

A guide to understanding variant classification

In a diagnostic setting, variant classification forms the basis for clinical decision making. Proper classification of variants is therefore critical to your ability to appropriately manage your patient and realize the best possible outcomes. Find out how we do it.





With a detailed explanation of the decisions made during our variant classification process, you can make more **confident diagnostic decisions** based on our results.

Clarity is crucial in diagnostic decision-making

In a diagnostic setting, variant classification forms the basis for clinical decision making. Proper classification of variants is therefore critical to your ability to appropriately manage your patient and realize the best possible outcomes.

Without thorough interpretation and evaluation of the evidence, sequencing results are not much more than meaningless data points. It is therefore crucial that the clinician is confident in the judgments made by their genetic diagnostics laboratory when it comes to variant classification.

It is standard practice across the genetic diagnostics industry for every company to develop and use its own in-house variant classification system. Most of the guidelines follow the recommendations of the ACMG guidelines, however, there are differences between the classification systems. This can be quite confusing, especially when it results in different classifications of the same variant between companies. Referring clinicians and genetic counselors should feel comfortable with the classification system used by their genetics diagnostics laboratory.

We aim to be transparent in everything we do, and that includes the decisions made during variant classification. When we share this information with you, you should have a clear understanding of how the sequencing results are evaluated and interpreted. We believe transparency provides assurance that variants are systematically and consistently classified according to established guidelines and practices. The end result is that you can make more confident diagnostic decisions based on our conclusions.

Founded in sound practices, built by the best

Using the ACMG guidelines as a framework, our classification system was built by an experienced, world-class team of laboratory geneticists and expert clinicians.

Blueprint Genetics has developed a variant classification system intended to classify variants in dominant monogenic disorders. These are rare diseases caused by single variants in single genes. Our system closely follows the guidelines and interpretation criteria established by the American College of Medical Genetics and Genomics (ACMG 2015), the industry standard for clinical genetic diagnostics laboratories.

Using the ACMG guidelines as a framework, our classification system has been built by an experienced, world-class team of geneticists and clinicians. It has also been greatly influenced by our experience in sequencing samples from thousands of patients.

Our 5-tiered scheme describes the quantity and quality of evidence needed to classify a genetic variant as pathogenic, likely pathogenic, a variant of uncertain significance (VUS), likely benign, or benign.

Systematic, clear, and sensible variant evaluation criteria

Assigning evidence-based criteria ensures that the decisions made by our entire variant review team are as accurate, traceable, and consistent as possible.

Variants are evaluated using evidence from population and gene/disease-specific databases, *in silico* prediction tools, our in-house variant database, and the relevant scientific literature. To this end, we use criteria to evaluate variants for potential pathogenicity, with evidence from the relevant databases and literature as the foundation for classification.

Assigning evidence-based criteria ensures all evidence is assessed and that the decisions made by our entire variant review team are as accurate, traceable, and consistent as possible. The use of criteria does not imply quantitative certainty in our evaluation. Rather, it establishes an objective checklist for assessing all of the available evidence.

In our professional opinion, the evidence based criteria system is the most straightforward way to ensure that everyone on the evaluation team comes to the same conclusion, and that you as the clinician can clearly understand the pathway of decisions that led to the classification. The system also ensures that the variants will always be classified based on the most up-to-date evidence available, regardless of the patient case. When a variant is reclassified, our follow-up report services make certain that all patients who tested positive for that variant receive an updated report. The follow-up report is accessible through our online portal, Nucleus, after a notification email is sent.

That said, comprehensive patient information and history plays an extremely important role in the variant review and classification process. With detailed clinical information, we are able to query the relevant medical literature for genotype-phenotype correlations, which has the potential to move a variant from the VUS or likely pathogenic to likely pathogenic or pathogenic.

Our 5-tiered variant classification system describes the amount and quality of evidence needed to classify a genetic variant.

- Pathogenic variant
- Likely pathogenic variant
- Variant of uncertain significance (VUS)
- Likely benign variant
- Benign variant

The 5 classification tiers explained

In a clinical setting, the main goal of genetic diagnostics is to determine whether the patient carries a pathogenic or likely pathogenic variant, as this knowledge can influence the care and treatment of the patient and their family members. Thus, we begin by evaluating the potential pathogenicity of variants by evaluating all existing evidence. If there is no or very little evidence to confidently support or rule out pathogenicity, the variant is classified as a VUS.

