INTRODUCTION

Lynch syndrome (also known as Hereditary Nonpolyposis Colon Cancer), is an autosomal dominant genetic syndrome that is responsible for 2-4% of colorectal cancers, as well as other cancers, within the United States. Over time, Myriad Genetic Laboratories, Inc. has included additional genes to the Lynch syndrome panel to increase sensitivity. Since 2011, testing has included five genes associated with Lynch syndrome: MLH1, MSH2, MSH6, PMS2, and EPCAM. Myriad’s Lynch syndrome test identifies both sequencing and large rearrangement changes (only large rearrangements are detected in EPCAM). The identification of deleterious mutations 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 that have an unknown effect on protein production or function. Myriad classifies these variants as Variants of Uncertain Significance (VUS). These mutations can present a challenge to the clinician in how to appropriately guide the medical care of their patient. Clinical laboratories can utilize various strategies to help clarify the significance of VUS in order to provide clinicians with more definitive risk information.

This poster presents a breakdown of the current deleterious mutation distribution, mutation type (sequencing vs. large rearrangement) and the VUS rate.

METHODS:

For sequencing analysis, genomic DNA was directly amplified utilizing the Polymerase Chain Reaction (PCR) to generate exon-specific amplicons for MLH1, MSH2, MSH6 and portions of the PMS2 gene. Due to the presence of pseudogenes in PMS2, specialized PCR techniques were used for the remaining portions of this gene, allowing for the detection of sequencing mutations along the entire PMS2 coding region. MLH1 and MSH2 large rearrangement analysis (CART) was performed using a quantitative multiplex PCR assay, which detects copy number variations indicative of a deletion or insertion. PMS2, EPCAM, and MSH6 large rearrangement analyses were performed using Multiplex Ligation-dependent Probe Amplification (MLPA) assays (MRC-Holland).

To determine the mutation distribution, clinical test results were analyzed from 2011 to 2012 for patients who were referred for clinical genetic testing of the Lynch syndrome associated genes and therefore were tested for Comprehensive Colaris with concurrent PMS2 analysis.

For the calculation of VUS rates, Myriad’s mutation database was analyzed periodically from 2006-2012 to establish the percentage of overall tests reported with an interpretation of VUS in the Lynch syndrome associated genes (MLH1, MSH2, MSH6, and PMS2).

RESULTS:

Of positive clinical tests since May 2011, 38.8% reported a deleterious mutation in MSH2, 30% in MLH1, 16.4% in MSH6, 11.9% in PMS2 and 3% in EPCAM (Figure 1). PMS2 and MSH6 have the highest VUS rates with 66.6% of all VUS occurring in these two genes (33.3% in each gene) (Figure 2). Only 14.6% of VUS were in MLH1 and 18.8% of the VUS were in MSH2.

Large rearrangements represent 18.5% of the mutations detected in the Lynch syndrome genes (Figure 3). These mutations would not be detected by standard sequencing but require a methodology that will detect large structural changes. Figure 4 shows the percentage of large rearrangements by gene. About one-fourth of mutations are rearrangements in the PMS2 and MSH2 genes. Of the MLH1 gene mutations 14% are rearrangements while only 3.6% of mutations are rearrangements in MSH6. All of the EPCAM mutations are rearrangements as this testing is designed to detect deletions in the EPCAM gene that negatively affect transcription of the MSH2 gene.

MLH1 and MSH2 were the first genes identified as being causative for Lynch syndrome. They are the most penetrant which may explain why they were most readily identified in linkage studies. MSH6 and PMS2 were subsequently identified and added to testing. These two genes have lower mutation rates but higher VUS rates.

DISCUSSION:

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. Although the overall rate of VUS has decreased, it is important to note that novel variants are still discovered in these genes on a regular basis. Myriad utilizes a costly labor and data intensive effort with multiple lines of evidence to evaluate and reclassify these variants, and new reclassification methods are continually being assessed by Myriad scientists. Current methodologies include:

- In-trans Deleterious Co-occurrences – Bi-allelic deleterious mutations in MLH1, MSH2, MSH6, and PMS2, (in-trans co-occurrence) produce very specific phenotypes. If a patient has a deleterious mutation and a variant on opposite alleles and lacks this bi-allelic phenotype, it provides strong evidence that the variant is not deleterious.

- Mutation Co-occurrence – A statistical tool unique to Myriad which analyzes expected mutation co-occurrence frequencies and allows for variant reclassification, powered by Myriad’s database. For example, deleterious mutations in both MLH1 and MSH2 are possible in the same patient, but are very rare. If a VUS is seen with multiple MLH1 and MSH2 mutations, the VUS becomes statistically unlikely to be deleterious.

- Phenotype Analysis – A statistical tool unique to Myriad that compares the severity of a variant’s associated personal and family histories to other benign and deleterious control populations.

- Segregation Analysis – Myriad actively works to determine whether or not variants track with cancer in families.

- Accurate Evolutionary Conservation Analysis – Myriad has developed a highly accurate way in which to use evolutionary conservation analysis, validating the technique against the Myriad database.

- Literature Evaluation – Literature evaluation incorporates the evaluation of all publicly available data. A varied team of research scientists including geneticists, biochemists, biologists, statisticians and other specialists allows for skilled evaluation of this data.

Lynch syndrome testing has improved over time with the addition of more genes. With newer sequencing technologies, we anticipate demand for including more genes in the interpretation of cancer risk. Increasing the number of genes analyzed will increase sensitivity but it will also demand greater effort towards the analysis of VUS to provide health care providers with the most useful information.

REFERENCES: