Title: Lynch Syndrome *GeneReview*: Microsatellite Instability (MSI) Testing
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Microsatellite Instability (MSI) Testing

Microsatellites are stretches of DNA with a repetitive sequence of nucleotides (e.g., AAAAA or CGCGCGCG) that are particularly susceptible to acquiring errors when the MMR gene function is impaired. Cancers arising in cells with defective MMR gene function exhibit an inconsistent number of microsatellite nucleotide repeats when compared to normal tissue, a finding referred to as "microsatellite instability" (MSI) (see Figure 1).

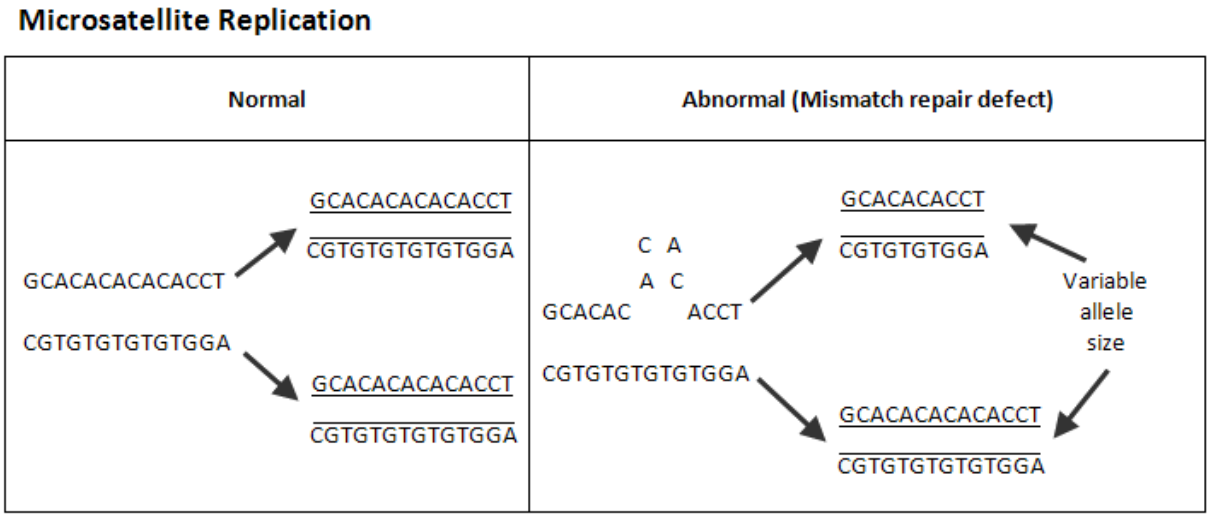

Figure 1. Microsatellite instability testing is used to identify tumors caused by defective MMR by comparing the number of nucleotide repeats in a panel of microsatellite markers in normal tissue with the number from tumor tissue from the same individual.

Microsatellite stability (MSS) is present if the same number of repeats is present in each marker in both the tumor and the normal tissue.

Microsatellite instability (MSI) is present if the number of repeats in the tumor and the normal tissue differs.

Adapted from Gruber & Kohlmann [2003]

MSI, assessed using a panel of microsatellite markers, is compared in tumor tissue and normal tissue. A 1997 NCI consensus meeting recommended testing a core panel of five markers: BAT25, BAT26, D2S123, D5S346, and D17S250 [Boland et al 1998]. This panel remains the most commonly used, and it includes two mononucleotide and three dinucleotide repeats. However, many labs are now using a variety of panels [Hegde et al 2014]. A tumor continues to be classified as follows [Boland et al 1998, Hegde et al 2014]:

- **MSI-high** if two or more of the five markers of the core panel show instability or more than 30% of markers show instability in other marker panels
- **MSI-low** if one of the five markers in the core panel shows instability or fewer than 30% of markers show instability in other marker panels
- **MSI-stable** if 0 (or 0%) of the markers show instability in the core panel or other marker panels

Note: Although some clinical laboratories use additional markers when performing MSI testing, there is a lack of consensus on the markers beyond the five designed by Boland et al [1998]. Next-generation sequencing (NGS) methods have been used to detect MSI-high tumor status [Nowak et al 2017, Hempelmann et al 2015, Stadler et al 2016].

