Variant reclassification and clinical implications

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ABSTRACT
Genomic technologies have transformed clinical genetic testing, underlining the importance of accurate molecular genetic diagnoses. Variant classification, ranging from benign to pathogenic, is fundamental to these tests. However, variant reclassification, the process of reassigning the pathogenicity of variants over time, poses challenges to diagnostic legitimacy. This review explores the medical and scientific literature available on variant reclassification, focusing on its clinical implications. Variant reclassification is driven by accruing evidence from diverse sources, leading to variant reclassification frequency ranging from 3.6% to 58.8%. Recent studies have shown that significant changes can occur when reviewing variant classifications within 1 year after initial classification, illustrating the importance of early, accurate variant assignment for clinical care. Variants of uncertain significance (VUS) are particularly problematic. They lack clear categorisation but have influenced patient treatment despite recommendations against it. Addressing VUS reclassification is essential to enhance the credibility of genetic testing and the clinical impact. Factors affecting reclassification include standardised guidelines, clinical phenotype-genotype correlations through deep phenotyping and ancestry studies, large-scale databases and bioinformatics tools. As genomic databases grow and knowledge advances, reclassification rates are expected to change, reducing discordance in future classifications. Variant reclassification affects patient diagnosis, precision therapy and family screening. The exact patient impact is yet unknown. Understanding influencing factors and adopting standardised guidelines are vital for precise molecular genetic diagnoses, ensuring optimal patient care and minimising clinical risk.

INTRODUCTION
The importance of accurate molecular genetic diagnoses for patients has been emphasised in light of the expansive range of genomic technologies available to clinicians and increasingly used genetic tests for clinical diagnosis. The Genetic Testing Registry records $74,313$ genetic tests for $24,204$ conditions, spanning coverage of $18,726$ genes (https://www.ncbi.nlm.nih.gov/gtr/ accessed 14 January 2024). The most widely adopted system for sequence variant classification remains the 2015 guidelines published by the American College of Medical Genetics (ACMG) and the Association for Molecular Pathology (AMP). Genetic variants that are uncovered during genetic testing are largely classified on a continuum from benign, likely benign, uncertain significance, likely pathogenic and pathogenic. As proposed by the 2015 ACMG/AMP guidelines, genetic variants should be classified as benign or likely benign variants if they are unlikely to be associated with disease (>90% certainty of not being associated with a disease); variants of uncertain significance (VUS) do not have enough evidence to be or classified as likely pathogenic/pathogenic or likely benign/benign; and genetic variants should be classified as pathogenic or likely pathogenic if they are considered to be disease causing (>90% certainty of being associated with a disease).

Variant classification is the process of reassigning the pathogenicity of variants over time. As genomic knowledge evolves and information for classifying a variant accrues, any variant that has been previously classified can be reclassified from one category to another (benign, likely benign, VUS, likely pathogenic, pathogenic). Variant reclassification is an idiosyncrasy of genomic medicine that represents an evolving body of literature.

With the mainstreaming of genetic testing and increasing use of next generation sequencing (NGS) for clinical diagnosis, variant reclassification is becoming a timely issue within clinical genetics. We aim to review the published medical literature to date that examines variant reclassification. Structural genome variants are also identified in clinical genetic testing, but their classification over time is outside the scope of this review. We reviewed the literature that describes gene sequence variant reclassification. A thorough PubMed search was performed using the Medical Subject Headings terms (“variant” AND “reclassification”) returning 358 results (accessed 29 June 2023). Abstracts were screened for variant reclassification with clinical implications published between January 2015 and June 2023 which resulted in 18 abstracts being chosen for the review to create a table summary of sequence variant reclassification literature (see online supplemental table).

VARIANT RECLASSIFICATION
Variant reclassification is based on an accumulation of information—from functional studies, in silico models, case reports, familial segregation studies—between first variant classification and later reclassification. Variant reclassification rates are not insubstantial ranging divergently from 3.6% to 58.8% in the current published literature when
assigned variants have been examined under different conditions and for different causes.

