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The Current State of Clinical Interpretation of Sequence Variants

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Abstract

Accurate and consistent variant classification is required for Precision Medicine. But clinical variant classification remains in its infancy. While recent guidelines put forth jointly by the American College of Medical Genetics and Genomics (ACMG) and Association of Molecular Pathology (AMP) for the classification of Mendelian variants has advanced the field, the degree of subjectivity allowed by these guidelines can still lead to inconsistent classification across clinical molecular genetic laboratories. In addition, there are currently no such guidelines for somatic cancer variants, only published institutional practices. Additional variant classification guidelines, including disease- or gene-specific criteria, along with inter-laboratory data sharing is critical for accurate and consistent variant interpretation.

Introduction

Molecular diagnostics requires accurate and consistent classification of sequence variants (i.e., single nucleotide variants (SNVs) and deletion/insertion (indels) variants). Detection of a pathogenic or “actionable” variant may influence a patient's diagnosis and/or prognosis, family planning, and lifelong health management. But variant classification has historically been inconsistent between clinical molecular genetic laboratories [1,2] and potentially even inaccurate [3]. These issues arose in part due to a lack of appreciation of the diversity and complexity of the human genome [4,5] and the differing classification methodologies used by individual clinical laboratories [2,6,7].

Prior to the onset of large-scale genomic studies, SNVs were often classified as disease-causing largely based on the absence of such variants in ethnically-matched control cohorts. However, these cohorts generally included, at most, hundreds of individuals [8]. The release of large population variant databases [9–12] revealed the unexpected prevalence of many rare variants, previously classified as disease-causing, in the general population. These rare

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variants appear at rates significantly higher than expected, given the associated disease prevalence [13–18]. For example, the p.G278E variant in the *MYBPC3* gene was initially categorized as presumably pathogenic, but recategorized to benign after release of these large general population databases [3]. It is now known that this variant is present in 2.6% (76/2968) of tested African chromosomes (data from the Exome Aggregation Consortium (ExAC) browser [11•]), a frequency higher than the prevalence of hypertrophic cardiomyopathy (~1:500) [19]. Furthermore, classification discrepancies often exist between clinical laboratories [1•,2]. Such discrepancies may arise from methodological differences, in particular differing weights assigned to certain data or reliance on unpublished in-house data [2,6]. In response to these discrepancies, the American College of Medical Genetics and Genomics (ACMG), joined by the Association of Molecular Pathology (AMP) and College of American Pathologists (CAP), updated the standards and guidelines for variant interpretation for Mendelian disorders to include recommendations for weighing particular evidence [20••]. In addition, AMP is currently developing a guideline for interpreting somatic cancer variants, taking into account previously published institutional practices [21••,22••]. Herein, we focus on current recommendations and methodologies used to classify the clinical significance of identified sequence variants for Mendelian disorders and somatic cancers as algorithms for variant calling have been described previously [23–25]. Classification of copy number variants and other structural variation is outside the scope of this review.

Interpretation of variants for Mendelian disorders

Classification of variants

Clinical assessments classify variants based on the probability of a variant to cause disease [26]. To clarify the effect of a nucleotide change, the term "mutation", which has a negative connotation but does not actually infer a deleterious effect, has been replaced with the neutral term "variant" that is modified to define its association to disease [27], with descriptors such as pathogenic or benign [20••]. These descriptors aid clinicians in understanding the clinical significance of variants.

