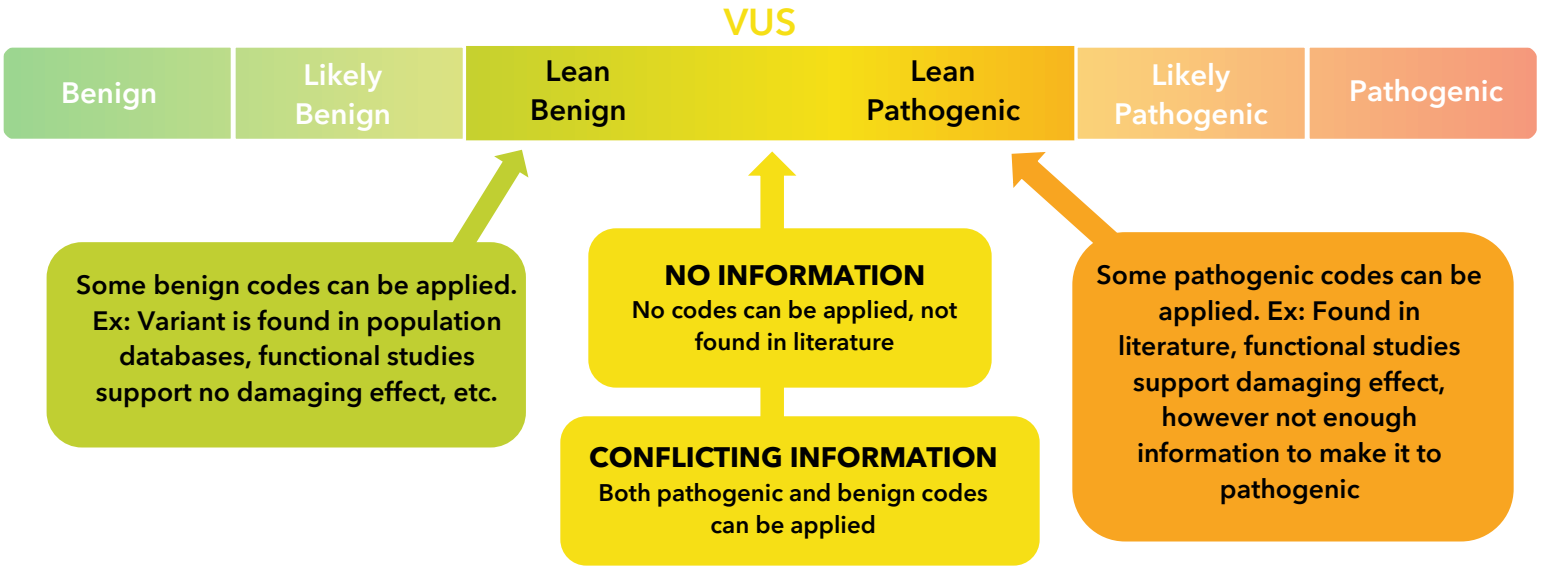
Assessing a Variant of Uncertain Significance (VUS)

If you have a received a VUS from clinical genetic testing and would like to understand its importance for your patient, this guide may be helpful for you.

- Consider the variant's **classification**
- Consider the **associated disorder** and your **patient's presentation**
- Consider **gathering additional information** that may alter the variant's classification

1 CONSIDER THE VARIANT'S CLASSIFICATION

Figuring out if a VUS falls more on the benign or pathogenic side is the first step in determining how to discuss it with a patient and whether to pursue further investigation.



How to determine where a variant falls on the VUS spectrum

- ✓ Read through **the clinical lab's variant summary** on the test report and determine what evidence was considered for the classification
- ✓ **Check ClinVar** to see if other labs have classified the variant differently. If other well-known clinical laboratories have classified your variant as likely benign or likely pathogenic, you can reach out to them to get more information about why they classified it as they did.
- ✓ **Use Franklin, Varsome, gnomAD**, etc. to collect additional information about the variant
- ✓ Most clinical laboratories know which side the variant leans, even if they did not indicate it on the report. You can contact the lab and ask for this information as well.

TIP: Refer to *Variant Curation Evidence Handout* for more information about how to apply codes. Depending on how many pathogenic or benign codes have been applied, your variant may lean in a direction on the spectrum.

2 CONSIDER THE ASSOCIATED DISORDER

How well established is the gene-disease association?

How well does the disorder fit with your patient's presentation?

Do the mode of inheritance and zygosity fit your patient's history?

Gene-Disease Association

Some genes found on exome or genome or included on panels may not have a Strong association to disease, and therefore may need additional research. To determine how strong the gene-disease association is, the following resources can be used. Please note: for more information on how gene-disease validity is scored, see ClinGen's guidelines here: https://clinicalgenome.org/site/assets/files/9232/gene_curation_sop_version_10_1_docx.pdf

- ✓ Check **ClinGen Gene-Disease Validity** to see if a classification for Gene-Disease Association has been made
- ✓ **GenCC** - an open database for submissions on gene disease associations (similar to ClinVar)
- ✓ **Medline Plus** - a database with disease descriptions in patient friendly language
- ✓ **OMIM** - Limited gene-disease associations may be present (indicated with a question mark), and it is not always frequently updated (check the dates at the bottom of the page)

- ✓ **GeneReviews** - a centralized resource provides clinically relevant and medically actionable information for inherited conditions in a standardized journal-style format, covering diagnosis, management, and genetic counseling for patients and their families. Most of the well characterized genetic conditions can be found in GeneReviews, suggesting a strong clinical validity gene.
- ✓ **Literature search / PubMed** - assess number of case reports in the literature to determine if the gene-disease association is strong. For scoring criteria please see ClinGen's guidelines linked above.

Patient's Presentation

Consider how well the disease fits with your patient's presentation.

- ✓ Are there features that are typically present in the disease that are not present in your patient? If so, how common are they in the disorder? If your patient's presentation does not match up with the disorder, it's less likely to be the explanation for your patient's disease. Please note: variable expressivity of a disease may also explain why a patient does not have the typical presentation.
- ✓ **Specific or rare phenotypic features** are more compelling. If your patient has very specific features of a rare disorder and there are few genes which have been found to cause this disorder, it is more likely that the VUS could be related to your patient's presentation.

Mode of Inheritance and Zygosity

Consider whether the variants found in your patient and the family history fit the mode of inheritance for the disorder.

- ✓ **X-linked or Autosomal Dominant:** one variant detected
- ✓ **Autosomal Recessive**: homozygous variant or compound heterozygous variants. If your patient only has one heterozygous variant found in a gene associated with a recessive condition, it is less likely to be causing disease in your patient. (Consider the methodology of the test and if all types of variants in the gene were tested for, ex. Copy number, deletion/duplication testing)
- ✓ **X-linked Recessive**: hemizygous variant in a male, homozygous or compound heterozygous variants in a female

Family History: does the mode of inheritance fit with other affected family members?

- ✓ **Dominant** - variant is present in other affected family members, not present in unaffected family members
- ✓ **Autosomal recessive** - both parents are carriers, unaffected siblings are carriers or do not have the variant, affected siblings are homozygous/compound heterozygous
- ✓ **X-linked recessive** - present in affected male relatives related through females, not present in unaffected male relatives related through females

TIPS: Keep in mind age of onset and the possibilities of variable expressivity and reduced penetrance, particularly for dominant disorders i.e. *C9orf72*.
Keep in mind the possibility of **manifesting female carriers** of X-linked recessive disorders

3 GATHER ADDITIONAL INFORMATION

If the above steps have not given you an answer for your patient's VUS, it may be possible to further investigate for additional evidence that may help upgrade or downgrade the classification. The following suggestions are more time intensive than the previous steps.

Patient Phenotyping

Segregation Data

Additional Cases

Functional Studies

Patient Phenotyping

Are there **rare clinical findings** that are common in the disorder that your patient can be tested for, for example an imaging or biochemical finding? If so, their presence may add evidence for **PP4**.

Segregation Data

Testing of family members may be able to add additional codes or increase the weight of the codes to either upgrade or downgrade the variant.

- ✓ For recessive disorders, testing of both parents can determine if the variants are compound heterozygous or biallelic and increase the weight of **PM3**.
- ✓ Testing family members who are **definitively affected**: A positive result in affected family members, particularly multiple affected family members is supportive of the variant (**PP1**). A negative result for an affected family member may exclude the variant (**BS4**)
- ✓ Testing family members who are **definitively unaffected** (*Note: this is typically less helpful for disorders with reduced penetrance or later onset*). Finding the variant in an individual who is unaffected may exclude the variant (**BS4**)
- ✓ **De novo**: If the variant is not present in either unaffected parent, the **PS2** or **PM6** code may be applied. (*Note - To be definitively de novo (PS2) and increase the weight of the code, the parents must also have been genetically confirmed for biological parentage.*

This may be done as part of trio exome and genome sequencing but is not typically performed.)

Additional Cases

Finding other individuals who have the same variant and a similar phenotype can help add evidence towards the classification. While the easiest way to find these is through the literature, you may be able to find additional cases through websites like Matchmaker Exchange (https://www.matchmakerexchange.org/). You can also contact any labs that have submitted the variant to ClinVar to determine if they have any internal cases.

Functional Studies

While often not feasible, functional studies may provide strong evidence for (**PS3**) or against (**BS4**) pathogenicity for a variant.

- ✓ If the variant is predicted to alter splicing, consider **RNAseq** (transcriptome analysis) to provide abnormal splicing and, potentially, nonsense mediated decay. (*Note: The appropriate sample type will vary by gene (not all genes are well expressed in blood). Few labs are currently offering this testing clinically, and insurance coverage may be a challenge.)*
- ✓ Consider reaching out to the authors of prior publications on the disorder that included functional studies of variants.
- ✓ Websites like **Model Matcher** (https://www.modelmatcher.net/) may also be an option to find researchers who have functional studies available for the gene of interest.

Revisit in the Future

In most cases, it will not be possible to reclassify a VUS, particularly one that was just reported. It will be important to reassess the classification on a routine basis, as new information that may impact classifications becomes available. Many labs will reassess the variant if you reach out to them. You may want to consider reassessing the variant a year after the initial classification was completed.

ADDITIONAL FAQs AND INFORMATION

Should you alter management based on a VUS?

"A variant of uncertain significance should not be used in clinical decision making. Efforts to resolve the classification of the variant as pathogenic or benign should be undertaken. While this effort is underway, additional monitoring of the patient for the disorder in question may be prudent." (ACMG Guidelines, PMID: 25741868)

However, clinical judgement may be used when considering if an intervention may be appropriate for a patient with a VUS. If you are highly suspicious of the VUS, the cost, efficacy and burden to the patient should be considered before suggesting an intervention.

How to discuss a VUS with your patient

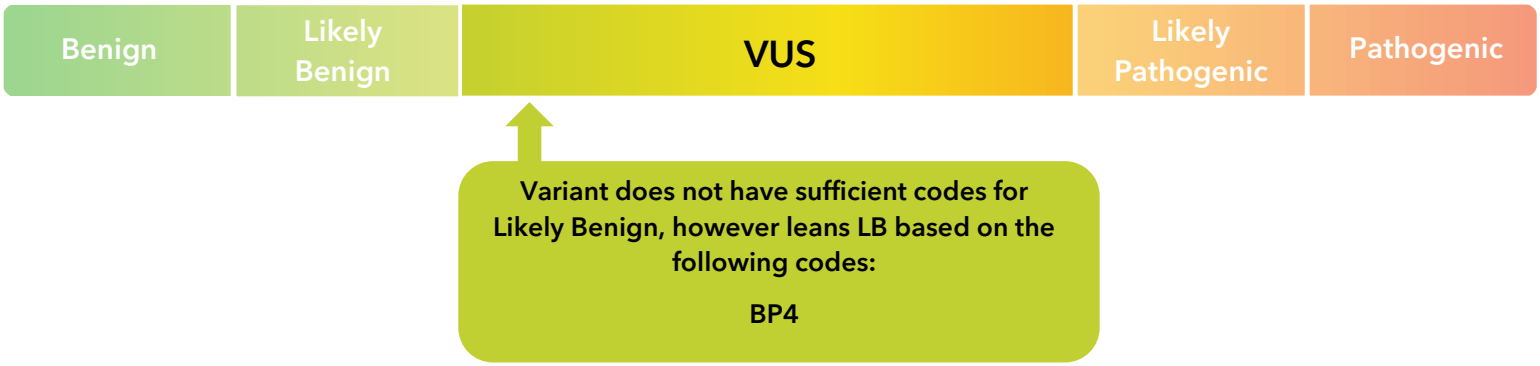
- ✓ The significant likelihood of identifying one or more VUSs should be discussed as part of **pre-test counseling**/consenting. It is often helpful to refer back to that prior conversation when discussing the finding of a VUS.
- ✓ Emphasize that a VUS is **not a diagnosis**, even when the disorder associated with the gene seems like a clear match to the patient's phenotype. It may be helpful to discuss with the patient where on the spectrum a VUS falls.
- ✓ In many/most cases, the only thing to do is to **wait and see** if the variant is ultimately reclassified.
- ✓ Most VUSs are ultimately reclassified, and the **majority are reclassified to likely benign** (PMID: 31752965).
- ✓ **Testing of unaffected family members** for the variant for their own health is not typically recommended, given the uncertainty of its significance.

EXAMPLE VUS CASES

Example 1 - evidence indicating may lean Likely Benign (LB)

CASE: A patient with congenital non-syndromic hearing loss had hearing loss gene panel which returned a single heterozygous variant in the OTOF gene, which is associated with autosomal recessive hearing loss. On the genetic testing report, the lab classified this as VUS.