- **Colon tumors.** When adequate tissue is available, studies of Lynch syndrome-associated adenomas suggest a slightly lower rate of MSI compared to invasive cancers, with approximately 80% of adenomas being MSI-high (see Table 3). Adenomas with high-grade dysplasia are more likely to exhibit MSI than early polyps [Iino et al 2000].
- **Endometrial cancers.** Approximately 20%-30% of endometrial cancers exhibit MSI, and as with CRC the majority are the result of somatic *MLH1* promoter methylation [Hampel et al 2006].

Advantages of MSI testing:

- MSI testing is an effective method for determining which tumors arise from MMR deficiency. Studies have demonstrated that the sensitivity of MSI testing for identifying tumors that arise in individuals with a germline MMR gene pathogenic variant is 93% [Shia 2008].
- MSI testing may be positive (i.e., MSI-high, identifying a tumor as arising from MMR deficiency) when the IHC studies are negative due a protein that is present, but nonfunctional) [Shia 2008].
- MSI testing requires very little tissue [Zhang 2008].
- MSI testing is highly reproducible [Zhang 2008].

Limitations of MSI testing:

- It may not be readily available at all centers because it requires microdissection and molecular analysis [Bellizzi & Frankel 2009].
- In some tumors, MSI cannot be detected because of technical challenges such as lack of DNA in extremely mucinous tumors [Hampel et al 2005].
- A small portion of Lynch syndrome-related tumors will not show evidence of MMR deficiency [Shia 2008].
- It may not reduce the cost of molecular testing because it does not help identify the gene that is most likely mutated, but this is less of a factor now that multigene panel testing is typically as cost effective as targeted, single-gene testing.

- Biallelic somatic (tumor) inactivation of an MMR gene can result in a sporadic tumor with MSI (see Genetically Related Disorders).

Literature Cited

Bellizzi AM, Frankel WL. Colorectal cancer due to deficiency in DNA mismatch repair function: a review. Adv Anat Pathol. 2009;16:405-17.

Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S (1998) A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res. 58:5248-57.

Gruber SB, Kohlmann W. The genetics of hereditary non-polyposis colorectal cancer. J Natl Compr Canc Netw. 2003;1:137-44.

Hampel H, Frankel W, Panescu J, Lockman J, Sotamaa K, Fix D, Comeras I, La Jeunesse J, Nakagawa H, Westman JA, Prior TW, Clendenning M, Penzone P, Lombardi J, Dunn P, Cohn DE, Copeland L, Eaton L, Fowler J, Lewandowski G, Vaccarello L, Bell J, Reid G, de la Chapelle A. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. Cancer Res. 2006;66:7810-7.

Hegde M, Ferber M, Mao R, Samowitz W, Ganguly A, Working Group of the American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee. ACMG technical standards and guidelines for genetic testing for inherited colorectal cancer (Lynch syndrome, familial adenomatous polyposis, and MYH-associated polyposis). Genet Med. 2014;16:101-16.

Hempelmann JA, Scroggins SM, Pritchard CC, Salipante SJ. MSIplus for integrated colorectal cancer molecular testing by next-generation sequencing. J Mole Diagn. 2015;17:705-14.

Iino H, Simms L, Young J, Arnold J, Winship IM, Webb SI, Furlong KL, Leggett B, Jass JR (2000) DNA microsatellite instability and mismatch repair protein loss in adenomas presenting in hereditary non-polyposis colorectal cancer. Gut 47:37-42

Nowak JA, Yurgelun MB, Bruce JL, Rojas-Rudilla V, Hall DL, Shivdasani P, Garcia EP, Agoston AT, Srivastava A, Ogino S, Kuo FC, Lindeman NI, Dong F. Detection of mismatch repair deficiency and microsatellite instability in colorectal adenocarcinoma by targeted next-generation sequencing. J Mol Diagn. 2017;19:84-91.

Shia J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I: The utility of immunohistochemistry. J Mol Diagn. 2008;10:293-300.

Stadler ZK, Battaglin F, Middha S, Hechtman JF, Tran C, Cercek A, Yaeger R, Segal NH, Varghese AM, Reidy-Lagunes DL, Kemeny NE, Salo-Mullen EE, Ashraf A, Weiser MR, Garcia-Aguilar J, Robson ME, Offit K, Arcila ME, Berger MF, Shia J, Solit DB, Saltz LB. Reliable detection of mismatch repair deficiency in colorectal cancers using mutational load in next-generation sequencing panels. J Clin Oncol. 2016; 34:2141-7.

Zhang L. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part II: The utility of microsatellite instability testing. J Mol Diagn. 2008;10:301-7.