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How and When to Consider Genetic Testing for Colon Cancer?

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Approximately 20% to 30% of patients with colorectal cancer (CRC) have a potentially definable inherited cause.[1] Furthermore, 3% to 5% CRCs are associated with hereditary cancer syndromes.[2] Individuals who harbor germline mutations are at an increased risk of developing early onset CRC as well as extracolonic tumors. Identifying individuals with germline mutations in CRC driver genes offers the potential to provide targeted prevention strategies and surveillance for the proband and their family.

The introduction into clinical practice of next-generation sequencing for the assessment of CRC risk has revolutionized clinical care and has led to more effective management for patients with potentially high-risk CRC. However, it has also presented with new challenges, given the unique issues involved with genetic testing. This article intends to provide practical recommendations for identifying patients who would benefit from undergoing genetic testing, provide recommendations for implementation, and discuss some of the challenges associated with genetic testing.

Advances in Genetic Testing

The inclusion of genetic testing into patient care has been a gradual process. Over time, we have acquired more robust evidence and established guidelines on how to best manage patients with germline mutations in cancer susceptibility genes, particularly high-penetrance genes. The traditional approach includes the evaluation of patients based on phenotypic criteria, including family history, patient-specific factors such as age at diagnosis, and tumor phenotype. Genetic testing would be offered to patients who meet the clinical criteria for a particular hereditary cancer syndrome. This finding led to germline testing of a single or a limited number of genes at a high cost. One of the limitations of this traditional approach is that it does not take into account the variability in diseases penetrance and potential overlap in clinical manifestations associated with known hereditary cancer syndromes.[3] With the advent of next-generation sequencing, there has been a shift in paradigm from genetic testing based on phenotypic criteria to multigene panel testing, which allows simultaneous testing of multiple susceptibility genes. These multigene panels have the advantage of detecting germline mutations that would not have been discovered based on the patient's clinical phenotype and family history. In addition, it can increase the yield of identifying germline mutations for syndromes with genetic heterogeneity and overlapping phenotypes.[4] Limiting germline testing based on phenotype may lead to missing mutations in actionable high penetrance cancer susceptibility genes.

Lynch Syndrome

Clinical Characteristics

Lynch syndrome (LS) is the most common inherited CRC syndrome and accounts for $\leq$3% of all newly diagnosed CRC cases.[5] In addition, it accounts for nearly 10% of CRC diagnosed at <50 years of age. LS results from germline inactivation of the mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6*, *PMS2*, or loss of expression of *MSH2* owing to deletion in the *EPCAM* gene. The lifetime risk of CRC in individuals with LS ranges between 40% and 80% and differs based on the specific MMR gene alteration.[5] Although CRC is the most common tumor, individuals with LS are at increased risk for extracolonic cancers of the endometrium, ovaries, gastrointestinal tract (stomach, small intestine, pancreas, biliary tract), urinary tract, brain, and skin. Approximately 90% of colorectal tumors exhibit microsatellite instability (MSI). In addition, most of LS tumors exhibit a unique histopathology characterized by the absence of expression of one of the MMR genes on immunohistochemistry (IHC). Specific MMR gene alterations are associated with different phenotypes and cancer risks.[5] Several clinical criteria including Amsterdam criteria I/II and the Revised Bethesda guidelines were developed in an attempt to identify individuals who are likely to have a germline mutation in one of the MMR genes. However, using clinical criteria to identify patients with LS has been debated for less than optimal sensitivity.[6] To improve the identification

