

# Current Variant of Uncertain Significance Rates in *BRCA1/2* and Lynch Syndrome Testing (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*)

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## Introduction

Deleterious (i.e. disease associated) mutations in the Hereditary Breast and Ovarian Cancer syndrome genes (*BRCA1/2*) and the Lynch syndrome associated genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*) confer a significantly increased risk for cancer. The identification of a deleterious mutation in these genes has important implications for the clinical management of patients in guiding strategies for prevention and early detection of cancers. However, testing of these genes frequently detects genetic Variants of Uncertain Significance (VUS). VUS present a challenge to the clinician in how to appropriately guide the medical care of their patient in the context of an inconclusive test result. Clinical laboratories can utilize various strategies to help clarify the significance of VUS in order to provide clinicians with definitive information. Here we report on the dramatic success achieved in our laboratory through the development and application of improved statistical techniques and a targeted program for data gathering through additional testing in patients and their relatives. Mutations in the *BRCA1/2* and Lynch syndrome associated genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*) that disrupt normal protein production and/or function are known to cause an increased risk for cancer. Myriad Genetic Laboratories Inc. (Myriad) classifies variants that have an unknown effect on protein or RNA function as VUS. While the majority of VUS are eventually discovered to be non-disease causing, some are pathogenic. Utilizing an approach similar to that described in Goldgar et al, Myriad makes a concerted effort to gather more information on VUS in order to reclassify the variants as deleterious mutations or polymorphisms (benign variants not associated with disease).<sup>1</sup> This approach incorporates segregation analysis in families, co-occurrences with deleterious mutations, evaluation of personal and family history, evolutionary conservation status, and data from the literature is utilized.<sup>1</sup>

Currently variants of uncertain significance are more common in non-European ancestries than in European groups. This is partially due to the lower utilization of genetic testing in non-European groups. As more testing is performed within a given ancestry, the uncertain variant rate within that group is predicted to decline.

## Methods

Myriad's mutation database was analyzed periodically from 2002-2012 to establish the percentage of overall tests reported with an interpretation of VUS (test offerings queried are defined in the tables/figures) and subdivided by ancestry. Inclusion criteria consisted of individuals who were referred for clinical genetic testing of the *BRCA1/2* or the Lynch syndrome associated genes. Patients for which no ancestry was selected or for which multiple ancestries were selected were excluded from the ancestry breakdown but were included in the total count.

## Results

**Variant of uncertain significance rates and ancestry for BRCA genes:** In 2002, 12.8% of all *BRCA1/2* tests resulted in a VUS results. By 2012, that rate had declined to 2.9% (77.3% decline, Table 1). From 2002 to 2012 there was a decline in the VUS rate across all ancestries. Of particular interest is the large decline in the African American (a decline of 87.0%) and Latin American populations (a decline of 85.1%; Figure 1). The dramatic decline in the VUS rate in these ancestries is due to both improved methods for establishing the clinical significance of variants and increased utilization of testing in these populations, which provides more data for analysis.

**Variant of uncertain significance rates and ancestry for Lynch syndrome associated genes:** Testing for Lynch syndrome has evolved over time. In 2002 testing at Myriad consisted of sequencing of *MLH1* and *MSH2* genes. As of 2012, testing includes evaluation of the mismatch repair genes associated with Lynch syndrome (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) and *EPCAM* (mutations in *EPCAM* can effect expression of *MSH2*). In order to compare variant rates in Lynch syndrome testing over time we compared *MLH1/MSH2* variant rate from 2005 to the 2012 overall variant rate of *MLH1/MSH2/MSH6/EPCAM*. The *PMS2* variant rate for 2012 is reported separately which reflects the way testing is conducted at Myriad at this time. While *PMS2* is conducted concurrently and included in Myriad's standard Lynch syndrome testing along with *MLH1/MSH2/MSH6/EPCAM* these tests are run separately. Similar to *BRCA1/2* the overall rate of VUS for Lynch syndrome associated genes has decreased over time with the current rate for *MLH1/MSH2/MSH6/EPCAM* being 6.6% (34.7% decline; Table 2). This decline was noted across multiple ancestries with the exception of the Latin American population (Table 2). The overall rate for VUS as of 2012 for *MLH1/MSH2/MSH6/EPCAM* genes is 6.6% and for *PMS2* is 4%.

Table 1: Decline in Rate of *BRCA1/2* Variants of Uncertain Significance

Ancestry	2002	2006	2008	2012	% Decline
All Patients	12.8%	7.0%	5.1%	2.9%	77.3
Middle Eastern	23.7%	10.0%	9.6%	5.9%	75.1
Asian	25.1%	13.6%	10.5%	7.8%	68.9
African	38.6%	21.0%	10.9%	5.0%	87.0
Native American	16.1%	7.4%	5.2%	2.7%	83.2
Latin American	26.1%	11.2%	6.6%	3.9%	85.1
Central European	11.8%	6.6%	4.8%	2.6%	78.0
Western European	11.1%	6.3%	4.3%	2.6%	76.6

Table 2: Decline in Rate of Lynch Syndrome Associated Genes Variants of Uncertain Significance