Another important focus in the variant reclassification literature is the interval between variant classification and reclassification. An incremental rate of variant reclassification over time passed since initial classification has been demonstrated. This may be suggestive of accruing information to test a previously identified variant against. Most conservative sources suggest that variant reclassification occurs within 2 years of initial classification. However, retrospective studies within cancer genetics have reported time to variant reclassification that occurs <1 year after initial variant classification.

Any variant, from benign to pathogenic, can be reclassified, although the reclassification of VUS to either benign or pathogenic category is a current imperative of genomic medicine to increase clinical impact of genetic testing. VUS are increasingly reported with widespread use of NGS, even outnumbering the reporting of clinically actionable variants (likely pathogenic/pathogenic) where gene panels are used to investigate clinical phenotypes. The 2015 ACMG/AMP guidelines state explicitly that a VUS should not be used in clinical decision-making, and recommend clinical investigation and correlation to aid in classification of the variant to likely pathogenic/pathogenic or likely benign/benign. Although, in practice, this does not always hold true and some studies demonstrate that patients and clinicians will pursue treatment, including surgical treatment, based on a VUS result.

Current recommendations to ameliorate clinical risk attendant with variant reclassification is that previously designated variants are routinely and consistently revisited to assess whether new information would prompt a reclassification of the variant. Variant reclassification has significant clinical implications within the practice of clinical genetics as reclassification can undermine the validity of diagnostic genetic testing at any point in a patient journey.

**FACTORS ASSOCIATED WITH VARIANT RECLASSIFICATION**

There is no gold standard test for pathogenicity when classifying (and reclassifying) a sequence variant. As such, scientists and clinicians rely on a range of resources to contribute evidence to an assessment of probability that a variant will fall within a certain category in relation to a specific disease. Some of the distinct considerations required for variant reclassification will be discussed below.

**Standardised guidelines**

Standardised guidelines improve variant classification across a wide range of literature. This evidence is important to reinforce the need for universal implementation of standardised guidelines. The most widely used guidelines for variant classification are the 2015 ACMG/AMP guidelines but these are voluntarily adopted guidelines and not enforced. Variant reclassification is documented to be reduced for variants classified after 2016 compared with variants classified within the same laboratory before 2016, which may be in part due to the release of the 2015 ACMG/AMP guidelines.

Older classifications are often discordant between individual laboratories, compared with recent classifications likely due to the application of the 2015 ACMG/AMP guidelines. Discordant classifications of variants between laboratories may pose as a predictor for later variant reclassification.

**Segregation analysis**

Family studies are identified in the 2015 ACMG/AMP guidelines as tools for aiding variant classification and reclassification, especially for de novo conditions, for identifying the phase of variants in recessive conditions, or for identifying variants within a family that co-segregate with disease. Accumulation of clinical phenotype-genotype correlation within a family for familial variants is known to help in reclassification of VUS.

**Clinical presentation**

Concordance in variant classification and reclassification are different between clinical phenotypes. Concordance in variant classification in patients with cancer are higher than those in cardiac presentations. Different fields are represented disproportionately in studies relating to genetic variant reclassification which likely contributes to a weight of evidence that can support phenotype-specific variant classification.

**Ancestry**

Ancestry has a role to play in variant reclassification. Non-European ancestry has been directly correlated with the higher prevalence of VUS in and age-matched and sex-matched population with European ancestry. Efforts are being made to sequence genomes from under-represented populations in order to make genomic testing more comprehensive.

**Databases**

The development of large-scale public databases has somewhat lessened the complexity involved in classifying rare variants. It has been proven that using publicly accessible variant databases improves variant classification in rare disease. Increased transparency and knowledge sharing enforces reproducibility of variant classification procedures across laboratories and clinical sites. Publicly available, open access databases are instrumental in streamlining reproducible genetic variant classification. Increasing information available from databases is currently used to good effect in variant reclassification.