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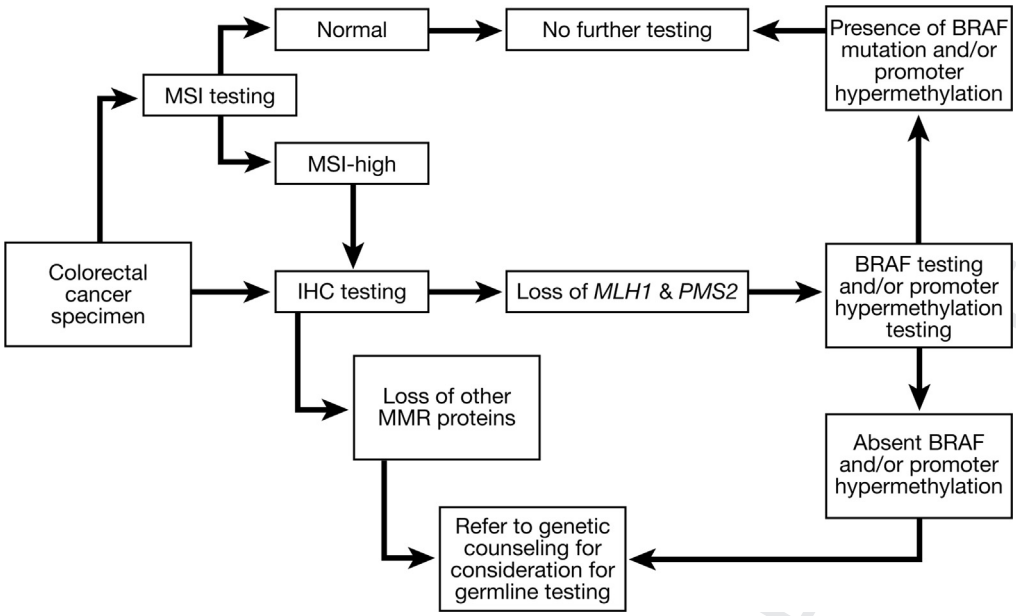

Figure 1. Algorithm for patients diagnosed with colorectal cancer: universal screening.[6]

of mutation carriers of LS several clinical prediction models have been validated including most recently $PREMM_5$, which had an area under the curve of 0.81 (95% confidence interval, 0.87-0.91) for the identification of carriers with LS pathogenic gene mutations, including those with weaker phenotypes and individuals unaffected by cancer. This risk assessment tool can be used in clinical practice.[7]

Tumor Testing

LS-associated CRC displays a characteristic molecular finding termed MSI, which is characterized by abnormal expansion or contractions of microsatellite repeats.[6] Accumulation of replication errors in microsatellite identifies tumors that have developed in MMR gene mutation carriers.[2] MSI is found in approximately 90% of CRC in patients with LS and in 12% of patients with sporadic CRC owing to hypermethylation of the *MLH1* gene.[8] A corresponding approach to MSI testing is IHC, which uses antibodies to the MMR (*MLH1, MSH2, MSH6, PMS2*) and evaluates for loss of MMR protein expression. Germline or somatic mutations in specific MMR genes are indicated by loss of or partial production of the MMR protein produced by that gene.[6] IHC has the advantage of indicating which MMR gene might carry the defect. The sensitivity and specificity of IHC for identifying an MMR gene mutation is 83% and 89%,[9] respectively. Limitations of IHC include decreased sensitivity for identifying carriers of pathogenic *MSH6* gene variants as well as carriers of pathogenic missense variants in *MLH1*.[10,11] It is important to note that hypermethylation of the *MLH1* promoter can also result in a loss of expression of *MLH1*. Many clinicians use both MSI and IHC testing because both methods are complementary.

Universal CRC Testing for LS

The National Comprehensive Cancer Network has endorsed screening all patients with newly diagnosed CRC at ≤70 years of age for either the absence of DNA MMR protein expression on IHC or MSI with the goal of reducing morbidity and mortality (Figure 1). Screening all individuals with CRC for LS has been shown to maximize sensitivity for identifying individuals with LS compared with selection based on age of diagnosis and/or family history[12] and compared with clinical criteria including the less stringent Revised Bethesda Guidelines.[6] A study by Ladabaum et al revealed that universal testing may provide significant clinical benefits at reasonable costs.[13]

Challenges of Genetic Testing

Although multigene panels have many advantages, they may also present challenges to the clinician. A limitation of testing larger number of genes simultaneously, particularly those that are less well-characterized, is the detection of variants of uncertain significance (VUS).[14] Limited knowledge about the risk of cancer associated with VUS is an important barrier to providing appropriate care for individuals found to carry these genetic mutations.[15] Recent studies examining VUS detection rates in clinical laboratories performing germline testing have shown VUS rates of ≤39.7%, highest for those with African American ancestry.[14] It is important to recognize that VUS should not be used to guide the management of a patient and should not be misinterpreted as deleterious mutations. Unfortunately, clinicians ordering genetic testing often misinterpret VUS results and make recommendations to patients that should be reserved for individuals carrying deleterious mutations.[16] Over time, VUS can be reclassified, but until more information is available the management of patients with VUS should be based on personal and family history.

Cancer genetic research has mainly focused on high-penetrance genes that result in autosomal-dominant syndromes such as LS, which are recognizable on a family