Ancestry	2005 ( <i>MLH1/MSH2</i> only)	2012 ( <i>MLH1/MSH2/MSH6/EPCAM</i> )	% Decline
All Patients	10.1%	6.6%	34.7
Middle Eastern	4.5%	2.3%*	N/A
Asian	20.6%	10.8%	47.6
African	35.4%	11.4%	67.8
Native American	13.9%	5.7%	59.0
Latin American	7.0%	7.1%	N/A**
Central European	10.1%	7.7%	23.8
Western European	9.4%	6.2%	34.0
Ashkenazi	4.1%	3.0%	26.8

\*Sample size is not sufficient for statistical significance  
\*\* % change not significantly different

Table 3: Rate of *PMS2* Variants of Uncertain Significance 2012

Ancestry	<i>PMS2</i>
All Patients	4.0%
Middle Eastern	N/A*
Asian	5.8%
African	5.0%
Native American	3.9%
Latin American	5.8%
Central European	4.1%
Western European	3.1%
Ashkenazi	5.8%

\* Sample size is not sufficient for statistical significance

Figure 1: Decline in Rate of *BRCA1/2* Variants of Uncertain Significance

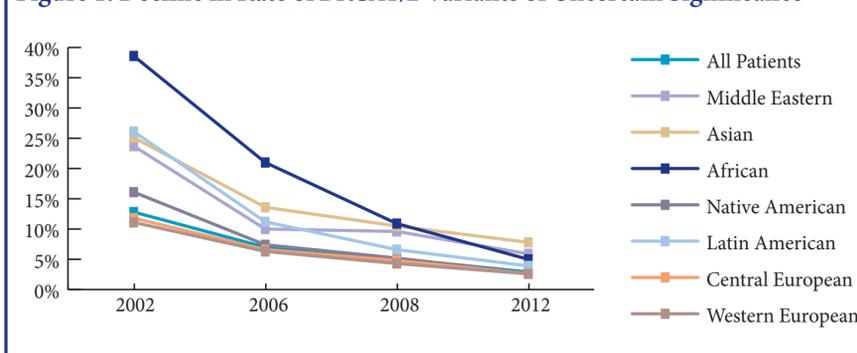
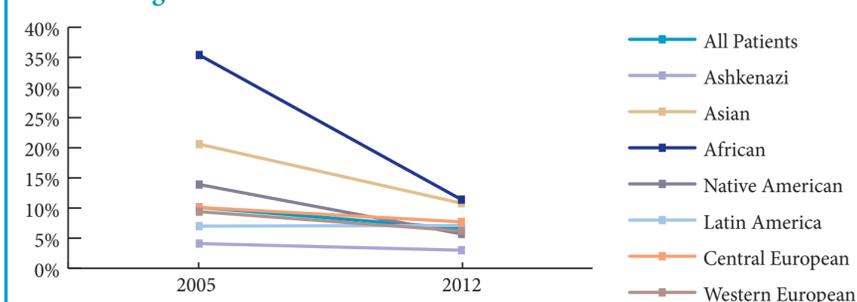


Figure 2: Decline in Rate of Lynch Syndrome Associated Genes Variants of Uncertain Significance



## Discussion

Interpreting the clinical significance of new variants is a challenge associated with any sequencing-based genetic test. This requires a costly labor and data intensive effort to analyze variants of uncertain significance using various lines of evidence. Of the many and varied types of mutations in the *BRCA* genes detected when testing first became clinically available in 1996, over 40% (data not shown) of results were originally of unknown significance. Myriad has been able to further characterize most of these variants and subsequently reduce the VUS rate to only 2.9% for the *BRCA* genes. Similarly, for the genes associated with Lynch syndrome, there has been a steady decline in the percentage of variants reported as being of uncertain clinical significance. The current VUS rate for *MLH1/MSH2/MSH6/EPCAM* is 6.6% and for *PMS2* is 4.0%. Although the overall rate of VUS has decreased, it is important to note that novel variants are still discovered in these genes.

Myriad utilizes multiple lines of evidence to evaluate and reclassify VUS, and new classification methods are continually being assessed by Myriad scientists.<sup>1,2,3</sup> Current methodologies include:

- Co-occurrence with a deleterious mutation in-trans for genes with known compound heterozygosity phenotype (i.e. Fanconi Anemia and *BRCA2*)
- Deleterious mutation co-occurrence with mutations in other genes associated with the same syndrome
- Personal and family history analysis (Phenotype analysis)
- Segregation analysis
- Evolutionary conservation
- Literature evaluation

Myriad has been able to apply methodologies and statistical techniques developed on a large *BRCA* mutation data set to Lynch syndrome associated genes thereby reducing the variant rates across all of the associated genes. Clarification of the meaning of a VUS gives more precise information to clinicians managing their patient and assists them in making medical management choices.

## References

- Goldgar D, Easton D, Byrnes G, et al. Genetic evidence and integration of various data sources for classifying uncertain variants into a single model. *Hum Mutat.* 11:1265-72, 2008.
- Tavtigian S, Samollow P, de Silva D, Thomas A. An analysis of unclassified missense substitutions in *BRCA1*. *Familial Cancer.* 5:77-88, 2006
- Easton D, Deffenbaugh A, Pruss D, et al. A systematic genetic assessment of 1,433 sequence variants of unknown clinical significance in the *BRCA1* and *BRCA2* breast cancer-predisposition genes. *Am J Hum Genet.* 5:873-83, 2007