**Bioinformatics**

Bioinformatics tools are increasingly used in genomics that further variant reclassification by providing in silico tools to test pathogenicity of a genetic variant against. Popular software tools include MutationAssessor, MutationTaster, PolyPhen among others. Variant reclassification has been shown to rise in direct proportion to the introduction of updated software tools for variant analysis.

**Genome reanalysis**

Genome reanalysis uses improved sequencing techniques, updated bioinformatics pipelines, including in silico prediction models, functional studies and statistical models in combination with updated variant classification guidelines to identify genomic variants that could be relevant to disease phenotypes. Improved classification of variants at the point of reanalysis using these methods results in reclassification of variants that were previously classified as VUS. Genome reanalysis therefore has a role in reclassification of variants that were previously identified on genomic testing.

Interval reanalysis of genomic tests which leads to variant reclassification has been shown to significantly increase clinically significant classification of variants. Just over 70% of total variants in one study were reclassified after genome reanalysis. Molecular diagnostic yield is thought to increase by ~10% with genome reanalysis. These studies add clinically impactful information to the variant reclassification literature as an indirect outcome of their results.
ETHICS AND LAW

There is little published work about the ethical duty that caregivers have towards patients receiving news of variant reclassification. Variant classification and reclassification does not fall entirely under medical negligence or malpractice as reclassification does not represent a breach of duty between a patient and a caregiver.

Who is responsible?
The current published literature does not agree on whether the physician has a duty of care to recontact a patient in the case of variant reclassification. In practice, laboratory processes do not include detailed operating procedures for data reanalysis that may lead to reclassification, but will provide an ad hoc service performing data reanalysis on request. Similarly, physicians have no standard operating procedure for how to follow-up genetic test but will variably request further analysis to aid reclassification of test results if prompted.

Early guidelines conferred responsibility of informing patients of updated sequence variants to the performing laboratory. The most recent policy statement from the ACMG states that, ‘recontact is fundamentally a shared responsibility among the ordering healthcare provided, the clinical testing laboratory and the patient’. Present policy recommendations from the European Society of Human Genetics also state that recontacting should be a shared process between the patient, clinician and laboratory. The continued development of such guidelines to address variant reclassification is important and would require multidisciplinary input from a wide range of professionals and societies.

More recent practicable recommendations emphasise the importance of continued genetic counselling within clinical genetics services in order to keep up to date with variant reclassification. Traditionally, clinical genetics services have been organised so as not to follow patients up once a diagnosis was concluded. New recommendations would represent a shift in the paradigm of clinical genetics services from a single point of service visit to ongoing caregivers.

Patient impact
At the fore of considerations in variant reclassification are the individuals and families who are impacted by uncertainty when faced with variant reclassification. Medically, the change in variant classification is impactful as it can change medical management of patients. This is important where genetic testing is used for predictive as well as diagnostic causes.

There is conflicting evidence in the literature that patients will experience either a favourable response to variant reclassification (relief, happiness) or will report overwhelmingly negative feelings on receiving news of variant reclassification (uncertainty, concern, distrust, confusion, misunderstanding). Importantly, patient understanding of variant reclassification is often incomplete. Careful counselling is required in these instances. Patient experience of variant reclassification is yet to be fully captured in the published literature.

DISCUSSION

There is new evidence that variant reclassification can occur within 1 year of initial classification. Sources that report a time to reclassification <1 year, agree that variant reclassification assessment should be carried out at minimum 2 years after initial classification. If a molecular diagnosis is not concluded within 2 years of initial genetic testing, variant reclassification attempts are recommended by some authors before further genetic testing is initiated.

Standardised guidelines play a crucial role in variant reclassification across a wide range of literature. Standardised methods of variant classification are evident in the published literature with most groups applying the 2015 ACMG/AMP guidelines for variant reclassification. Adoption of the 2015 ACMG/AMP guidelines contributes to reduced discordance and aids in more consistent variant classification. Variability between individual classifiers may also be reduced by the introduction of stringent protocols and competency assessments within labs.