The classifications, primary criteria for evaluation, and suggestions for application in a clinical setting can be found on the following pages. These descriptions are only a summary of the evaluation criteria. For a full explanation, please refer to the “criteria needed” section under

A note about disease modifiers

Some variants could function as disease modifiers: variants that do not cause the disease, but which may worsen the outcome. Classification as a disease modifier can be applied when extensive scientific evidence has been established for a

Pathogenic variant

The variant is considered the cause of the patient’s disease.

Main evaluation criteria

The variant is well established as disease causing in the databases and literature, and a wide consensus on the variant’s pathogenicity exists. In these cases, significant family segregation has been verified and several publications support pathogenicity.

Additional criteria are shown to the right.

Recommendations for clinical usage

This genetic information can be used independently in clinical judgment and in evaluating risks for family members. We recommend family member testing and genetic counseling.

CRITERIA NEEDED

1 point

1. Well-established mutation and wide consensus in the field on pathogenicity of the mutation. (Typically significant family segregation has been established and several publications support pathogenicity.) **1 Point**
2. In the setting of a novel disease in the family, *de novo* truncating variant absent from control populations in a gene where loss of gene function has been established as a mechanism of pathogenicity for patient’s disease (both paternity and maternity confirmed.) **1 Point**
3. Loss-of-function (LOF) variant seen in at least 3 patients with the same phenotype (one of which can be the current patient) with cautious interpretation of the variants located in the last exon or in the last 50 base pairs of the penultimate exon. **1 Point**

or at least 5 points

Compulsory a or b:

- d. Positive segregation with the disease (≥ 2 families) and at least 5 unrelated patients (one of which can be the current patient) with the same variant and phenotype. **2 Points**
- e. ≥ 5 cases (one of which can be the current patient) with the same variant and phenotype reported.

Additional points:

1. Variant is novel or very rare in control populations (cannot be applied for ethnic backgrounds absent from control populations). **1 Point**
2. Loss of gene function has been established as a mechanism of pathogenicity; scientific evidence for a genotype-phenotype association exists. **1 Point**
3. A missense variant predicted deleterious by a majority of *in silico* tools applied and/or a well-established paralog mutation exists. **1 Point**
4. *De novo* alteration in the setting of a novel disease in the family (paternity and maternity unconfirmed). **1 Point**
5. Variants considered deleterious (a substitution or indel in consensus splice site (+/-1, 2), nonsense, and frameshift variants). **1 Point**
6. Deficient protein function in appropriate functional assay(s), eg, an animal model with an equivalent mutation or splice site defect confirmed on the mRNA level. **1 Point**
7. Well-characterized other mutation at the same codon or same splice consensus site (+/-1, 2). **1 Point**

Likely pathogenic variant

The identified variant is considered the probable cause of the patient's disease. This information should be used cautiously for clinical decision-making, as there is still a degree of uncertainty.

Main evaluation criteria

A clear genotype-phenotype correlation exists. In these cases, it is essential to have thorough background information from the referring clinician about the patient's phenotype, which helps to determine the probable pathogenicity. The variant typically results in premature truncation (an incomplete protein product) in a gene where loss of function has been established as a mechanism of pathogenicity for the patient's suspected disease. Alternatively, the variant is an amino acid substitution (missense), which is predicted deleterious by the majority of *in silico* tools applied. In addition, the variant is novel or very rare in control populations.

Additional criteria are shown to the right.

Recommendations for clinical usage

We recommend family member testing and genetic counseling, but the variant alone should not be used for family risk stratification. That said, we believe that a likely pathogenic variant could be used to rationalize family member risk stratification and a follow-up strategy on a case-by-case basis. This could include additional genetic counseling after 2 to 5 years to evaluate the status of the variant. Family member testing may offer new evidence to support further classification of the variant as pathogenic.

CRITERIA NEEDED

2 points

1. Alterations resulting in premature truncation (eg, frameshift, nonsense, or consensus splice site (+/-1, 2) in a gene where loss of gene function has been established as a mechanism of pathogenicity for the patient's disease.
1 Point
2. Variant is novel or very rare in control populations (cannot be applied for ethnic backgrounds absent from control populations).
1 Point

