WHITE PAPER

Driving Down the Rate of Variants of Uncertain Significance as the Myriad myRisk® Multigene Panel Grows

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Introduction/Background

Genetic variation in the human genome is extensive, and although there is clear evidence of the benign or pathogenic impact of many variants, current scientific knowledge is lacking for others. Variants of uncertain significance (VUS) are changes in the typical sequence of a gene for which the clinical significance and/or association with disease is unclear. VUS are reported on Myriad's myRisk® genetic test result, and in accordance with professional society guidelines, individuals with VUS identified on germline testing should be managed based on personal and family history.[1]

VUS are a normal and expected outcome of genetic testing. Nonetheless, at Myriad we recognize that definitive classifications allow for the most appropriate management and care and reduce the uncertainty of genetic testing. Accordingly, VUS identified at Myriad are continually evaluated through Myriad's Classification Program, myVision®. Myriad's robust reclassification program has led to significant reductions in the VUS rate over the years. The combined VUS rate for the 25-gene Myriad myRisk test was 41.7% in 2014[2] and decreased to 28.6% in 2016.[3] With the continued enhancement of reclassification tools, including the development and improvement of novel classification methodologies, additional testing experience, and further knowledge of the role of these genes in hereditary cancer, the VUS rate has further decreased to 23.8% (December 2019) for the original 25 myRisk genes.

As multi-gene panel testing is increasingly utilized in hereditary cancer, the number of genes tested for has also increased. Since 2016, ten additional genes have been added to the myRisk panel. These include three additional colorectal genes (*GREM1*, *POLD1*, *POLE*) added in 2016, prostate cancer associated *HOXB13* in 2018, and six more colorectal genes (*AXIN2*, *GALNT12*, *MSH3*, *NTHL1*, *RNF43*, *RPS20*) in 2019. The combined VUS rate for the expanded 35-gene myRisk panel is 37.1%, which is an expected increase given the addition of ten genes with emerging literature and knowledge. Despite the overall increase in the VUS rate following the introduction of ten additional genes, the VUS rate for these new genes is already decreasing (**Figure 1**) and this trend is expected to continue.

In this review, we discuss the variant classification methods used at Myriad that have contributed to the reduction in the VUS rate over time.

myVision®
Myriad Variant Classification Program

Methods

The American College of Medical Genetics (ACMG) and Association for Molecular Pathology (AMP) published classification guidelines[4] are the baseline for variant classification at Myriad; however, our classification team builds upon these guidelines by developing and enhancing our own classification tools based on the knowledge gained through our testing experience. Myriad employs eight classification methods in variant classification (**Figure 2**). These methods include literature review (LitView), homozygous/*in trans* co-occurrence, mutation co-occurrence (M-Co®), segregation analysis, structural analysis, cancer history weighting algorithm (Pheno®), population frequency, and RNA/splice analysis (InSite®). Although each of these tools has played an important role in the decline of the overall VUS rate, some have been especially impactful.

The VUS rate for a given time point is estimated by counting the total number of clinical myRisk reports with one or more VUS (and no pathogenic variants) and dividing this by the total number of clinical myRisk reports issued during that time period. Therefore, the calculated VUS rate reflects the actual probability that a clinician will receive a VUS result for a patient rather than the percent of VUS observed overall. Variation in the VUS rate per gene is dependent on the size of the gene, the classification tools available for the gene, and the existing knowledge base of the gene. Amended reports are issued to healthcare providers when any reported variant is reclassified.

FIGURE 2. Myriad Classification Methods.

