Invitae's method of variant classification

Executive summary

Invitae's team of scientists, physicians, and genetic counselors works together to provide high-quality and rigorous variant classifications. Invitae's method of variant classification is a systematic process for assessing the evidence gathered during variant review and applying a formal variant classification based on this evidence.

Our method of variant classification adheres closely to the recommendations from the American College of Medical Genetics (ACMG).[1,2] These guidelines for variant interpretation represent the industry standard among clinical genetic testing laboratories. Invitae communicates the evidence and logic behind the classification to the clinician through an interpretive report.

Variant research

The first step of variant interpretation is to collect and research evidence related to each sequence change.

If available, the following types of evidence are reviewed during this process:

Strong to moderate evidence. Considered most informative for variant classification.

- Previous observations of the sequence change in individuals
  - Is the sequence change found in control populations at a frequency above the reported incidence of its associated disease?
  - Is the change reported in affected individuals?
  - Does the change segregate with disease in family pedigrees?
  - Is there evidence from case-control studies that this change increases disease risk?
  - Does the change co-occur with a pathogenic variant in affected individuals?

- The type of sequence change and mechanism of disease
  - Is it a truncating mutation (i.e., nonsense, frameshift) in a loss-of-function gene?
  - Does the sequence change alter the protein sequence (i.e., missense) or a consensus splice site?

- Experimental evidence
  - Does this sequence change impact protein function or disrupt splicing?
  - Is the impact deleterious or neutral?
  - Are there in vivo animal experiments demonstrating a physiological effect?
  - Are there in vivo cellular or in vitro molecular assays that demonstrate indirect effects, i.e., morphological, viability, or molecular interactions?

Databases reviewed during variant research:

- Population databases:
  - 1000 Genomes (http://browser.1000genomes.org)
  - NHLBI GO Exome Sequencing Project (ESP) (http://evs.gs.washington.edu/EVS)
  - dbSNP (http://www.ncbi.nlm.nih.gov/snp)
  - dbVar (http://www.ncbi.nlm.nih.gov/dbvar)

- Disease databases:
  - ClinVar (http://www.ncbi.nlm.nih.gov/clinvar)
  - ClinVitae (http://clinvitae.invitae.com)
  - Online Mendelian Inheritance in Man (OMIM) (http://www.omim.org)
  - Human Gene Mutation Database (HGMD) (http://www.hgmd.org)
  - Human Genome Variation Society (HGVS) (http://www.hgvs.org)
  - Leiden Open Variation Database (LOVD) (http://www.lovd.nl)
  - DECIPHER (http://decipher.sanger.ac.uk)

- Sequence databases:
  - NCBI genome (http://www.ncbi.nlm.nih.gov/genome)
  - RefSeq Gene (http://www.ncbi.nlm.nih.gov/refseq/rsg
  - Locus Reference Genomic (LRG) (http://www.lrg-sequence.org)
  - MitoMap (http://www.mitomap.org/MITOMAP/HumanMitoSeq)

Supporting evidence. These evidence types are considered secondary to those listed above, but they may provide supporting information or additional context.

- Indirect and predictive evidence
  - Does the sequence change alter a highly conserved amino acid(s) or a functionally important residue?
  - Are there other reported missense mutations nearby?
  - Does the sequence change disrupt a functionally or structurally important protein domain?
  - Do multiple computational algorithms consistently suggest an impact on protein function or splicing?

- Clinical interpretations from other laboratories
  - Is the sequence change in ClinVar or a locus-specific database?
  - Is the interpretation consistent with our interpretation?

Variant classification

After the sequence change has been thoroughly researched and the relevant information has been gathered, a formal variant classification is assigned to the sequence change.

A formal classification is meant to help answer two questions about the clinical significance of the sequence change.

- From a **diagnostic** perspective: Does this sequence change, in the correct genetic background, provide an explanation for disease in an affected individual?
- From a **predictive** perspective, narrowly applied to highly penetrant conditions: Is an individual who inherits this sequence change likely to develop disease?

The ACMG has recommended a five-tier classification system.[1] According to this system, a sequence change can be classified as:

- **Pathogenic**: This sequence change directly contributes to the development of disease. Some pathogenic sequence changes may not be fully penetrant. In the case of recessive or X-linked conditions, a single pathogenic sequence change may not be sufficient to cause disease on its own. Additional evidence is not expected to alter the classification of this sequence change.

- **Likely pathogenic**: This sequence change is very likely to contribute to the development of disease; however, the scientific evidence is currently insufficient to prove this conclusively. Additional evidence is expected to confirm this assertion of pathogenicity, but we cannot fully rule out the possibility that new evidence may demonstrate that this sequence change has little or no clinical significance.

