Myriad's Multidisciplinary Approach and Consistent Investment in Variant Classification for Clinical Decision Making

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Genetic testing for hereditary predisposition to cancer was introduced almost 30 years ago.

While originally focused only on Sanger sequencing of highly penetrant cancer genes such as *BRCA1* and *BRCA2*, oncology genetics now routinely includes next generation sequencing multigene panels for both germline and somatic tumor testing. These advances have led to more defined gene-related cancer risks, improved medical management recommendations, personalized cancer therapies, and treatments, allowing for more informed decision making.

Variant classification is essential for accurate test results and appropriate care. Variant classification programs and teams must stay current with the advancements to testing and indications, as definitive variant classification is more important now than ever given the many decisions that are based on test results. A robust and accurate classification/reclassification program is essential for lowering the rate of variants of uncertain significance (VUS) to identify clinically meaningful results. Myriad's classification program continues to utilize a multidisciplinary expert committee approach with increased automation to accurately and efficiently classify variants, reduce the VUS rate, and provide more definitive answers for treatment and management. Here we describe Myriad's multidisciplinary team approach to classification, some examples of automation in our classification process, and updated VUS rates by gene.

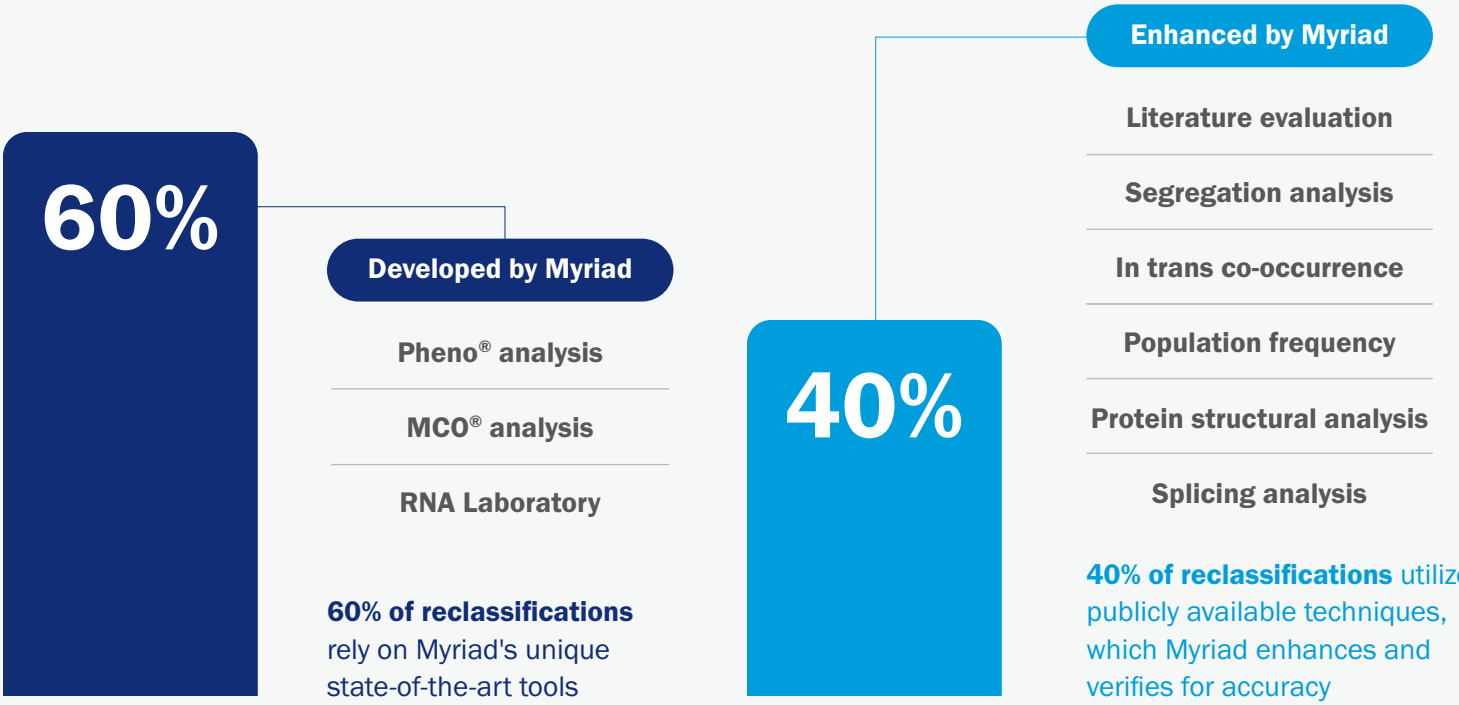
Myriad Variant Classification (Team and Tools)