Variants are classified into five distinct categories: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign (Figure 1) [20••,26,28,29]. Originally, the International Agency for Research on Cancer (IARC) defined likely pathogenic and likely benign variants as a 95–98% probability of being pathogenic or non-pathogenic [26]. The ACMG/AMP guideline expanded this definition to 90% certainty [20••]. However, most evidence is non-quantitative, and therefore these percentages are actually estimates [20••]. The uncertain significance category is a default classification for variants lacking sufficient evidence to determine their clinical significance [20••]. Some academic and commercial molecular diagnostic laboratories further subcategorize variants of uncertain significance (VUS) into “leaning pathogenic” or “leaning benign”, if supportive data exist but is insufficient to meet criteria for likely pathogenic or likely benign [30,31]. For example, a variant that segregates with disease in an affected relative and is absent from general population databases may warrant the variant to be classified as VUS “leaning pathogenic”, but is insufficient to classify it as likely pathogenic. Alternatively, a variant with a frequency

in the general population higher than expected for the associated disease prevalence, but not high enough to meet criteria for a likely benign classification may be used to designate it as VUS "leaning benign".

The ACMG/AMP guideline attempts to ensure evidence-based interpretation of variants by dividing classification into a two-step process. First, multiple categories of data are considered, including (1) frequency in affected and unaffected populations; (2) computational prediction tools; (3) in vitro and/or in vivo functional studies, such as direct assays on patient samples, studies in animal models or cell lines, and/or minigene splicing assays; (4) co-occurrence and segregation studies; and (5) gene- or disease-specific information (reviewed in [32]). Next, these data are classified into "levels of evidence": stand-alone, very strong, strong, moderate, or supporting (Supplementary Table 1) [20••]. These levels of evidence are then combined to determine a variant classification according to a formula applicable to all Mendelian variants. For example, "a null variant in a gene where loss of function is a known mechanism" is categorized as "very strong" evidence, and a variant may be classified as "pathogenic" given one instance of "very strong" evidence plus two instances of "supporting" evidence. This evidence-based approach attempts to replace a subjective estimation of pathogenicity with a dispassionate mapping of variant data to levels of evidence.

Building concordance across clinical laboratories

Prior to the ACMG/AMP guidelines, individual clinical laboratories developed their own criteria for variant classification [26,29–31,33,34], which led to many inter-laboratory discrepancies [6,35–37]. The recent ACMG/AMP guidelines were intended to reduce the subjective nature of variant classification and increase consistency between laboratories. But mapping variant data to levels of evidence is inherently subjective, and the ACMG/AMP guidelines merely confine to this first step the subjective aspects of variant classification. Furthermore, the mapping from variant data to levels of evidence is intentionally flexible to accommodate multiple inheritance and penetrance patterns (Table 1). As a result, the ACMG/AMP guidelines alone may not promote the desired consistency between laboratories.

The Clinical Sequencing Exploratory Research (CSER) consortium investigated the performance of the recent ACMG/AMP guidelines across nine clinical genetic laboratories. Initial variant classifications using the new guidelines had an overall inter-laboratory concordance rate of 34%, which was not significantly different than using prior laboratory-developed methods [1•]. Discordance between classifications was primarily due to differences in how individual laboratories interpreted the mapping of variant data to levels of evidence, though inaccurate usage of the ACMG/AMP guidelines also contributed. Imposition of further disease- and gene-specific criteria governing the first level of evidence step as well as additional clarification of the guidelines increased this inter-laboratory concordance rate to 71% [1•]. Some of the recommendations that increased the concordance rate included defining new disease-specific minor allele frequency thresholds for stand-alone benign criteria, defining which functional studies constituted "well-established", and developing quantitative thresholds for the number of segregations needed to meet criteria

[1•]. This study suggested that the first stage of the current guidelines, the mapping of data to lines of evidence, may trade consistency for the flexibility necessary for broad applicability.

Ongoing initiatives are attempting to improve the current guidelines by refining the first mapping step. While the CSER consortium provided some clarity for mapping data to lines of evidence [1•], additional initiatives are defining disease- and gene-specific criteria for mapping data to lines of evidence. The Clinical Genome Resource (ClinGen) consortium, a National Institutes of Health (NIH)-funded entity, approves disease-specific expert panels to perform high-level curation and determine specific gene- and disease-specific recommendations for variant interpretations [36,38]. These expert panels – such as InSiGHT for Lynch syndrome [39], CFTR2 for cystic fibrosis [40], and ENIGMA for hereditary and ovarian breast cancer [41] – consist of a combination of researchers, genetic counselors, molecular genetics, and clinicians from multiple institutions, who review available evidence to establish consensus [27].

