Piloting the Forthcoming ACMG/AMP/CAP/ClinGen Standards for Sequence Variant Classification

Introduction: The American College of Medical Genetics and Genomics (ACMG), Association for Molecular Pathology (AMP), College of American Pathologists (CAP) and Clinical Genome Resource (ClinGen), are working on updated standards for sequence variant classification (SVC), referred to as SVC v4.0. SVC v4.0 uses a Bayesian, points-based system and flow diagrams that guide curators through evidence application. To evaluate the validity and usability of the draft standards, we launched a pilot involving clinical laboratories with expertise in variant classification.

Methods: Twenty variants of varying impact and evidence were selected to test specific components of the v4.0 framework. For all variants, supporting evidence, including mechanism of disease, prevalence and penetrance estimates, and clinical data were provided to avoid variation due to access to evidence. All curations were performed in a pilot curation interface developed by ClinGen and results evaluated for concordance.

Results: Over 40 curators from 25 different institutions participated. On average each variant had 22 classifications (range 17-28) as not all curators assessed each variant. We assessed concordance on three different scales: three-level (P/LP, VUS, LB/B), the standard five-level, and a seven-level scale with VUS broken into VUS-high, VUS-mid, and VUS-low. At the three-level scale, 14 variants (70%) were 100% concordant, with only one <80%. With the seven-level scale, eight variants (40%) reached 100% concordance, with a further six (30%) >=90%, four (20%) >=80% and two (10%) <80% concordant (68% and 70%). Of the two variants with the lowest concordance on the seven-level classification scale (68% and 70%) - one is a frameshift variant in a gene where LoF is a suspected but not an established mechanism for the disease. For this variant all 17 classifications reached VUS but the VUS subtype was split between VUS-High and VUS-Mid. The other variant is an inframe deletion of 2 exons where curations were split among LP, VUS-High, and VUS-Mid. Based on curator comments, the differences were primarily based on inclusion of P/LP missense variants as informative for the region being critical and weighting proband occurrences when the variant allele frequency was higher than expected for a P/LP variant. Lastly, for each variant, curators were asked if they agreed with the classification reached based on the evidence provided. For ten variants (50%) curators all agreed with the

classification and for nine variants (45%) only one or two curators indicated disagreement with the classification. For the remaining variant, curators reached concordance with regards to the LB classification however 42% (8/19 curators) felt that population frequency data alone was not high enough to warrant LB.

Conclusions: Twenty variants were selected to test various workflows and scenarios for the forthcoming ACMG/AMP/CAP/ClinGen standards with 85% (17/20) reaching >90% concordance on the three-level classification scale. We will next examine curations in the pilot curation interface to identify which components of the curations differed and discuss if updates or further guidance on use of the v4.0 framework is necessary. Afterward, we will add ten more variants to this pilot, make necessary adjustments and then proceed with a final pilot of the same 30 variants that includes all interested members (>100) of the community. By piloting SVC v4.0 we will ensure that the framework is valid and instructions for use are clear before the final stages of development.

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