The American College of Medical Genetics (ACMG) and Association for Molecular Pathology (AMP) published guidelines[1] are the baseline for all variant classifications at Myriad and most other commercial genetic testing laboratories. These guidelines are internationally accepted and provide a framework for classifying variants into one of five tiers (benign, likely benign, VUS, likely pathogenic and pathogenic) by defining classifying criteria by weight and type of evidence. Despite these well-defined guidelines, additional clinical and/or functional evidence is often needed to classify genetic variants. Some degree of automation is necessary for efficient variant classification, given the vast amounts of published scientific literature and patient samples processed at genetic testing laboratories. While Myriad utilizes many automated processes in our classification workflow, we passionately believe that a detailed review and discussion of the available evidence by our variant classification team is also a critical component of variant classification. We believe that this combination of automation and multidisciplinary team review is essential for appropriate variant classification and sets Myriad's Variant Classification team apart from other genetic testing laboratories.

Myriad's multidisciplinary variant team includes individuals with different experiences and expertise. This diversity helps to ensure each piece of evidence is reviewed, weighed, and applied appropriately in the classification of each variant. This team is composed of lab directors, PhD trained scientists, genetic counselors, and variant scientists. Each group has its own specific role in the variant classification process for increased accuracy and quality.

The variant classification team meets daily to assign classifications to new variants and to discuss and analyze new evidence for previously classified variants. The team utilizes an internally developed, state-of-the-art application that pulls in all information about a variant. This automation

Myriad's Classification Techniques



makes the process more efficient and accurate as individuals do not have to find each piece of information on their own.

Classification decisions are based on specific, well-defined rules but discussions with the team are essential to applying the rules in each situation appropriately. While the team members typically agree on how evidence can be applied, there are times where there are conflicting opinions. For example, reports of an *ATM* variant in combination with another *ATM* mutation in patients with a clinical diagnosis of ataxia-telangiectasia can be used to upgrade an *ATM* variant per ACMG guidelines; however, if there are concerns about the clinical diagnosis, consanguinity, overlapping patients among studies, and/or phase of the variants, there may be differences of opinion on the strength of this evidence. Complexities like this show the importance of a team discussion rather than just taking the evidence at face value. Some caveats can be overlooked by one person, but the committee/team approach makes this less likely, adding to the accuracy of the classification.

Myriad's committee approach is combined with automation by including use of an important classification tool, Pheno®, which is an internally developed and validated history weighting algorithm.[2] This powerful statistical tool compares the personal and family cancer histories of individuals with a variant of interest to both the histories of individuals with a known pathogenic variant and to individuals with no pathogenic variants to determine if a variant is pathogenic or benign. When a variant is identified in a new test/individual, Pheno automatically combines the new data with that of all other individuals carrying the same variant.

In addition, when the Pheno score reaches statistical significance allowing it to make a classification call, an automatic notification is sent to Myriad's classification team. This automation allows for great efficiency as the tool is doing the work in the background when variants are seen again. Pheno has been validated with greater than 99.5% positive and negative predictive values making it a highly accurate tool and allowing for reclassifications based on this evidence alone.[6,7] However, to help ensure accuracy, the Myriad variant classification team reviews all evidence for each variant, looking for any conflicting evidence.

Therefore, when Pheno makes a classification call, the variant is always reviewed and discussed by the variant classification committee. Pheno is a strong, accurate, and useful classification tool, but Myriad's team will not reclassify a variant without the multidisciplinary team review. Pheno has been used to reclassify many variants[3] and has proven invaluable for classifying VUSs more quickly as often variants with a Pheno call do not have any additional evidence to aid in classification.

Myriad combines automation with detailed manual review of evidence in several other areas of variant classification. Automated literature searches are used to identify publications that may be used as evidence for variant classification or reclassification. Although the literature search is automated, each piece of literature identified is carefully reviewed by Myriad's PhD-level scientists with unique experience and expertise in genetics and biology. The diverse team of scientists evaluates each piece of evidence in the literature thoroughly. Factors such as the design of the study, study population, and sample size are considered. In addition, whether the model or assay used is appropriate for the gene/variant in question, if appropriate ethnically, matched controls were utilized, and how other known pathogenic/benign variants perform in the assay are analyzed. Only studies that are vetted and meet Myriad's thresholds are used as sufficient evidence for variant classification at our laboratory.