Reliance on unpublished, in-house data when interpreting variants constitutes another source of inconsistency between laboratories. When laboratories have different information about variants, they will interpret the variants differently. Thus, consistent variant classification requires public availability of all relevant variant-specific data. Currently, too much data resides in private in-house databases, due to the lack of interest or merit to publish [6,42]. Laboratories should be encouraged to make variant- and case-level data available by depositing unpublished data into public repositories, such as ClinVar (<http://www.ncbi.nlm.nih.gov/clinvar/>) [38,43], Leiden Open Variation Database (LOVD; <http://www.lovd.nl/>) [44], or Universal Mutation Database (UMB; <http://www.umd.be/>) [45]. Alternative distribution avenues include collecting variant classifications directly from the patients or physician by the "Free the Data" campaign (www.free-the-data.org) or Sharing Clinical Reports Project (www.sharingclinicalreports.org), respectively. While these resources may encourage information-sharing and transparency, the existence of multiple independent repositories creates other issues. For example, instances may be duplicated across these repositories, resulting in an overestimation of pathogenicity for some variants. Ideally, there would be one common global repository for the aggregation of all of these data [6].

Interpretation of variants in somatic cancers

Classification of variants

While governing bodies, such as ACMG or AMP, have yet to promulgate guidelines for the analysis of somatic cancer variation in the clinical setting, several studies have recently highlighted various institutional practices, including algorithms for calling and annotating variants [7,24,25,46] and somatic variant classification [21•,22•]. These approaches differ considerably from the ACMG/AMP guidelines of Mendelian disorders, classifying somatic cancer variants based on clinical "actionability" – defined as the prognostic, diagnostic, or therapeutic implications, interpreted in a disease-specific context – rather than variant effect (e.g., benign versus pathogenic) (Figure 1). Notably, the clinical actionability of a somatic variant depends on the specific tumor type it was identified in.

Many tools exist to annotate somatic variants, though there is currently no consensus with regards to the quality of evidence used in the assessment of their significance. Upon removal of apparently normal germline variation, data mining of knowledge bases designed to capture variant frequency – the Catalogue of Somatic Mutations in Cancer (COSMIC; <http://cancer.sanger.ac.uk/cosmic>) and The Cancer Genome Atlas (TCGA; summarized in cBioPortal; <http://www.cbioportal.org/>) – or clinical effect – MyCancerGenome (<https://www.mycancergenome.org/>) and the Clinical Interpretation of Variants in Cancer (CIVIC) database (<https://civic.genome.wustl.edu>) – may aid in determining the significance of a given variant. While preclinical and in silico analyses can be helpful in assessing the possible functional impact of variants detected, the clinical significance of these findings should be made with caution [21••,22••]. Continual review of new publications, clinical trials, and databases should be performed to ensure that the variant annotation is up to date [21••]. Finally, it is important to note that the clinical effect of any single variant may be influenced by the presence or absence of other variants [22••], and thus familiarity with the gene of interest in any given disease is critically important to the success of the interpretation performed.

