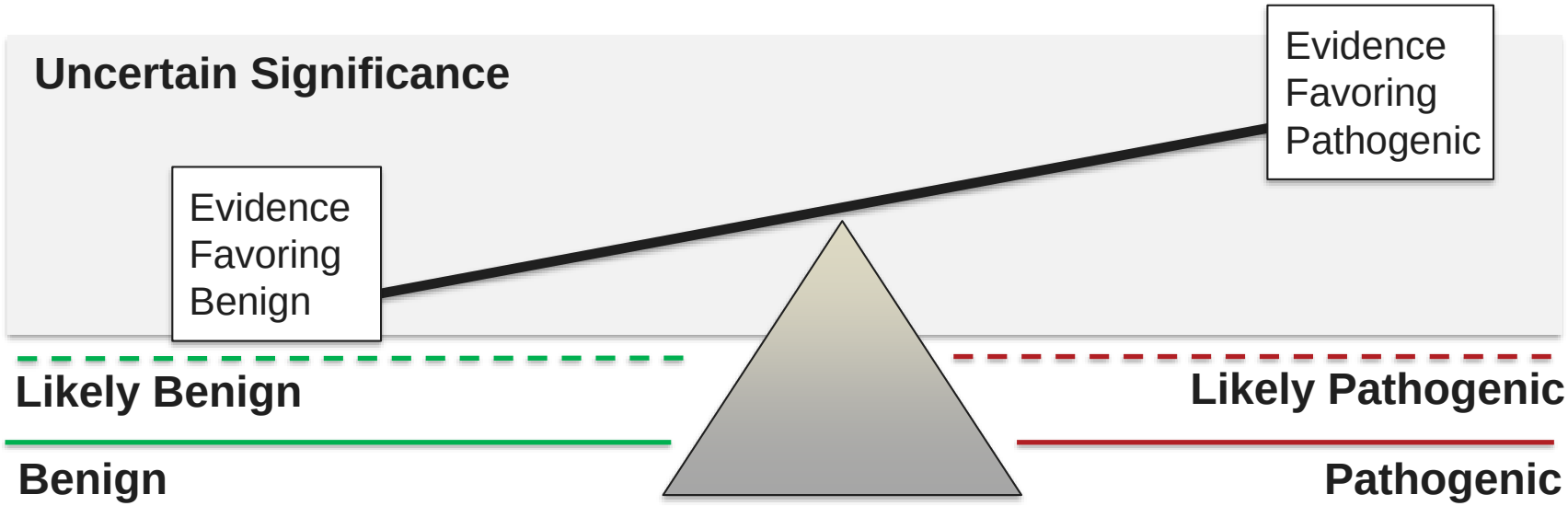
Compiling the evidence for classification