RNA Analysis

Myriad has offered reflex testing for RNA analysis since 2015. While other labs incorporate internal RNA analysis into their variant classification, Myriad's RNA testing includes allele specific quantification to assess the potential for incomplete (aka "leaky") splicing.

This allele specific quantification is essential for Myriad's classification committee to understand the full impact of the splice defect. Without additional significant evidence, it is unclear how an incomplete splice defect will impact overall function.[4,5] Some genes can tolerate a substantial reduction in normally spliced transcript. For example, some *BRCA2* variants cause 90% of the transcript to be aberrantly spliced. However, in combination with the wild-type allele in a heterozygous carrier, the remaining 10% of normally spliced transcript is sufficient for normal protein function.[4,5] If an incomplete splice defect is observed or cannot be ruled out, Myriad's classification team needs additional strong lines of evidence such as clinical phenotypes and/or segregation with disease to confirm a variant is truly pathogenic. Without allele-specific quantification, RNA data may be incorrectly used to upgrade a variant.[8]

Given the need for allele-specific quantification, not all individuals are eligible for RNA studies at Myriad.

The RNA program is a targeted program to select the variants most likely to impact splicing as well as to test individuals that will be informative (allow for allele-specific quantification). In this way we can provide more clinically actionable results to the provider and patient.

Since Myriad has implemented internal RNA studies, a total of 329 RNA samples have been analyzed. On average, we conduct five RNA studies per month. To date, our RNA studies have resulted in 126 variant reclassifications from VUS to likely pathogenic/pathogenic impacting over 3,600 patient reports.

Rates of Variants of Uncertain Significance at Myriad

The Myriad team understands that even with a team of experts and excellent classification tools, VUSs will continue to exist. Understanding the value of definitive variant classifications to clinicians, patients, and families, we continually focus and invest in the classification process with the goal of providing a definitive, and highly accurate classification for each variant identified.

Myriad's robust reclassification process and uniquely enhanced automated classification tools have allowed for the reclassification of many VUSs,[3] reducing the VUS rate over time for genes on the MyRisk® panel.

Tracking these rates for genes over time demonstrates the effectiveness of a laboratory's reclassification program.

The Myriad VUS rate is calculated by counting the total number of clinical MyRisk reports with one or more VUS in a specific gene and dividing this by the total number of clinical MyRisk reports issued in the same period. **Table 1** shows the change in VUS rates for genes from 2019 to 2023. Of the 30 genes with a reported VUS rate (*EPCAM* testing includes specific large rearrangement testing only and no VUS were detected as of December 2023), 16 of the 30 genes showed a decrease in the VUS rate over four years. In addition, updated VUS rates showed that 18 of the 30 genes have a VUS rate of less than 1%.

There have been several gene additions and gene enhancements to the MyRisk panel since its initial launch in 2013. In 2022, Myriad added 17 genes to the MyRisk gene panel (4 genes were removed for a new total of 48 genes). The current version of MyRisk includes enhanced gene coverage for some original cancers and incorporates genes associated with new cancer sites including skin, renal, endocrine/thyroid, and lung. The VUS rates for 16 of these additional genes (*MITF* not included) are shown in **Table 2.** These VUS rates were calculated after approximately 18 months of testing so there is no comparison to previous VUS rates. Although we expect the VUS rates of these newer MyRisk genes to decrease over time based on the trend observed with previous MyRisk expansions, we are pleased that 13 of these 16 genes have a VUS rate of less than 1%.

While Myriad strives to achieve low VUS rates for all genes tested at our laboratory, low VUS rates for the *BRCA1* and *BRCA2* genes are extremely important to us given the therapeutic implications for patients. Newly diagnosed breast cancer patients often undergo MyRisk testing to aid with surgical decisions. Given the increased risks for developing second primary breast cancers associated with mutations in *BRCA1* and *BRCA2*, professional society guidelines recommend *BRCA1/BRCA2* mutation carriers consider bilateral mastectomy to treat their current breast cancer as well as to reduce their risks for developing subsequent breast cancers in their lifetime.

