Performance of ACMG/AMP variant interpretation guidelines among nine laboratories in the Clinical Sequencing Exploratory Research consortium

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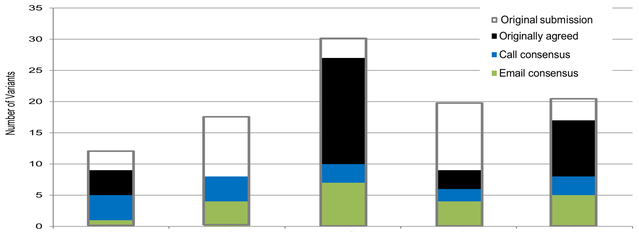
Introduction

- There is a need to standardize pathogenicity classification of genomic variants in medical sequencing.
- We have previously reported both inconsistency across labs in variant classification and a bias towards overcalling pathogenicity.[1, 2]
- The ACMG recently published guidelines for variant classification for Mendelian disorders designed to increase consistency among labs.[3]
- The Clinical Sequencing Exploratory Research (CSER) Consortium piloted the use of these rules by 9 of the CLIA labs supporting CSER projects.

Methods

- 99 variants were considered, representing all categories (pathogenic, likely pathogenic, uncertain significance (VUS), likely benign, and benign).
- 9 were classified by all 9 labs, 90 variants were classified by 3 labs using both the lab's own classification system and also the ACMG guidelines.
- We evaluated both intra-laboratory and inter-laboratory differences among variant classifications using the labs' criteria vs. adopting ACMG criteria.
- Discrepant ACMG classifications were discussed via phone conferences (including the 9 variants classified by all 9 labs) and over email to identify sources of discordant variant classifications and clarify correct use for the ACMG lines of evidence.

Figure 2. Distribution of 99 Variants Submitted for Assessment. Gray outlines illustrate the distribution of variant classifications submitted. Green bars indicate calls that were agreed upon after initial review, blue indicates calls agreed upon after email exchange and the black bars indicate calls agreed upon after discussion on conference calls.



Results

Figure 1A. Intra-laboratory Concordance of Lab vs. ACMG/AMP Classification Systems. 275/347 (79%) variant classifications were concordant across labs; 48/72 (67%) discordant calls, the ACMG/AMP system calls were closer to VUS, mostly due to labs more often calling likely benign and benign with their own criteria.

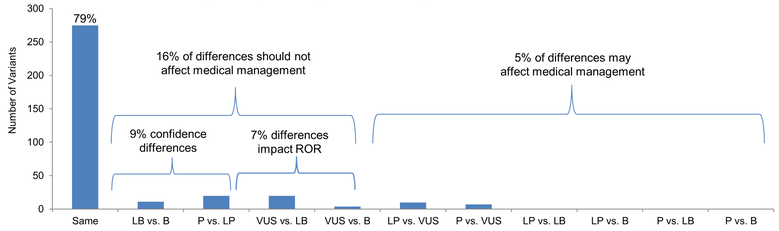


Figure 1B. Inter-laboratory Concordance of 97 Variants. Labs fully agreed 34% of the time. There was no statistically significant difference in concordance whether the lab used their own criteria vs ACMG criteria. (K Alpha Lab = 0.77, 95% CI [0.73, 0.80] vs. ACMG/AMP = 0.70, 95% CI [0.66, 0.74])

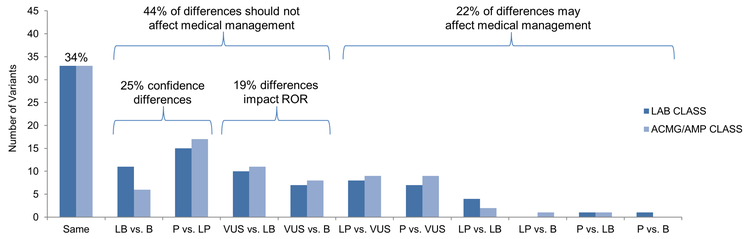


Figure 1C. Inter-laboratory Concordance after Consensus Efforts. After either email or telephone conference calls, consensus was achieved for 70/99 (71%) variants.