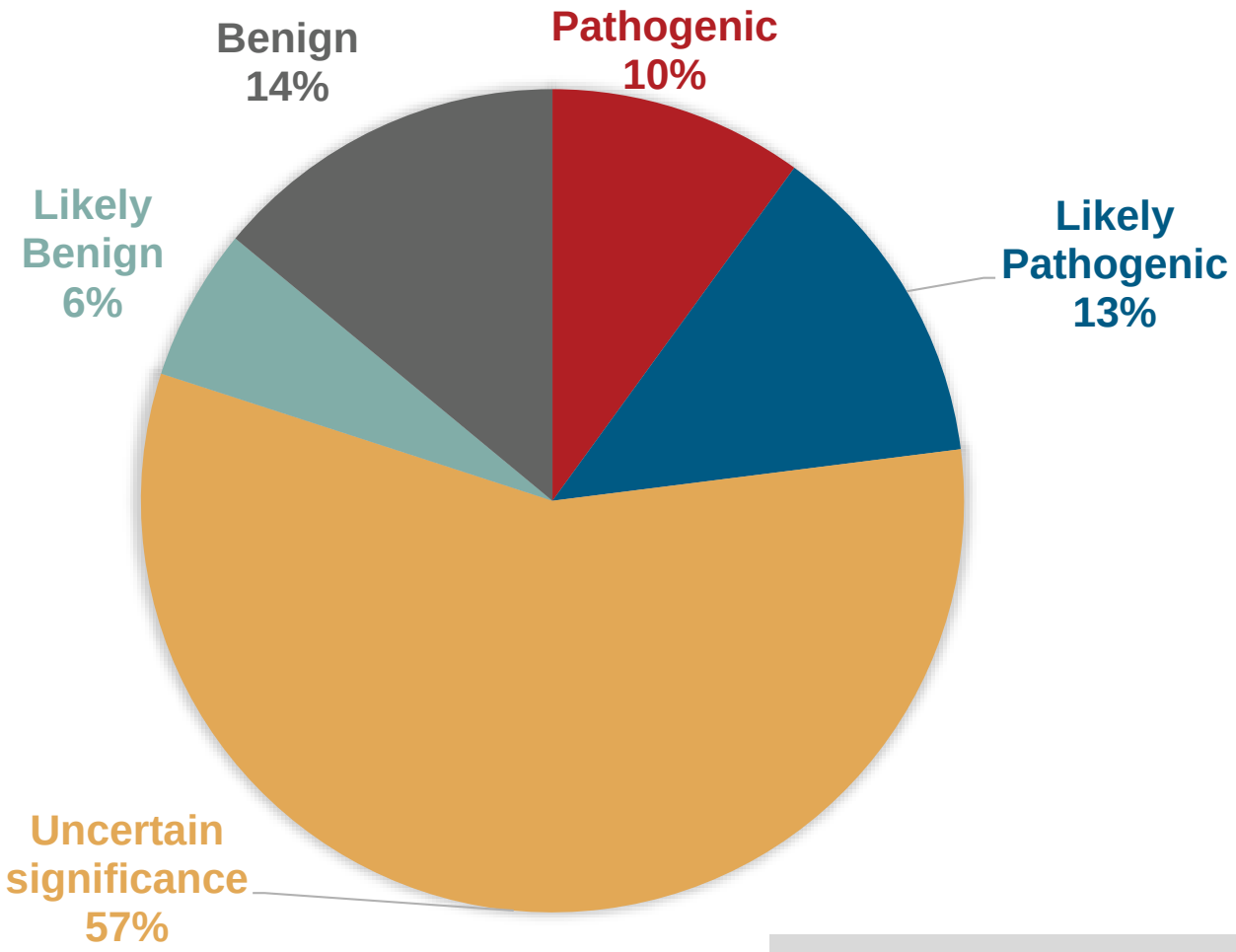


VUS example – *PRSS1*: c.623G>C (p.G208A)

The patient has a history of idiopathic pancreatitis; *PRSS1* encodes trypsin-1 which is commonly implicated in hereditary pancreatitis

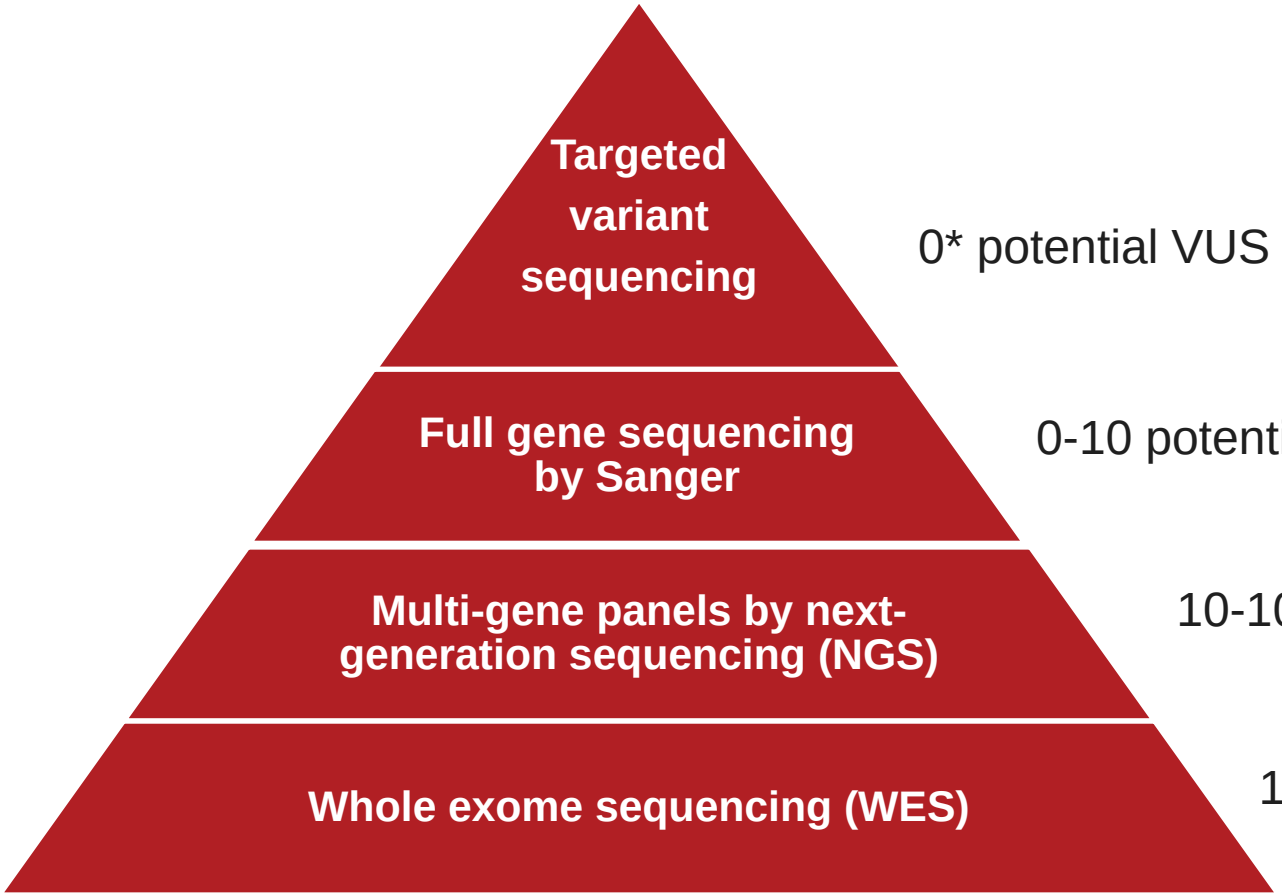
Evidence	Favors
Novel missense mutation not seen in laboratory previously	**VUS**
In one family study the variant is present in multiple unaffected family members	**Benign**
In another family study the variant co-segregates with affected family members in a gene known to cause disease	**Pathogenic**
Functional studies are inconclusive how this variant might affect protein function	**VUS**
Minor allele frequency is >1% in certain populations	**Benign**
Strong enrichment of variant in the affected population	**Pathogenic**
Highly conserved amino acid with 2 of 3 in silico programs predicting damage to the protein function	**Pathogenic**