In addition, there are targeted treatments that may be considered for breast, ovarian, prostate, and pancreatic cancer patients found to carry germline mutations in *BRCA1* and *BRCA2*. Even in the absence of a germline *BRCA1/BRCA2* mutation, identifying a somatic (tumor genomic) *BRCA1/BRCA2* and other gene mutation in the homologous recombination repair pathway on Myriad's MyChoice® CDx or Precise Tumor® testing impact the utilization of PARP inhibitors in treating a variety of cancers. Therefore, definitive variant classification on both germline and tumor genomic testing is critical to aid patient treatment. Myriad takes immense pride in our *BRCA1* and *BRCA2* VUS rates, 0.30% and 0.70% respectively, given that these low VUS rates mean more informed decision making for patients.

Table 1. VUS reporting rate in 2019 and 2023 by gene.

Gene	2023 VUS Rate	2019 VUS Rate	2016 VUS Rate
APC	2.70%	2.80%	
ATM	2.70%	3.10%	
BARD1	0.60%	0.80%	
BMPR1A	0.40%	0.50%	
BRCA1	0.30%	0.30%	0.50%
BRCA2	0.70%	0.90%	1.10%
BRIP1	1.90%	1.90%	
CDH1	1.10%	1.20%	
CDK4	0.40%	0.40%	
CHEK2	1.30%	1.50%	
MLH1	0.40%	0.40%	
MSH2	0.60%	0.70%	
MSH6	1.10%	1.30%	
MYH	1.40%	1.40%	
CDKN2A (p14ARF)	0.20%	0.40%	
CDKN2A (p16INK4a)	0.80%	1.10%	
PALB2	0.50%	0.70%	
PMS2	1.40%	1.50%	
PTEN	0.10%	0.20%	
RAD51C	0.30%	0.90%	
RAD51D	0.90%	0.90%	
SMAD4	0.20%	0.20%	
STK11	0.50%	0.50%	
TP53	0.60%	0.60%	
GREM1	0.02%	0.01%	
POLD1	0.67%	0.67%	
POLE	1.30%	1.28%	
AXIN2	6.10%	4.50%	
HOXB13	0.40%	0.50%	
MSH3	4.60%	3.50%	
NTHL1	1.40%	1.50%	

Table 2. 2023 VUS detection rate for newly added MyRisk genes added after 2019.

Gene	2023 VUS Rate
BAP1	0.40%
CTNNA1	0.60%
EGFR	0.20%
FH	0.50%
FLCN	0.70%
MEN1	0.60%
MET	1.80%
RET	0.50%
SDHA	2.10%
SDHB	0.80%
SDHC	0.20%
SDHD	0.20%
TERT	0.10%
TSC1	0.90%
TSC2	2.20%
VHL	0.70%

ACMG guidelines encourage institutions to share their variant classifications, and the evidence used for these classifications to help with the resolution of discordant classifications. Myriad contributes oncology variants to ClinVar on a quarterly basis. Myriad has fully committed to submitting all new clinically actionable variants as well as all reclassified variants each quarter. Other variants are also included with the quarterly submissions. Myriad contributed over 20,000 oncology variant classifications in 2023-2024.

Myriad's robust variant classification program is also well recognized among providers in the oncology genetics space. Many providers contact Myriad's Medical Service team to inquire about Myriad's variant classification upon receiving a VUS classification from another clinical genetic testing laboratory. In addition, classification scientists from other laboratories also regularly contact members of Myriad's team asking about variant classifications and the evidence used in the classifications.

It is clear providers and classification scientists acknowledge and appreciate that Myriad's decades of experience in the field combined with our variant classification tools and processes often lead to a faster definitive variant classification.

Conclusion

Since variants of uncertain significance from genetic and genomic testing cannot be acted upon clinically, accurate definitive classification is essential for patient care and targeted treatments. The uncertainty of a VUS on a genetic test report may be frustrating to healthcare providers given there is little guidance on how to manage these patients. A VUS on a genetic testing report may place the patient in a medical as well as possibly psychological limbo until the variant is definitively reclassified. Variants cannot be reclassified in a timely manner if the laboratory where the testing was performed is not proactively evaluating and investing in the reclassification process and in some cases, still in business. The gene specific VUS rate is influenced by several factors, including the length of the coding regions, penetrance of associated disorders, severity of phenotypes, and our knowledge through peer-reviewed published research. Given this complexity and the growing impact that this information has for personalized patient care, a close partnership between clinicians and laboratory experts is essential to optimizing the impact of genetic and genomic testing to improve patient outcomes.

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