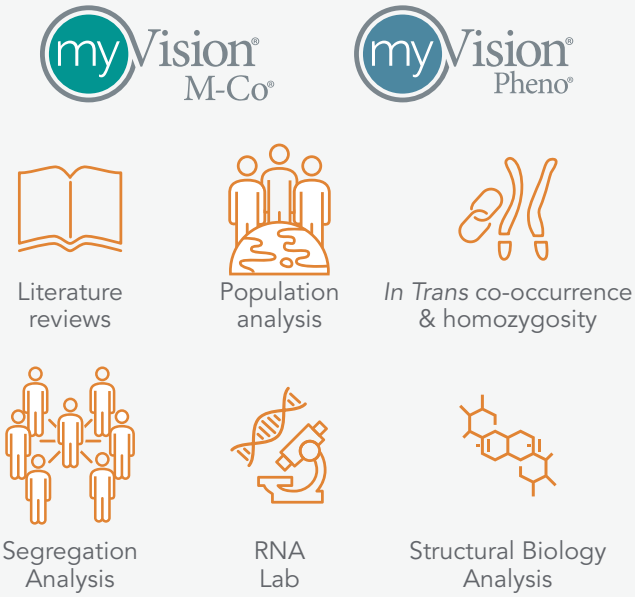
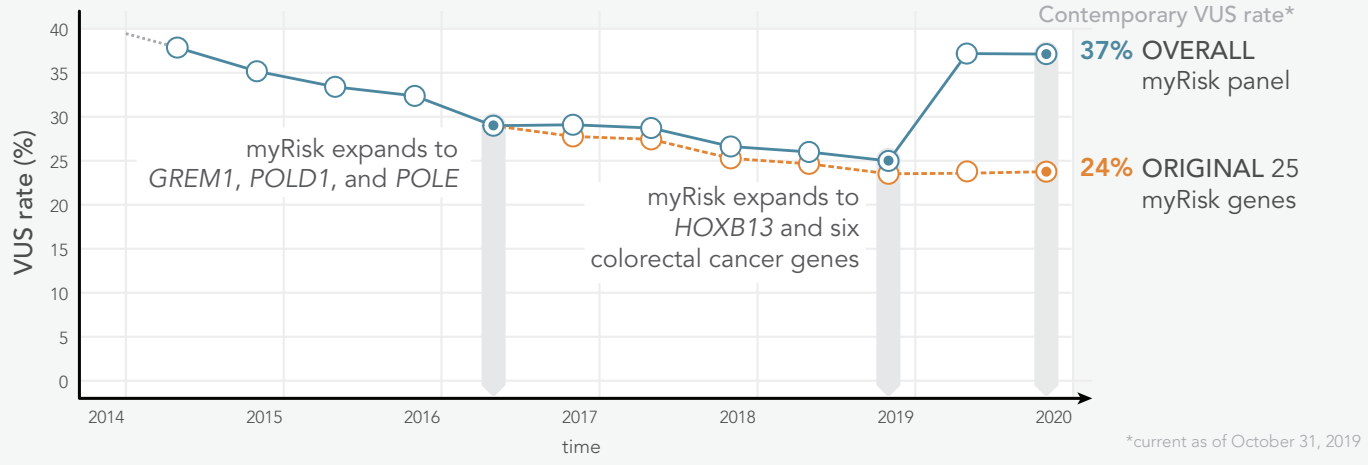


FIGURE 1. VUS Rate by Panel Over Time. 35-gene panel includes all genes on smaller panels.



Discussion

Pheno, a personal and family history weighting statistical algorithm, has long been used at Myriad for upgrading and downgrading variants. It measures the strength of cancer histories associated with a particular variant and compares it to those of pathogenic and benign control variants in the same gene. It controls for ancestry and inconsistencies in physician-reported histories and can determine the clinical significance of a variant with a high degree of confidence. Pheno has been used for several years to upgrade and downgrade VUS in *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *MSH6*, as well as to downgrade VUS in *ATM*, *CHEK2* and *PALB2*. Improvements and enhancements are continuously being made to Pheno with each followed by additional validations, and in 2017, Pheno was validated for use in upgrading variants in *ATM* and *CHEK2* as well as downgrading variants in *BARD1*. As illustrated in Table 1, Pheno has had a significant impact on the VUS rate for many of these genes from 2016-2019.

Any VUS has the potential to be pathogenic or benign; however, benign variation is more common. In fact, a review of 10 years (2006-2016) of reclassification data at our laboratory showed that 91.2% of reclassified VUS were downgraded to likely benign/benign while 8.7% were upgraded to pathogenic.[5] Although this is the expected overall trend with VUS reclassifications, the ratio of upgrades to downgrades fluctuates at different time points with the use of new evidence or introduction of a new classification tool. Recently, Myriad's increased utilization of enhancements to the myVision classification program that generate or aggregate crucial sources of functional evidence to help upgrade VUS to likely pathogenic/pathogenic has led to a substantial increase in the number of upgrades from VUS to likely pathogenic/pathogenic. Specifically, during the two-year time period from January 2018 through December 2019, 36.9% of VUS reclassifications were upgrades to likely pathogenic/pathogenic. Most of these upgrades are attributed to two enhancements to the myVision classification program: Myriad InSite RNA lab and the use of internally validated published functional evidence.

TABLE 1. VUS Rate by Gene.