After annotating somatic variants, these variants should be clinically classified before reporting back to treating oncologists. Currently, there are two published institutional practices for classifying somatic variants: the SVC method proposed by Sukhai et al. [21••] and the PHIAL method by Van Allen et al. [22••]. Both describe tier-based classification schemes to classify variants identified in clinical oncology cases (Table 2). These methods assign the highest priority (Class 1) to variants shown to influence the diagnosis, prognosis, or treatment of the specific tumor being evaluated, such as a p.V600E variant in the *BRAF* gene in a melanoma sample, which confers sensitivity to tyrosine kinase inhibitor therapies [47] and thus is actionable. Variants with potential, but yet unproven, therapeutic utility are classified as Class 2 for the SVC method and Class 3 for the PHIAL method. For example, the p.V600E variant in the *BRAF* gene in a low grade glioma would be classified as Class 2 by the SVC method or Class 3 by the PHIAL method as there is currently no compelling evidence that identifying this variant in low grade gliomas has clinical utility. The SVC and PHIAL methods further diverge at lower classification tiers, which concern VUSs. The SVC method classifies VUSs depended on the "actionable" of the gene in which they are identified. If a VUS is identified in a gene considered "actionable" for that tumor type then it is classified as Class 3, and Class 4 if the gene is considered "actionable" for a different tumor type. However, not all variants in an actionable gene will drive disease. The PHIAL method, the basis for our method (described below), takes a more evidence-based approach, classifying variants based on limited (Class 2), pre-clinical (Class 4), or inferential (Class 5) evidence. Currently, both published methods lack a separate classification for variants of known clinical insignificance (i.e., benign).

At Brigham and Women's Hospital (BWH) and the Dana-Farber Cancer Institute (DFCI), variant classification for clinical oncology cases, which is used to guide patient care and prognosis, builds upon the PHIAL method, but also incorporates aspects of the SVC method. Similar to the SVC method, variants with established potential to modify treatment, diagnosis, or prognosis in the specific tumor being evaluated are deemed Class 1, while the same variant in a different tumor where such effect remains unproven is classified as Class 2.

Such variants may be considered clinically actionable by the treating oncologist as they may predict prognostic/diagnostic implications, or therapeutic agents available off-label or in clinical trials [24]. Like the PHIAL method, Class 2 also includes variants with limited evidence. For Class 3, we combine Class 4 and 5 from the PHIAL method, and Class 4 is the equivalent of the Class 5A from the SVC method (Table 2).

Unlike the SVC and PHIAL methods, the BWH/DFCI method explicitly includes a separate classification for benign variants (Class 5 as shown in Table 2). This classification serves two purposes: (1) informs the clinician it is clinically irrelevant and (2) reduces the burden of interpretation on laboratories. VUSs must be continuously reviewed and updated, a process that is both expensive and time-consuming. The most effective means of reducing benign variants, specifically normal germline and age-related benign somatic variants, includes profiling matched tumor and normal tissue [7,21••]. However, due to financial or biological constraints, this is rarely accomplished. Thus, interpretation of the genetic results from a tumor sample likely includes many clinically irrelevant variants [7]. In the current reimbursement environment, laboratories have limited resources and, therefore, a category for benign variants allows such variants to be excluded from the reviewing and updating process, and also simplifies inter-laboratory data sharing.

Differences in these three methods appear driven in part by differences in input data sets. The SVC and BWH/DFCI methods were developed for use with targeted NGS panels, which typically consist of 10s to 100s of previously curated genes. Because genes are in effect prescreened for relevance, even variants significant to another tumor may be allotted a higher classification. In contrast, the PHIAL method was developed for use with whole exome sequencing. The resulting data would likely include a far greater number of variants, and more variants significant to diseases unrelated to the current tumor type. Thus, this method depreciates variants unrelated to the disease or tumor in question. However, a unified method that can classify variants from any input data should be established for consistent variant classification.

Comparison of somatic cancer to Mendelian classifications

The ACMG/AMP guidelines for Mendelian variants and the published institutional practices for somatic cancer variants are more similar than they may immediately appear (Figure 1). Both methods aim to identify clinically significant variants to aid in clinical management. Both methods also classify variants based on the actionability or pathogenicity in a disease-specific manner. For example, a gain-of-function variant in *PTPN11* may be pathogenic for Noonan syndrome or clinically actionable for Juvenile myelomonocytic leukemia [48], while a loss-of-function variant in *PTPN11* may be pathogenic for Metachondromatosis [49]. However, for Mendelian disorders, variant classification is independent of the phenotype of a given patient [20••,32]. As such, a previously categorized pathogenic variant in the *MYH7* gene will be pathogenic in an individual with hypertrophic cardiomyopathy or an unaffected individual. So while a somatic cancer variant may be clinically actionable in one tumor and a VUS in another tumor (see the p.V600E variant example above), the clinical significance of a Mendelian variant should be the same whether or not it explains that patient's disease.

