Harmonization of the ACMG–AMP Scoring Rules and Experiences in Clinical Adoption Within Opal™ Clinical, a Genome Interpretation Software Platform

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ABSTRACT

The ACMG-AMP Standards and Guidelines for the interpretation of sequence variants, published in March 2015, provides a rubric for those who attempt to classify variants in the context of Mendelian disease (pathogenic, benign, uncertain significance). This rubric has 28 criteria to be answered, and rules for how the answers aggregate to a classification.

We introduce a tool that steps users through the criteria, presenting appropriate contextualized information to be considered, and updating the inferred classification. We demonstrate its use on a SCN1A variant missense.

The tool is in use clinically, and here we show a subset of ~11,700 variants classified using it. For the vast majority of variants (~95%) the classification implied by the Guidelines was the one chosen for reporting; in a small minority the classification was changed, as allowed for by the Guidelines.

Focusing on missense variants within this data, we discuss the issue that variants with previous cases reported are both more common (and hence less likely to be deleterious) and are much more likely to be classified as Pathogenic or Likely Pathogenic (because there are several criteria that only apply if a variant has previous cases reported).

The ACMG-AMP Guidelines of March 2015
Provide a Framework for Variant Interpretation

The ACMG-AMP Standards and Guidelines for the interpretation of sequence variants (hence the Guidelines), published in March 2015, provides a rubric for the clinical interpretation of germline variants involved in Mendelian disease. The rubric has 28 criteria, which are answered using all available evidence. Each piece of evidence is given a certain weight (e.g. strong, medium, and these weights are aggregated, using a set of rules, into one of five classifications: Pathogenic, Likely Pathogenic, Variant of Uncertain Significance (VUS), Likely Benign, and Benign.

The Guidelines were designed to reduce misclassification of variants by:
- Improving communication within and between labs, and with clinicians
- Introducing standardization within and between labs
- Aggregating evidence using the “scorecard” approach

In practice, discordance in classification remains even when the Guidelines are followed, due to the subjective process of deciding which criteria are met.

One recent study (Collins et al, 2014) found discordance remained high, but that the Guidelines provided a valuable tool for discussing differences in classifications. Currently, best practice would be to ensure that all available evidence is utilized, and to address and standardize gray areas in the criteria.

Introducing a Tool to Speed up the Variant Interpretation Process

We introduce an advanced scoring capability with Opal™-Clinical that steps users through the criteria, presenting appropriate contextualized information for consideration. This new tool:
- Shows the variant’s previous scoring history, both in the lab and in ClinVar
- Allows for the interpretation in the context of a given condition, as specified by the Guidelines
- Steps through each criterion in a logical order
- Provides a simple check-box per criterion
- Provides resources and tools specific to each criterion
- Automatically calculates and updates the Classification based on the Guidelines’ rules
- Allows for the calculated classification to be overridden
- Collates associated references, and allows these to be reported out
- Shows a summary of the criteria met

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An Example Variant: SCN1A Tyr413Asn

Here we show the tool in action interpreting a variant in SCN1A, the Tyr413Asn missense mutation. This variant has been in the limelight as its interpretation is the subject of a pending lawsuit brought against a diagnostic lab. The variant was reported as a VUS; the case turns on whether the laboratory originally misclassified the variant. Although the variant was originally reported before the Guidelines were published, it can still be used to illustrate aspects of the Guidelines, as done in a recent Medscape paper.

There are four criteria which are fairly clearly met (see Figures):
- PM1: Absent from controls (or at extremely low frequency if excessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
- PM2: Absent from controls (or at extremely low frequency if excessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
- PM3: Absent from controls (or at extremely low frequency if excessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
- PM4: Absent from controls (or at extremely low frequency if excessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

The tool pulls this information through, such that it is clear the criteria are met.

PM1: Located in a mutational hotspot and/or critical and well-established functional domain without benign variation.

The tool presents two main data sources to help users answer this criterion:
- The missense score from Samocha et al, downloaded from ExAC, which indicates how evolutionary constrained the gene is for missense variation, and B) links out to the protein domains where the variant falls in. This variant meets the functional domain part of the criteria.

PM2: Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease

The tool displays two main data sources to help users answer this criterion:
- The missense score from Samocha et al, downloaded from ExAC, which indicates how evolutionary constrained the gene is for missense variation, and B) links out to the protein domains where the variant falls in. This variant meets the functional domain part of the criteria.

PM3: Variants with previous cases reported are both more common (and hence also may be pathogenic).

The tool presents two main data sources to help users answer this criterion:
- The missense score from Samocha et al, downloaded from ExAC, which indicates how evolutionary constrained the gene is for missense variation, and B) links out to the protein domains where the variant falls in. This variant meets the functional domain part of the criteria.

PM4: Pathogenic and Likely Pathogenic classification.

The tool presents two main data sources to help users answer this criterion:
- The missense score from Samocha et al, downloaded from ExAC, which indicates how evolutionary constrained the gene is for missense variation, and B) links out to the protein domains where the variant falls in. This variant meets the functional domain part of the criteria.

One criteria is possibly met, PM4: The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls.

There are several places where cases can be found. One of these is the primary literature, and the tool structures searches using different transcript aliases to maximize the return of papers. The applicability of a paper to the phenotype needs to be addressed by the reader. The tool does not do this for the reader, and the reader should make this determination themselves.

If the reader were to do this, they would see that the工具 would have solidified the classification.

CONCLUSIONS

The ACMG Guidelines give a solid framework for interpreting sequence variation in a clinical context.

The subjectivity inherent in the criteria makes the classification progress hard to automate.

We present a tool designed to make following the ACMG Guidelines as straightforward and as quick as possible. The tool also provides transparency for all those using it, via tracking all the evidence and its assessment.

The ACMG Guidelines heavily weight evidence that a variant has been previously reported. Even with the latest databases such as ClinVar, it will be hard for very rare missense variants to receive a non-VUS classification.

TABLE 1: Evidence from previous cases plays a crucial role in shifting missense variants away from VUS towards either benign or pathogenic.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Novel rare missense variants</th>
<th>Novel common variants</th>
<th>VUS with previous cases</th>
<th>VUS with previous cases</th>
</tr>
</thead>
</table>

TABLE 2: The rarer the variant the more likely it is to be deleterious, but also the less likely it is to have been reported in a case (see Table 2). Hence the conundrum: those variants more likely to be pathogenic (the rare ones) can’t be harder to classify as pathogenic, because the Guidelines place such weight on previous cases.

These data suggest the limitations of a focus on building up databases of previous cases, and an important future role for better high throughput computational and experimental approaches.

1. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, Sue Richards et al, Genetics in Medicine, March 2015
3. The Athena Diagnostics Lawsuit: a Teaching Moment, Jeanette J. McCarthy, and Bryce Mendelsohn, Medscape

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