- **Uncertain significance**: There is not enough information at this time to support a more definitive classification of this sequence change.

- **Likely benign**: This sequence change is not expected to have a major effect on disease; however, the scientific evidence is currently insufficient to prove this conclusively. Additional evidence is expected to confirm this assertion, but we cannot fully rule out the possibility that new evidence may demonstrate that this sequence change can contribute to disease.

- **Benign**: This sequence change does not cause disease.

One additional classification has also been developed at Invitae:

- **Pathogenic (low penetrance)**: This sequence change is commonly accepted as a contributing factor of disease. However, the penetrance of this particular change is sufficiently low (<25%) that it is often seen in individuals without disease. As a result, the predictive value of this information is considered to be low.

Variants are assigned a formal classification based on:

- **Weighted evidence**: The evidence gathered during variant review is assessed based on the relative importance of the evidence type. Certain types of evidence may support a pathogenic classification while others may support a benign classification.
- **Grouping by basic argument**: If multiple pieces of evidence point to the same basic argument, only the strongest piece of evidence is considered.
  - Example: A functional study that demonstrates reduced protein levels and an in silico algorithm that predicts that the variant is deleterious, both contribute to the general argument that "protein function may be disrupted."
- **Additive vs. non-additive evidence**: Certain types of evidence can be considered more than once, and certain types cannot.
  - Example: Independent observations of the variant in multiple affected individuals are additive.
  - Example: Multiple experimental studies demonstrating that different aspects of protein function are disrupted are non-additive.
- **Professional judgment and review**: Each variant classification undergoes extensive review by our clinical reporting team. Invitae has developed proprietary software tools to efficiently record and share information throughout this process.
  - A PhD scientist with a medical or human genetics background initially researches and classifies the sequence change.
  - A licensed genetic counselor summarizes the clinical significance of the sequence change for the patient.
  - A second individual with a PhD or MD degree reviews the evidence and logic behind the original variant classification.
  - The report as a whole is given final review and approval by a lab director.
  - Interesting and/or complicated variants are discussed at a case review conference.

Reporting

Our clinical report documents the evidence and logic supporting each variant interpretation to enable the ordering clinician to evaluate and, if appropriate, discuss the evidence with their patients. All language used to describe genetic variants and variant interpretation is systematic and consistent with ACMG[1] and Human Genome Variation Society (HGVS)[3] recommendations.

The report includes:

- **Clinical summary**: a description of the relevance of the genetic results to the patient based on their clinical and family history
- **Variant details**: a summary of the evidence and logic used to justify the interpretation of the sequence change

The field of genetics is constantly evolving, often revealing new evidence relevant to variant interpretation. When new evidence on a variant becomes available, we review our variant interpretation and, if indicated, we will reclassify the variant and issue an amended report to the ordering clinician. If a report is amended, the ordering clinician will receive a notification via phone or email.

Genetic testing can have health implications not only for an individual, but for an entire family. To help resolve variants of uncertain significance (VUS) in our test results, Invitae offers complimentary follow-up testing to select family members of patients tested at Invitae when informative data can be obtained. We also offer affordable targeted family variant testing to help identify other family members who have the same sequence change as the original patient.

To learn more about these programs, please visit www.invitae.com/vus-resolution and www.invitae.com/family-testing.

Clinical validation

Invitae offers high-quality genetic testing services for a broad menu of genes, including BRCA1 and BRCA2. To measure the performance of our BRCA1/2 test, Invitae has embarked on a rigorous study to validate both our next-generation sequencing (NGS) assay results and our clinical variant classifications.

To learn more, visit www.invitae.com/validation-studies.

Invitae has also collaborated with Stanford University researchers, James Ford, MD, and Allison W. Kurian, MD, MSc, to demonstrate the value of multi-gene panels in hereditary cancer risk assessment.[4] Invitae's variant classifications for the study participants were validated as part of this research.

To learn more, visit www.invitae.com/clinical-studies.

References

1) Richards CS, et al. ACMG recommendations for standards for interpretation and reporting of sequence variations: Revisions 2007. *Genet Med*. 2008; **10**(4): 294-300. (2013 revision in press)

2) Duzkale H, et al. A systematic approach to assessing the clinical significance of genetic variants. *Clin Genet*. 2013; **84**: 453-463.

3) den Dunnen JT and Antonarakis SE. Recommendations for the description of protein sequence variants (v2.0). *Hum Mutat*. 2000; **15**:7-12.

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INVITAE

+1 415-374-7782 or 800-436-3037
458 Brannan Street, San Francisco, CA 94107
clinical@invitae.com www.invitae.com