Deletions of TACSTD1 Account for a Significant Fraction of MSH2-Associated Lynch Syndrome Cases

K. Rumilla¹, K. V. Schowalter¹, B. C. Thomas¹, K.A. Mensink¹, S. N. Thibodeau¹
¹Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

Lynch Syndrome is an autosomal dominant cancer predisposition syndrome characterized by loss of function of DNA mismatch repair enzymes MLH1, MSH2, MSH6, or PMS2, with mutations in MLH1 and MSH2 accounting for ~80% of the inherited cases. Of the mutations identified in MSH2, ~2/3 are point mutations or small insertions and deletions, while the remaining ~1/3 are large gene rearrangements or deletions. Of interest, germline mutations in MSH2 have not been identified in up to 20% of cases expected to have such an alteration. Recently, deletions involving the 3-prime end of TACSTD1 have been shown to result in hypermethylation of MSH2 and subsequent loss of MSH2 transcription. This study was performed to examine the frequency of this novel mechanism for MSH2 inactivation among those cases in which an expected germline mutation was not previously identified. From August 2001 to May 2008, 21 cases were identified from the Molecular Genetics Laboratory at Mayo Clinic for further analysis. For each of these patients, tumor analysis showed loss of staining of MSH2 and MSH6 by immunohistochemistry and the presence of microsatellite instability. Follow-up germline testing, including DNA sequencing and large deletion/duplication analysis by southern blot or Multiplex Ligation-dependent Probe Amplification (MLPA), was performed on MSH2 in all 21 cases and for MSH6 in 12 cases. No germline mutations were identified. The 21 cases were tested using additional MLPA probes located in TACSTD1 directly upstream of MSH2 (including probes for exons 3, 8, 9, one 3 kb downstream and one 2.5 Kb from the MSH2 gene). The methylation status of the MSH2 promoter was confirmed on a subset of 8 cases by methylation sensitive PCR primers on bisulfite treated DNA from the tumor and normal paraffin-embedded tissues. Of the 21 cases tested, 4 (18%) demonstrated deletions of TACSTD1. Two of these mutations encompassed at least exons 8 and 9, while 2 were larger deletions that extended 3 kb downstream of the gene’s coding region. None of the deletions involved exon 3. Hypermethylation of MSH2 was confirmed on all 4 cases in DNA from tumor and normal tissue. Approximately 18% of HNPCC cases with abnormal MSH2 and MSH6 staining but no identified germline mutation, were found to have deletions in TACSTD1. In the available cases, the methylation sensitive PCR confirmed that the deletion resulted in MSH2 promoter hypermethylation.