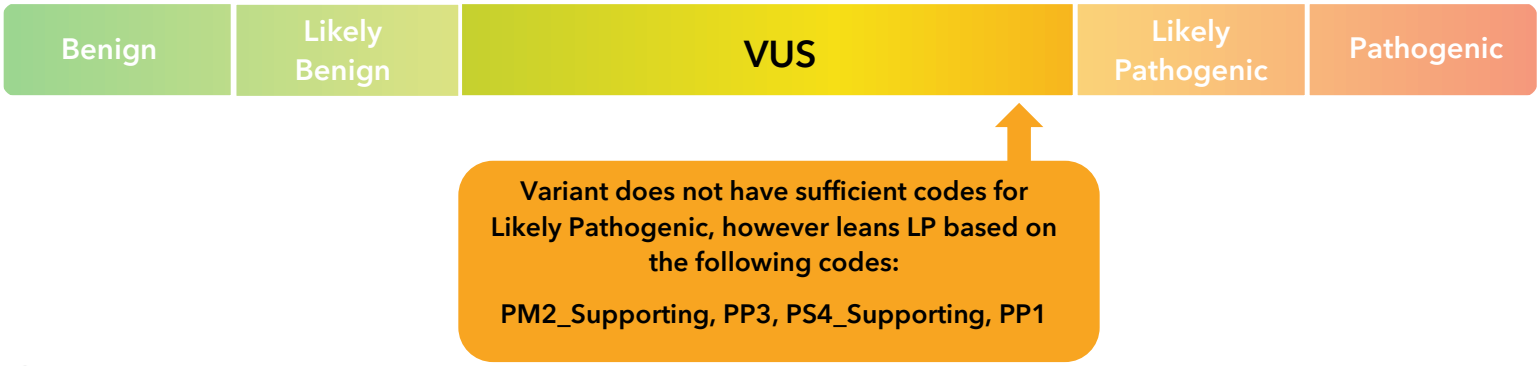
- ✓ The following evidence is found for the variant:
  - The variant is **present in the gnomAD** population database, but the allele frequency is not greater than expected for the disorder. **(No code)**
  - It has **not been reported** in literature in individuals with hearing loss. **(No code)**
  - In silico predictions suggest the variant will **NOT impact protein structure**. **(BP4)**
  - A second variant in *OTOF* was not identified in this individual, and other testing strategies for the gene (such as duplication and deletion) were performed.



Example 2: evidence indicates variant may lean Likely Pathogenic (LP)

CASE: A patient with ALS had genetic testing which returned a single heterozygous missense variant in *SOD1*. On the genetic testing report, the lab classified this as VUS:

- ✓ The following evidence is found for the variant:
  - The variant is **absent from the gnomAD** population database. **(PM2_Supporting)**
  - It has been **reported in the literature in one individual with ALS who was shown to have** reduced CuZn-SOD activity in erythrocytes. **(PS4_Supporting)**
  - In silico predictions suggest the variant will **impact protein structure,** and the residue is highly conserved in vertebrates. **(PP3)**
  - This variant is also present in **another affected individual** in the patient's family. **(PP1)**
  - *SOD1* gene is a well characterized gene in association with ALS, which is consistent with our patient's clinical diagnosis. However, there is no clinically available tests to distinguish SOD1-related ALS from other forms of ALS.



Example Scenarios and Next Steps

Variant less likely to be cause of disease

Patient has a sibling with the same condition; however the sibling was found to not have the VUS

Caveat: be careful of phenocopies

Loss of function variant found in a gene in which gain of function is the established disease mechanism

Second variant was NOT found, and the gene is associated with an autosomal recessive disease

Caveat: consider alternative testing methodologies to identify alternative variant types

Variant was inherited from an unaffected parent and, the gene is associated with an autosomal dominant disorder

Caveat: be careful of reduced penetrance and variable expressivity

Additional follow-up is needed

Patient is not known to have a common and specific finding of the disease

Follow-up: when possible, pursue additional, targeted phenotyping of patient presenting features of disorder

Patient has clearly affected and clearly unaffected relatives who have not been tested

Follow-up: Explore family members' willingness to be tested for the variant

Variant more likely to be cause of disease

Patient has a pathognomonic imaging or biochemical finding for the disorder

In the case of a gene associated with autosomal recessive disease, the patient has a second variant that is known to be pathogenic

Follow-up: When possible, test parents to determine if the two variants are in trans

If you have questions or feedback on this handout, would like to suggest changes or inquire about additional training, please contact the Variant Curation Task Force at variantcurationrequests@mgb.org.

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