or at least 4 points

1. Clear genotype-phenotype correlation exists (eg, MFS and *FBNI*).
1 Point
2. Variant is novel or very rare in control populations (cannot be applied for ethnic backgrounds absent from control populations).
1 Point
3. Missense variant predicted deleterious by a majority of *in silico* tools applied.
1 Point
4. A variant predicted to have an effect to the splicing by majority of *in silico* tools applied.
1 Point
5. An inframe deletion affecting conserved aa in a functional domain.
1 Point
6. Variant has been identified in ≥ 2 individuals (one of which can be the current patient) with the same disease manifestation.
1 Point
7. Evidence of a well-established paralog mutation exists.
1 Point
8. *De novo* alteration in the setting of a novel disease in the family (paternity unconfirmed).
1 Point
9. Variants considered deleterious (a substitution or indel in consensus splice sites (+/-1, 2), nonsense, and frameshift variants) identified in a gene with weak evidence for causativity in the disease type.
1 Point
10. Deficient protein function in appropriate functional assay(s), eg, an animal model with an equivalent mutation or splice site defect confirmed on the mRNA level.
1 Point
11. Well-characterized mutation at the same codon or same splice consensus site (+/-1, 2).
1 Point
12. Other strong data supporting pathogenic classification.
1 Point

Variant of uncertain significance (VUS)

The variant has characteristics of being an independent disease-causing mutation, but insufficient or conflicting evidence exists.

Main evaluation criteria

The variant is typically very rare, predicted to be deleterious, and the gene has an association with the patient's phenotype.

Recommendations for clinical usage

The management of the patient and their family should be based on clinical judgment. This genetic information should not be used for family risk stratification, and we do not recommend family member testing in a diagnostic setting. However, in some cases family member testing may be useful, especially when the disease affects multiple individuals in the family and the variant has several characteristics that suggest it is disease causing. In these cases, a segregation study may help to gain the information needed to reclassify the variant as likely pathogenic or likely benign. Find out more at: blueprintgenetics.com.



Likely benign variant

The variant is not likely to be the cause of the tested disease.

Main evaluation criteria

Taking disease prevalence and penetrance into account, the minor allele frequency (MAF) in control populations is considerable (MAF < 0.001).

Additional criteria are shown to the right.

Recommendations for clinical usage

Genetic tests with only likely benign variants are considered a negative test result.

CRITERIA NEEDED

1 point

Control population minor allele frequency (eg, gnomAD) is considerable (MAF > 0.001) (disease prevalence must be taken into account).

1 Point

or at least 2 points

1. MAF < 0.001 in control populations but the variant is detected in healthy controls with no disease association in a case-control study/studies.
1 Point
2. Homozygous variant in a gene with no association with the disease.
1 Point
3. Co-occurrence with a pathogenic mutation in the same gene (phase unknown) or in another gene that clearly explains the proband's phenotype.
1 Point
4. The majority of *in silico* tools predict the substitution to be benign.
1 Point
5. Intact protein function observed in appropriate functional assay(s), eg, a splice-region variant without abnormal splicing.
1 Point
6. Other data supporting benign classification.
1 Point

Benign variant

The variant is not considered to be the cause of the tested disease.

Main evaluation criteria

It is evident that the variant does not segregate with the disease in families with 2 or more affected individuals.

Additional criteria are shown to the right.

Recommendations for clinical usage

Genetic tests with only likely benign variants are considered a negative test result.

CRITERIA NEEDED

2 points

Does not segregate with the disease in a family or families with two or more affected individuals.

1 Point

Any additional criteria described in this section.

1 Point

or at least 4 points

1. Control population minor allele frequency (eg, gnomAD) is considerable (MAF > 0.001) (prevalence of the disease must be taken into account).
1 Point
2. Homozygous variant in a gene with no association to the disease.
1 Point
3. Intact protein function observed in appropriate functional assay(s), eg, a splice-region variant without abnormal splicing.
1 Point
4. Co-occurrence with a pathogenic mutation in the same gene (phase unknown) or in another gene that clearly explains the proband's phenotype.
1 Point
5. No disease association in small case-control study.
1 Point
6. Majority of the *in silico* tools predict the substitution to be benign.
1 Point
7. Other data supporting benign classification.
1 Point

Our **transparency** helps to drive future developments

Not every genetic testing company shares their findings in public databases such as ClinVar. By reporting our findings, we aim to contribute to the advancement of the field of genetics. With this ever-growing body of research, as well as new analytical tools and reference databases, the field of genetics is advancing rapidly.

This growth of knowledge also has an impact on variant classification. In the event that new insights prompt variant reclassification, we consider it our responsibility to inform you so that you can re-evaluate diagnoses and care choices for your patients and their families.

We also hope our transparent contributions will

We are continuously developing our services and offering. We may amend service descriptions from time to time by posting new versions on our website. For up-to-date information, please visit blueprintgenetics.com.

We empower clinicians and geneticists with the most **accurate diagnostics** possible, helping them to better serve their patients.

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