Gene	VUS Rate 2016 → 2019	
APC	3.50% →	2.80%
ATM	4.70% →	3.10%
BARD1	1.60% →	0.80%
BMPR1A	0.60% →	0.50%
BRCA1	0.50% →	0.30%
BRCA2	1.10% →	0.90%
BRIP1	1.90% →	1.90%
CDH1	1.60% →	1.20%
CDK4	0.40% →	0.40%
CHEK2	2.50% →	1.50%
EPCAM	0.00% →	0.00%
MLH1	0.60% →	0.40%
MSH2	1.00% →	0.70%
MSH6	1.70% →	1.30%
MYH	1.50% →	1.40%
NBN	2.40% →	2.40%
P14ARF	0.40% →	0.40%
P16	1.10% →	1.10%
PALB2	1.20% →	0.70%
PMS2	2.10% →	1.50%
PTEN	0.30% →	0.20%
RAD51C	1.10% →	1.10%
RAD51D	1.00% →	0.90%
SMAD4	0.50% →	0.20%
STK11	0.70% →	0.50%
TP53	0.60% →	0.60%
GREM1	–	0.01%
POLD1	–	0.67%
POLE	–	1.28%
AXIN2	–	4.50%
GALNT12	–	1.20%
HOXB13	–	0.50%
MSH3	–	3.50%
NTHL1	–	1.50%
RNF43	–	2.40%
RPS20	–	0.01%

RNA/SPLICE ANALYSIS IN THE INSITE LAB

It is estimated that 10-15% of VUS have the potential to impact normal RNA splicing.[6] These are changes in the sequence of the DNA that affect how the RNA is spliced for translation into the final functional protein. Although there are many predictors of if/how a variant will impact splicing, functional evidence is often needed to prove a splice defect. Myriad's InSite RNA lab was established in 2015 to provide such functional evidence. InSite is an IRB-approved research laboratory protocol allowing for the collection and analysis of research samples from individuals with variants that have the potential to impact splicing. It is essential to distinguish between full and partial defects through quantification. If a VUS causes a fully penetrant splice defect, it can be reclassified to likely pathogenic or pathogenic. If a partial splice defect is identified, the variant is not reclassified without additional evidence. If no splice defect is identified, some variants may be downgraded from VUS to likely benign or benign. Since its inception, InSite has provided data leading to the reclassification of 87 unique VUS resulting in hundreds of amended reports for patients. Variants that are likely to impact splicing are targeted for analysis, so it is not surprising that 82% (71/87) of these reclassifications were upgrades from VUS to likely pathogenic or pathogenic. RNA/splice analysis is not limited to specific genes and evidence from the InSite lab is used in the classification of variants in most genes on the myRisk panel.

VALIDATED FUNCTIONAL EVIDENCE

Although determining when and how to use published functional and biochemical studies as evidence in classification can be challenging, there are robust and important functional studies that when, after careful vetting, can be informative for variant classification. Myriad validates all published studies thoroughly before considering the data in classification by conducting extensive work by our scientific and statistical teams to compare data from the published studies to variants classified with other validated methods (including Pheno, RNA lab data, etc.). This validation determines the specific rules and thresholds that meet Myriad's expected confidence level for use in classification. Even with the thorough validation and set threshold, functional evidence from a single source is not used in isolation to upgrade a VUS to likely pathogenic. There must be additional support from another classification tool such as structural review, segregation or an additional validated functional study.

The recent publication of studies of large datasets has greatly impacted the increase in the rate of reclassification from VUS to likely pathogenic/pathogenic. The Kotler et al. (2018)[7] paper is an example of one of these key studies. This study measured the impact of almost 10,000 *TP53* variants in the DNA binding domain providing essential information regarding the function of these variants. Myriad's internal validation proved the study provides accurate and informative data that meets our high confidence threshold and can therefore be used in variant classification. This has led to the reclassification of many VUS and provided over 300 patients with important information regarding their medical management plan. Studies of large datasets like Kotler et al. (2018)[7] can have a significant impact on reclassification and the ratio of VUS upgrades versus downgrades.

Conclusion

Variants of uncertain significance are routinely identified in genetic testing. However, even when incorporated appropriately into pre- and post-test counseling, VUS can result in uncertainty, anxiety, or misinterpretation. Therefore, the importance of accurately classifying and reclassifying variants cannot be overstated. Myriad's experience and continued investment in variant classification has allowed for a dynamic approach that integrates ACMG guidelines and unique classification tools with a high degree of accuracy. This approach has led to a consistent reduction in the overall VUS rate, which is currently 37.1% for the expanded 35-gene myRisk panel. Although new variants are observed daily and new genes are continuously evaluated for potential additions to the myRisk panel, it is expected that the VUS rate will continue to decrease. Definitive classifications allow for clinically significant results to be reported, reduced uncertainty for patients and families, and confidence for providers counseling their patients.

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