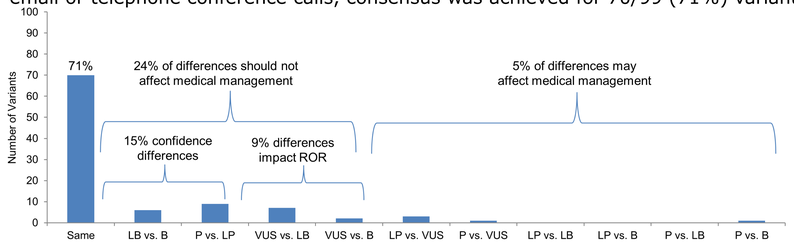


Table 1. ACMG/AMP rule clarifications and suggestions for modification

Rule	Description	Clarifications/Suggestions
PVS1	Predicted null where LOF is a mechanism for disease	• Do not apply to variants near the 3' end of the gene that escape nonsense mediated decay
PS1	Same amino acid change as previously established pathogenic variant, regardless of nucleotide change	• Does not include the same variant you are assessing as its not yet pathogenic and the rule is intended for variants with a different nucleotide change
PS2	De novo variant with confirmed maternity and paternity	• Suggest applying as moderate or supporting if variant is mosaic and frequency in tissue is consistent with phenotype
PS3	Well established functional study	• Suggest reduced strength for assays that are not as well-validated or less well linked to phenotype
PM1	Located in mutational hot spot and/or critical/well established functional domain	• Not meant for truncations, more clarification needed for application of rule
PM2/ BS1	Absent in population databases/allele frequency is too high for the disease	• Cannot assume longer indels would be detected by NGS • Suggest published control dataset can be used if size is ≥1000 • Cannot be applied for low quality calls or non-covered regions • Must define the condition and inheritance pattern
PM3	For recessive disorders, in trans with a pathogenic variant	• Suggest invoking as supporting if phase is not established • Can upgrade if more than one proband reported
PM4	Protein length changing variant	• Applicable for in-frame deletions, insertions or stop loss variants, not frameshifts, nonsense and splice variants
PM5	Novel missense at amino acid with different pathogenic missense change	• Ensure pathogenicity of previously reported variant • Suggest changing "novel" to "different" as some variants that are not novel may require assessment with this rule
PP3/ BP4	Multiple lines of computational evidence	• All lines must agree
PP4	Patient's phenotype or family history highly specific for genotype	• Not to be used for genetically heterogeneous or unsolved etiology conditions • Not typically applied for an incidental finding analysis but may be applied for prior observations
PP5/ BP6	Reputable source calls pathogenic/benign	• Only applicable when evidence is not available, (e.g. Sharing Clinical Reports Project)
BS2	Observed in healthy adult for disorder with full penetrance at an early age	• Populations may not have been screened or excluded for phenotype
BP1	Variant in a gene in which truncations primarily cause disease	• Clarify the meaning of 'primary'. Suggest >90%.
BP2/ BP5	In trans with dominant pathogenic variant vs. Found in a case with alternate molecular basis for disease	• Clarify that one should apply BP2 when the pathogenic variant was seen in the same gene as the variant being evaluated vs. BP5 when the pathogenic variant was in a different gene.

Observations and Lessons Learned

- The majority of variant classification **differences** are **resolvable** through consensus & data sharing
- Variant classification often requires **professional judgment** (even when using the same rules) and therefore **complete consensus may not occur**
- But all **evidence** must be **accessible** and rules should be applied correctly
- The ACMG/AMP rules would benefit from added **quantitative guidance** and **gene specific guidance**

1. Amendola et al., Genome Res. 2015 Mar;25(3):305-15.
2. Rehm et al, NEJM, 2015;372, 2235-42.
3. Richards et al., Genet Med. 2015 May;17(5):405-24.

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