Clinical Laboratories Implement the ACMG/AMP Guidelines to Resolve Differences in Variant Interpretations Submitted to ClinVar

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Background and Methods

- The ClinVar database allows labs to share variant interpretations that previously had been unpublished or unavailable to the larger community.
- ACMG published guidelines for variant interpretation provide a framework to classify variants; however, given the complexity of variant interpretation, application of the guidelines still require subjective interpretation
- Through a ClinGen initiative, 4 clinical labs, Ambry Genetics, GeneDx, Laboratory for Molecular Medicine (LMM), and University of Chicago, worked to resolve variants with interpretation differences in ClinVar with the following process:
  - Compare labs' interpretation previously submitted to ClinVar to labs' most recent interpretation
  - Reassess variants using the ACMG/AMP guidelines
  - Share evidence & internal data used, when applicable
  - Identify persistent interpretation differences due to varying application of ACMG/AMP rules
  - Update variant classifications in ClinVar as needed

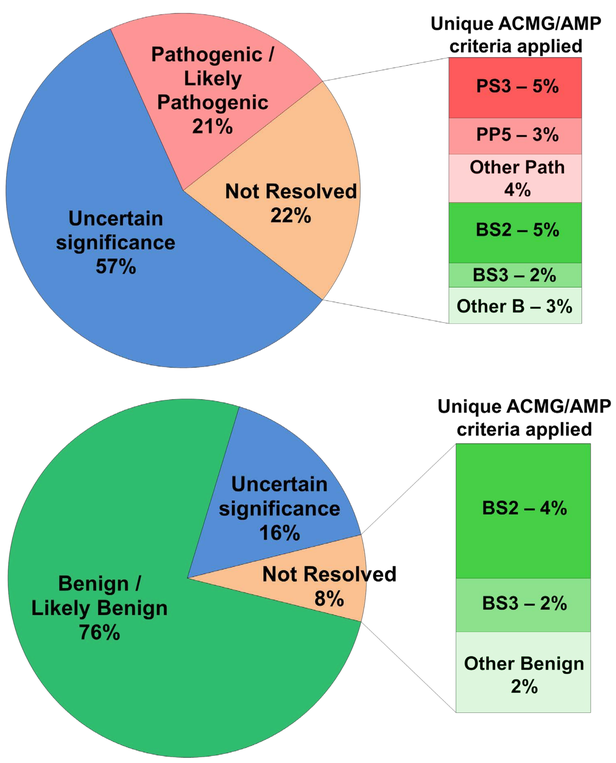
ClinVar and Interpretation Data

- **49,734** unique variants have been submitted to ClinVar by ≥1 of 4 participating labs (1/1/2016)
- **6169** variants were submitted by ≥2 participating labs allowing interpretation comparisons
  - **724 (12%)** had one or two-step differences between: Pathogenic/Likely pathogenic (P/LP), Uncertain significance (VUS), and Likely benign/Benign (LB/B)

Table 1: Interpretation differences in ClinVar from participating labs

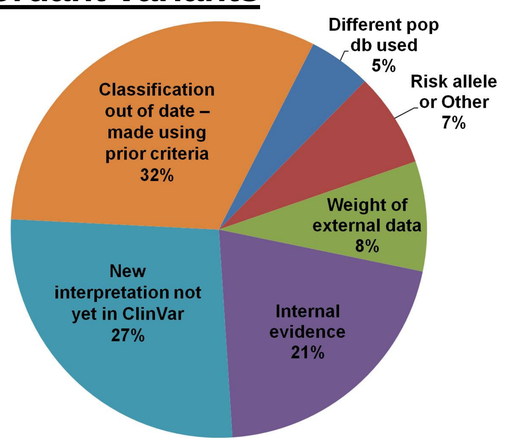
Submitted by	# shared variants	# Agreed (%)	# VUS vs. LB/B differences	# P/LP vs. VUS/LB/B differences
Lab 1 / Lab 2	2318	2035 (88%)	125 (5%)	158 (7%)
Lab 3 / Lab 1	2312	2068 (89%)	200 (9%)	44 (2%)
Lab 1 / Lab 4	1256	1086 (86%)	160 (13%)	10 (1%)
Lab 4 / Lab 2	513	478 (93%)	30 (6%)	5 (1%)
Lab 3 / Lab 4	86	77 (90%)	9 (10%)	0
Lab 3 / Lab 2	65	62 (95%)	2 (3%)	1 (2%)
All 4 Labs	6169	5445 (88%)	508 (8%)	216 (4%)

Figure 1: Resolution outcome of A) 104 reassessed P/LP vs VUS/LB/B differences and B) 128 reassessed VUS vs LB/B differences



PS3: Well established functional study; PP5: Reputable source calls pathogenic; BS2: Observed in healthy adult; BS3: Functional studies suggesting no impact

Figure 2: Basis of interpretation differences for discordant variants



Resolution Results

P/LP vs VUS/LB/B Reassessments (104 variants)

- Majority (57%) resolved as VUS; 21% resolved as P or LP
- Consensus not reached for 23 P/LP vs VUS/LB/B differences (22%):
  - For 8 variants, labs applied the same pathogenic criteria but differed on the application of benign criteria, specifically deciding how to account for observations of the variant in controls (BS2) or data suggesting no functional impact (BS3), despite other evidence for a pathogenic interpretation
  - For 15 variants, labs differently applied pathogenic criteria included functional studies (PS3), reputable source (PP5), and hotspots/functional domains (PM1)

VUS vs LB/B Reassessments (128 variants)

- Majority (76%) resolved as B or LB; 16% resolved as VUS
- Consensus was not reached for 10 VUS vs LB/B differences (8%), mostly due to differences in how labs applied benign criteria for observation in controls (BS2) and functional studies suggesting no impact (BS3)

Summary

- Labs reached concordance on 86% of the 232 reassessed variants
- Figure 2 shows the breakdown of reasons for discordant interpretations (87 variants)
- Sharing internal evidence, such as segregations, co-occurrences, and de novo observations, facilitated resolution of 23 interpretation differences

Table 2. Total Differences AFTER sharing data between labs (n=6169 variants)

# Agreed (%)	# VUS vs. LB/B differences	# P/LP vs. VUS/LB/B differences
5645 (92%)	398 (6%)	126 (2%)

Conclusions

- 78% of clinically actionable differences & 92% VUS vs. LB/B differences were resolvable after consensus efforts were applied; 492 differences have not yet been assessed
- Interpretations in ClinVar do not always represent a lab's current interpretation of a variant; more frequent submissions to ClinVar are needed
- Further specification regarding functional assays and weighting of conflicting pathogenic and benign criteria may further facilitate resolution of interpretation differences with ACMG/AMP guidelines
- **Application of the ACMG/AMP criteria and sharing internal evidence and classification rationales increased the overall concordance rate between these four labs from 88% to 92%**