Phenotype-specific modifications to the 2015 ACMG/AMP guidelines have been made by expert groups that improve concordance in variant classification across individual labs and clinical centres. Important factors influencing reclassification are multifactorial, comprising clinical phenotype-genotype correlations, family studies, depth of phenotyping, population-specific databases, functional analyses, bioinformatics tools and databases of human genetic variation. Once a variant is reclassified, submitters to databases are encouraged to update the classification of the variant within the database. The responsibility of updating databases with reclassification of a variant lies with the submitter but there is evidence in the literature that this is occurring.

Variant reclassification appears to have a widely disparate patient impact affecting patient medical management in up to 41.3% of patients where variants have been reclassified (see online supplemental table). Clinical management remained unchanged for patients who had pre-existing clinical diagnoses when VUS were reclassified into a benign/likely benign or pathogenic/likely pathogenic category. Variant reclassification affects diagnosis and precision therapy, and can change medical surveillance recommendations for patients affected by variant reclassification.

Variant reclassification is likely to result in reclassification of VUS to benign variants rather than increase diagnostic yield as it appears that VUS are more commonly reclassified as benign or likely benign than pathogenic or likely pathogenic. The downgrade in classification of VUS to benign or likely benign has been reported across disciplines examining different patient phenotypes. This trend of VUS reclassification may relieve some psychological impact experienced by unaffected patients.

Not all studies provide a comprehensive overview of the direction of reclassification. Some sources report variants that have been reclassified more than once, suggesting there may be a cycle of reclassification that the clinician should be mindful of. Variants that are reclassified more than once are a documented phenomenon, but there are no known risk factors for this occurrence.

Notably, frequency of variant reclassification is variable ranging from 3.6% to 58.8% in the current published literature. The higher end of the frequency of variant reclassification (58.8%) may be a relic of overclassifying rare variants in complex diseases as this study specifically examined previously identified variants in arrhythmogenic cardiomyopathy. Historically, clinical guidelines for variant interpretation were not robust in complex diseases, as in arrhythmogenic cardiopathy, and there is poor correlation between variant classification and disease pathogenesis.
The medical literature that covers variant reclassification focuses on variant reclassification frequency in geographical and disease-specific cohorts. Further investigation is needed to determine variant reclassification changes over time and identify timepoints and interventions that may signal a stabilisation of genetic variant reclassification. Although there are many known features associated with genetic variant reclassification, there are no current robust predictors of variant reclassification that could identify probability that a variant will be reclassified at the point of initial variant classification. Studies to identify VUS within certain phenotypes that are at increased probability of reclassification exist but these are not generally applicable across all variant reclassification.13 The ability to reclassify variants is often limited by the restricted operational capacity of laboratory staff to reassess variants. One study reports an average of 105 min per variant required to perform analysis necessary for variant reclassification.14 Therefore, further research is needed to explore variant-specific features that may predict reclassification outcomes.

Ethical considerations regarding recontacting patients after variant reclassification remain to be fully elucidated. Where the responsibility for recontacting patients and informing them of updated variant classifications lies is not well-defined. Clinical genetics services are evolving to address ongoing variant reanalysis and patient follow-up. This change in diagnostic genetic testing stresses that continued genetic counselling within clinical genetics services is essential to stay up to date with variant reclassification and ensure optimal patient care.

CONCLUSION
Variant reclassification is an uncertain science based on current evidence and standards, but it is improving. The clinical implications of reclassification are disparate: reclassification can enforce lifelong medical surveillance, prompt prophylactic surgical or medical treatment, lend information to precision medicine and prolong the time to diagnosis of patients living with a rare disease. Increasing understanding of the human genome in line with evolving genomic technologies will surely lend to more accurate variant classification soon, honing molecular diagnoses with evolving genomic technologies will surely lend to more disease. Increasing understanding of the human genome in line with evolving genomic technologies will surely lend to more accurate variant classification soon, honing molecular diagnoses earlier during investigations. Until then, the phenomenon of variant reclassification may represent a shift in the paradigm of how clinical genetics services are provided with long-term follow-up of patients rather than point-of-service appointments.

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