Conclusion

The rapid expansion of molecular genetics and the promise of Personalized Medicine relies on consistent variant interpretation for the purpose of informing diagnosis, treatment, or informed family planning. The interpretation of Mendelian variants may benefit from the development of more disease- and gene-specific guidelines for mapping data to levels of evidence. These expanded guidelines may reduce the subjectivity of variant classification and increase inter-laboratory consistency. Variant- and case-level data sharing should be encouraged, and mechanisms for preventing duplication of results in existing independent databases should be developed. Unified guidelines for classifying somatic cancer variants should be promulgated, similar to the guidelines developed for Mendelian variants. These guidelines should be assay-independent and include a separate category for benign variants, to inform clinicians and patients and to reduce the burden on laboratories.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- of special interest
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Figure 1.

This figure compares and contrasts variant classification between Mendelian disorders and somatic cancers. The somatic categories are based off of the SVC method (further described in Table 2) [21•], and the Mendelian categories are from the ACMG/AMP guidelines [20•]. Clinically significant, uncertain, and insignificant categories are common between Mendelian and somatic cancer classification systems. However, within these larger

categories there are subcategories that are unique to either the Mendelian or somatic variant classification system and consist of different levels and/or types of evidence.

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Table 1

Flexibility allotted for in "Lines of Evidence"

Lines of Evidence*	Type of Data	Flexibility
BS1/PM2	Allele frequency in general population	Determining what frequency is consistent with "greater than expected for disorder" or "at extremely low prevalence for recessive disorders"
BS2/PP4	Observed in unaffected/affected	Determining the extent of medical work-up necessary to conclude disease status
BS3/PS3	Functional studies	Determining what constitutes "well-established" functional studies
BS4/PP1	Segregation studies	Determining the extent of medical work-up necessary to conclude disease status Determining number of non-segregations-or segregations needed to meet criteria
BP2/BP5	Other pathogenic variant identified	May need to take account phenotype or disease severity
BP6/PP5	Reputable source classification	Determining what constitutes a "reputable source"
PM1	Gene-specific information	Determining what constitutes "a critical or well-established" functional domain

* Lines of evidence category from [20••]

Table 2

Classification methods for somatic cancer variants

Classification *	SVC Method [21••]	PHIAL Method [22••]	BWH/DFCI Method
Class 1	Clinically actionable for therapeutic, prognostic, or diagnostic purposes for same tumor type	Validated therapeutic, prognostic, or diagnostic implications for same tumor type	Validated therapeutic, prognostic, or diagnostic implications for same tumor type
Class 2	Clinically actionable for therapeutic, prognostic, or diagnostic purposes for a different tumor type	Limited evidence of therapeutic, prognostic, or diagnostic implications for same tumor type	Validated therapeutic implications for a different tumor type, or limited evidence of prognostic or diagnostic implications for same tumor type
Class 3	Other variants in this gene in this primary tumor are established as actionable for same tumor type	Clinical evidence of therapeutic response from another tumor type	Preclinical or inferential therapeutic, prognostic, or diagnostic implications
Class 4	Other variants in this gene in this primary tumor are established as actionable for a different tumor type	Preclinical association to therapeutic response	Novel or unstudied in cancer
Class 5	A) Gene is not actionable for any tumor type B) Established as benign	Inferential association to therapeutic response	Established as benign

BWH/DFCI= Brigham Women's Institute/Dana Farber Cancer Institute,

* PHIAL method classifications referred to as Levels A–E, while other methods refer to as Tiers