Introduction

With increasing use of high-throughput sequencing and multi-gene panels for diagnostic purposes, there is growing concern about the potential for inconsistent variant classifications among clinical labs. The American College of Medical Genetics and Genomics (ACMG) recently drafted new standards and guidelines for the interpretation of sequence variants (ISV) to address this concern (Richards et al., Genet Med 2015). Draft versions of these guidelines were shared broadly with the clinical genetics community, feedback was incorporated, and an evidence-based checklist for interpreting Mendelian disease variants has now been published. This checklist represents a major step toward evidence-assessment standardization and variant-classification consistency. However, many ISV criteria are quite expansive, which could result in inconsistency in their application. To date, a rigorous study has not been published to examine the impact of these new guidelines on variant classification and clinical reporting. It also remains to be seen whether the new evidence checklist results in increased interpretation concordance between clinical laboratories. We considered these issues when validating our laboratory’s classification procedures, which are based on the new ISV guidelines.

A Score-Based System for Interpretation

We developed, validated, and implemented a score-based variant classification system based on the ISV guidelines. This implementation, Sherloc, adheres to the guidelines as published while adding refinements to ensure consistent use and accurate conclusions when applied broadly. This implementation adds greater guidelines as published while adding refinements to ensure consistent use and system based on the ISV guidelines. This implementation adds greater

Defining Principles

1. Five major categories of evidence are considered in a hierarchical approach.
2. A point score is given to evidence (EV) criteria within each category.
   • A null variant (i.e., nonsense, frameshift) in LOF gene is 5 points for Pathogenic.
   • Very high MAF (i.e., much greater than expected) is 5 points for Benign.
   • All other EV scores reflect the relative importance along this scale.
   • Miscellaneous EV for Benign or Pathogenic can be applied as necessary.
3. Only the strongest EV is counted if two criteria contribute to the same basic argument (e.g., protein function is disrupted, either STRONG or WEAK).
4. Certain EVs can be counted more than once (e.g., single co-occurrences) and others cannot (e.g., 4+ case reports).
5. EV scores are calculated in Pathogenic and Benign directions independently.
6. Classification thresholds (P, LP, LB, B) are predetermined, and point scores above these thresholds receive a “calculated” classification.
7. Calculated classifications are suggestions only. The final interpretation is manually performed by a trained genomic scientist. All variant interpretations are reviewed by a 2nd experienced genomic scientist and an ABMG-certified medical geneticist.

A Consensus-Based Method for Evaluating an Interpretation Schema

To evaluate concordance of this method with community standards, we constructed a consensus of Sherloc and all ClinVar interpretations for variants where at least 2 other diagnostic lab reports were available. Consensus was defined as at least 60% of laboratories (i.e., 2 out of 3 labs, 3 of 4, etc.) agreeing on the 5-class scale. We then conducted an “odd man out” analysis to see how often any lab disagreed with this consensus. This particular methodology was chosen to facilitate an identical comparison for all labs using the same variants and same consensus interpretations. 814 variants could be compared, and methodology were excluded because no consensus was formed.

Re-evaluation of Discordant Variants

To evaluate these discordant variant classifications and at the same time assess the robustness of the Sherloc system, we selected 42 variants to be reinterpreted by a different scientist blinded to the original interpretation. Importantly, these 42 variants are strongly enriched for difficult-to-interpret cases and are not representative of most variants. Nevertheless, our reinterpretations of these variants showed high reproducibility:

• 39 (92.5%) matched the previous interpretation exactly.
• 2 matched within the same pathogenicity group:
  - 1 to LP change with inconsistent use of Clinical Findings.
  - 1 to LB change with omission of Misc. Benign evidence.
• 1 changed (VUS to LP) with addition of Misc. Pathogenic evidence.

Comparison with Individual Submissions

While a large number of ClinVar records had multiple submissions and a derived consensus interpretation, many did not. We thus also compared Sherloc against individual ClinVar submissions from diagnostic labs to evaluate the type and direction of disagreements that occur. This analysis included 1886 variants, of which 623 had one or more disagreements with Sherloc.

Conclusions

With the 2015 ACMG ISV recommendations for variant interpretation as our guide, we developed Sherloc, a score-based classification system with detailed evidence criteria, inherent logic for handling interdependent evidence, and comprehensive notes outlining caveats, various use cases, and evidence considerations for each criteria. This system has been implemented in our clinical-testing workflow and refined over the past 15 months. Importantly, Sherloc interpretations are highly consistent with those submitted to ClinVar. The methods used in this study to evaluate the concordance of Sherloc may be widely applicable to any clinical molecular laboratory looking to evaluate the concordance and consistency of their own interpretations.