Molecular pathology selected abstracts

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Assessment of AMP/ASCO/CAP guidelines used as a somatic variant classification system

March 2023—The Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists published a formalized somatic variant classification system in 2017. The tiered system stratifies variants based on clinical importance, taking into account how variants affect cancer diagnosis, prognosis, or treatment strategies. Somatic variants with strong clinical significance, including those that are associated with FDA-approved therapies or included in professional guidelines, are tier one; variants with potential clinical significance are tier two; variants of unknown significance are tier three; and benign variants are tier four. The authors, members of the AMP Variant Interpretation Across Testing Laboratories Working Group, assessed how laboratories are using the AMP/ASCO/CAP guidelines and whether there is good concordance among laboratories in applying the guidelines to variant interpretation. A somatic variant interpretation challenge was sent to participating laboratories. The challenge included four clinical scenarios—three solid tumor cases and one hematologic case—and 11 variants for participants to interpret. Participants were asked to classify the variants by tier and type of evidence (diagnostic, prognostic, or treatment based) used to make that decision. One hundred and thirty-four participants responded to the challenge. Each variant was considered independently, so the algorithms included 28 to 44 participants, depending on the variant, for a total of 362 classifications. Of those 362 classifications, only 59 percent agreed with the working group's consensus classification for tier and type of evidence used. The consensus improved to 65 percent when examining tier classification only and to 86 percent when distinguishing clinically significant variants (tier one or two) from variants of unknown significance and benign variants (tier three or four). A limitation of the study was that many of the participants did not complete all of the challenges. Therefore, it is possible that participants selectively avoided addressing the variants that were more difficult to classify, potentially leading to an overestimation of consensus. Participants were also surveyed about how they implemented the AMP/ASCO/CAP guidelines. Of 220 survey respondents, 71 percent had implemented the guidelines for variant classification. Participants were also surveyed about the resources they used for somatic variant classification. They most often used somatic variant databases such as the Catalogue of Somatic Mutations in Cancer (COSMIC), Clinical Interpretations of Variants in Cancer (CIVIC), BioPortal, The Cancer Genome Atlas, and American Association for Cancer Research Genie. The study showed that many laboratories are implementing the AMP/ASCO/CAP somatic variants guideline and that there is a good consensus for identifying clinically significant variants when using the classification system. However, the study also identified areas that need improvement. Respondents requested clearer guidance on classifying variants of uncertain significance and certain types of variants, including potential germline variants, structural variants, copy number variants, and fusions. Other requests included more granular definitions for specific tier categories and more educational resources related to clarifying the guidelines.

Li MM, Cottrell CE, Pullambhatla M, et al. Assessments of somatic variant classification using the Association for Molecular Pathology/American Society of Clinical Oncology/College of American Pathologists guidelines: A Report from the Association for Molecular Pathology. *J Mol Diagn*. 2022. doi:10.1016/j.jmoldx.2022.11.002

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Determining parent of origin for inherited alleles without sequencing parents

Understanding from which side of the family an inherited allele originates allows genetic counselors and clinicians to better classify potential pathogenic variants and better determine which family members need additional genetic testing. Trio sequencing—sequencing the patient and the mother and father—has been used to determine from which parent a patient inherited an allele. However, there are regions across the human genome that are differentially methylated based on whether they originate from the mother or father. These regions are consistent across individuals and tissues and persist throughout adulthood. The authors conducted a study that leveraged these regions to distinguish which alleles originated from which parent. They employed nanopore sequencing (Oxford Nanopore Technologies), a long-read technology that can call nucleotide sequence and methylation status, for their study. However, this approach cannot be used alone. One hundred and ninety-two differentially methylated regions have been identified across the genome. This is a small fraction of the genome, and even the long reads of nanopore sequencing cannot span the distances from one differentially methylated region to another. Consequently, the authors used a second technique—single-cell template strand sequencing (Strand-seq)—to complement nanopore sequencing. Strand-seq separates one parental DNA template from the other and performs sequencing using single-cell technology. This technique does not cover every allele and does not determine parent of origin, but it can determine which alleles are inherited together across the length of the chromosome. In this manner, the authors combined the chromosome-length haplotype scaffold of Strand-seq with the methylation status and coverage of nanopore sequencing to determine the parent of origin for virtually every allele across the autosomal genome. To validate their technique, they tested five well-characterized trios of diverse genetic backgrounds and compared their parent-of-origin analysis with the ground truth. The parent of origin was correctly identified for 99.7 percent of single nucleotide variants and 98.1 percent of indels. This study demonstrates a novel technique for determining parent of origin without having to sequence both parents. The approach can address immediate clinical needs by improving variant curation and estimates of disease penetrance and by providing more efficient genetic testing of potentially affected family members.

Akbari V, Hanlon VCT, O'Neill K, et al. Parent-of-origin detection and chromosome-scale haplotyping using long-read DNA methylation sequencing and Strand-seq. *Cell Genomics*. 2023. https://doi.org/10.1016/j.xgen.2022.100233

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