# 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 missense variant.

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)[1], 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

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

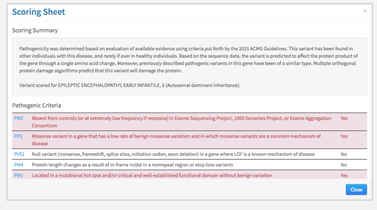
e.g. PS4: Prior observation of the variant in multiple unrelated patients with the same disease and absence in controls. What does "multiple" mean? What does "same disease" mean? Where should you look for the information, and how hard should you look?

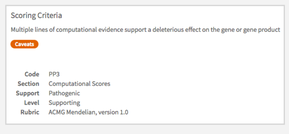
A recent study[2] found discordance remained high, but that the Guidelines provided a vocabulary for discussing differences in classifications. Currently, best practice would be to ensure that all the available evidence is utilized, and to address and standardize grey areas in the criteria.

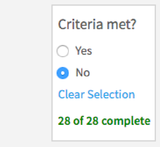
## Introducing a Tool to Speed up the Variant Interpretation Process

We introduce an advanced scoring capability within 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 overriden
- Collates associated references, and allows these to be reported out
- Shows a summary of the criteria met



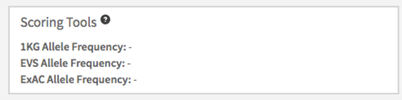

## An Example Variant: SCN1A Tyr413Asn

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 is done in a recent Medscape paper[3].

There are four criteria which are fairly clearly met (see Figures): **PM2, PP2, PM1, PP3**. These criteria (two moderate and two supporting) would give a classification of Likely Pathogenic.

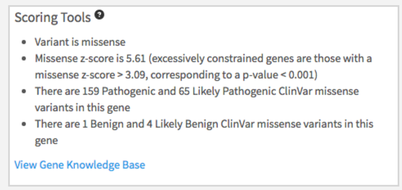
**PM2:** Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

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



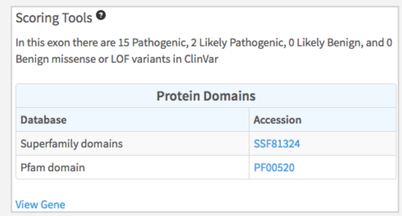

**PP2:** Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease

The tool displays two main data sources to help users answer this criterion: A) The missense z-score from Samocha et al, downloaded from ExAC, which indicates how evolutionary constrained the gene is for missense variation, and B) The counts of variants in the gene in ExAC and across the spectrum of ClinVar classes. The combination of these two data sources suggests the criteria is met.



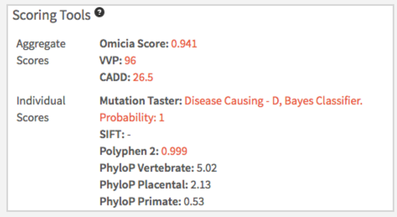

**PM1:** Located in a mutational hot spot and/or critical and well-established functional domain without benign variation

The tool presents two types of data, A) The counts of variants in the exon in ExAC and across the spectrum of ClinVar classes, and B) links out to the protein domains that the variant falls in. This variant meets the functional domain part of the criteria.



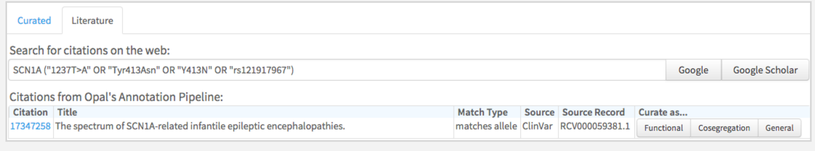

**PP3:** Multiple lines of computational evidence support a deleterious effect on the gene or gene product

The tool displays multiple computational scores and color codes them appropriately. The fact that all are agreeing that the variant falls in the "red" indicates that this criteria is met.




**One criteria is possibly met, PS4:** 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 allows the user to save citations and pull them through to the final report. In this case, there were two cases of other individuals with this variant at the time of the original report, one appearing in the paper shown in the screen shot of the tool's Citation Manager, and the other in a paper retrieved as the top Google Scholar search.




If met, this criteria would take the classification to Pathogenic. Parental testing to confirm whether the variant was de novo would have solidified the classification.

## Analyzing Use of the Tool Gives Insights into How the Guidelines Are Followed in Practice

We look at a subset of ~11,700 variants classified in our system. For all the variants, all 28 criteria were answered.

Of these variants, the calculated classification implied by the Guidelines was overriden in ~5% of cases. Five times as many variants were changed from Pathogenic/Likely Pathogenic to VUS as vice versa, reflecting the "downgrade if in doubt" mantra.

## The Novel Variant Conundrum

The Guidelines heavily weight evidence of previous cases. Indeed, there are five pathogenic criteria that are based largely on this evidence: PS1, PM5, PS4, PP5 and PP1.

In the absence of previously reported cases, it can be hard for variants to gain enough evidence to be classified as Pathogenic or Likely Pathogenic. Here we focus on missense variants which do not have previous cases – let us call these novel variants. Such variants can meet the four criteria clearly met by the SCN1A variant discussed above (PM2, PP2, PM1, PP3), and possibly PP4 (Patient's phenotype or family history is highly specific for a disease with a single genetic etiology). In aggregate, these criteria could never obtain a Pathogenic classification. The only way such variants could obtain a Pathogenic classification is if convincing functional data existed (criteria PS3), or further information was known about the patient (e.g. de novo status of the variant).

Given the weight the Guidelines place on previously cases, it is unsurprising that in our data set missense variants with previous cases are over three times as likely as novel missense variants to end up with a Pathogenic/Likely Pathogenic classification (**see Table 1**).

The rarer a 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 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.

| Classification | # Novel missense variants | Novel missense variants % | # Missense variants with previous cases | Missense variants with previous cases % |
|---|---|---|---|---|
| Pathogenic | 0 | 0% | 1 | 0% |
| Likely Pathogenic | 16 | 0.6% | 81 | 2.1% |
| VUS | 2086 | 85% | 1552 | 40% |
| Likely Benign | 352 | 14% | 2232 | 57% |
| Benign | 10 | 0.4% | 41 | 1.0% |

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

| | # Missense variants | % In CV |
|---|---|---|
| Absent | 1121 | 16% |
| AF ≤ 0.0001 | 843 | 32% |
| 0.0001 ≤ AF < 0.001 | 1152 | 52% |
| 0.001 ≤ AF < 0.01 | 1732 | 84% |
| AF > 0.01 | 1523 | 89% |

**TABLE 2:** The rarer the variant, the less likely it is to be in ClinVar. Allele Frequency, AF, is largest frequency reported in the 1000 Genomes Project, the Exome Sequencing Project, or the Exome Aggregation Consortium

## 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 growth of databases such as ClinVar, it will be hard for very rare missense variants to receive a non-VUS classification.

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
2. Performance of ACMG-AMP Variant-Interpretation Guidelines among Nine Laboratories in the Clinical Sequencing Exploratory Research Consortium, LM Amendola et al, AJHG, May 2016
3. The Athena Diagnostics Lawsuit: a Teaching Moment, Jeanette J. McCarthy, and Bryce Mendelsohn, Medscape

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