Distribution of variants from clinical testing



Karbassi I et al. 2015

Clinical sequencing – growing numbers of VUS



0* potential VUS

0-10 potential VUS

10-100s of potential VUS

1000s of potential VUS

* Assay troubleshooting may uncover a VUS

Managing VUS in large gene panels & WES

Exome sequencing at 100X coverage

- Estimated to detect on average 20,000-30,000 variants per individual
- A majority of these would be categorized as VUS

Variant filtration

- Remove less reliable variant calls (using quality metrics)
- Family trios (biological parents and proband) can be used to remove variants which do not fit the mode of disease inheritance
- Eliminate high frequency germline variants (>1% allele frequency)

Variant prioritization

- Variants impacting the canonical splice site acceptor/donor sites and nonsense alterations are, in general, more likely to impact genes and/or gene products versus missense alterations
- Other useful information includes *in silico* predictions, experimental studies, nucleotide/amino acid conservation

How is a VUS managed clinically?

- **Pretest Counseling:**
  - During the initial discussion of the possibility of genetic testing, the patient should be informed of the potential of encountering a VUS
- **Posttest Counseling:**
  - In the event a VUS is found by the laboratory and this result is reported to the healthcare provider, medical management should be based on personal and family history in the context of the clinical presentation
  - Targeted testing in family members should be limited to studies aimed at clarifying the meaning of the VUS as part of reclassification efforts
- **Reclassification:**
  - Over time (often years), laboratories may review VUS classifications and potentially reclassify these variants when new evidence is made available
  - Laboratories may seek to notify healthcare providers, who may then notify patients, of these changes

The dangers of a VUS

Overtreatment

- Inappropriate irreversible treatment decisions (prophylactic surgery) where a VUS is later reclassified as a benign variant

Patient anxiety

- No resolution whether a devastating disease might be present
- There is risk for a serious psychosocial impact

Misunderstanding

- The patient or healthcare provider may recommend targeted testing to other family members based on a VUS

The VUS challenge and database solutions

The availability of well-maintained and vetted databases documenting the genotype-phenotype relationship can dramatically reduce the number of VUS calls and improve standardization

- The number of VUS classification for *BRCA1/2* variants by Myriad Genetics Laboratory declined from ~13% to an estimated 2%. This is a much lower rate than other institutions, due to the development of a proprietary database of nearly 1 million patients who have had *BRCA1/2* testing.

- The International Society for Gastrointestinal Hereditary Tumours (InSiGHT) created a central repository for variant classification for *MLH1, MSH2, MSH6,* and *PMS2,* and re-examined classification. This led to reclassification of ~25% of variants, including a large number of VUS.

ClinVar – a variant classification resource

Composite from ClinVar submitters

- Basic researchers
- Clinical laboratories
- Expert groups
- Patient registries
- Disease-specific databases

Levels of Support - Assertion Criteria

- Practice Guideline ★★★★
- Expert Panel ★★★
- Multiple consistent entries ★★
- Single submitter with criteria provided ★
- Single submitter with no criteria provided

ClinVar – A variant classification resource

ClinVar Disclaimer

"The information on this website is not intended for direct diagnostic use or medical decision-making without review by a genetics professional. Individuals should not change their health behavior solely on the basis of information contained on this website. NIH does not independently verify the submitted information. If you have questions about the information contained on this website, please see a health care professional…"

Limitations to ClinVar

- Submitters may not update classifications when additional information is available that alters the interpretation of a variant
- Submitters traditionally have not used standardized classification criteria
- Incomplete entries – lacking components of evidence
- Only a small percentage of entries have more than 2 submitters
- ~ 1 in 5 submissions have multiple entries that are in disagreement

Summary

- Finding a VUS doesn't provide a solution to the problem the healthcare provider and/or patient was hoping to solve
- In the short term as new disorders are uncovered and new genes are sequenced clinically there will be an invariable increase in VUS encounters
- Classification of variants is a costly component of genetic testing and requires standardization to optimize value
- Healthcare providers should interpret a VUS cautiously and seek advice from the laboratory or a genetic counselor if there are any concerns wtih how to handle a VUS

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