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Molecular Background of Colorectal Tumors From Patients With Lynch Syndrome Associated With Germline Variants in *PMS2*

Q6 S. W. ten Broeke,[1] T. C. van Bavel,[1] A. M. L. Jansen,[2] E. Gómez-García,[3] F. J. Hes,[1] L. P. van Hest,[4] T. G. W. Letteboer,[5] M. J. W. Olderode-Berends,[6] D. Ruano,[2] L. Spruijt,[7] M. Suerink,[1] C. M. Tops,[1] R. van Eijk,[2] H. Morreau,[2] T. van Wezel,[2,§] and M. Nielsen[1,§]

[1]*Department of Clinical Genetics, Leiden University Medical Center, Leiden, the Netherlands;* [2]*Department of Pathology, Leiden University Medical Center, Leiden, the Netherlands;* [3]*Department of Clinical Genetics, Maastricht University Medical Center, Maastricht, the Netherlands;* [4]*Department of Clinical Genetics, VU University Medical Center, Amsterdam, the Netherlands;* [5]*Department of Clinical Genetics, University Medical Center Utrecht, Utrecht, the Netherlands;* [6]*Department of Clinical Genetics, University Medical Center Groningen, Groningen, the Netherlands;* [7]*Department of Clinical Genetics, Radboud University Medical Center, Radboud, the Netherlands*

BACKGROUND & AIMS: Germline variants in mismatch repair genes *MLH1*, *MSH2* (*EPCAM*), *MSH6*, or *PMS2* cause Lynch syndrome. Patients with these variants have an increased risk of developing colorectal cancers (CRCs) that differ from sporadic CRCs in genetic and histologic features. It has been a challenge to study CRCs associated with *PMS2* variants (*PMS2*-associated CRCs) because these develop less frequently and in older patients than CRCs with variants in other mismatch repair genes. **METHODS:** We analyzed 20 CRCs associated with germline variants in *PMS2*, 22 sporadic CRCs, 18 CRCs with germline variants in *MSH2*, and 24 CRCs from patients with germline variants in *MLH1*. Tumor tissue blocks were collected from Dutch pathology departments in 2017. After extraction of tumor DNA, we used a platform designed to detect approximately 3,000 somatic hotspot variants in 55 genes (including *KRAS*, *APC*, *CTNNB1*, and *TP53*). Somatic variant frequencies were compared using the Fisher exact test. **RESULTS:** None of the *PMS2*-associated CRCs contained any somatic variants in the catenin-β_1 gene (*CTNNB1*), which encodes β-catenin, whereas 14 of 24 *MLH1*-associated CRCs (58%) contained variants in *CTNNB1*. Half the *PMS2*-associated CRCs contained *KRAS* variants, but only 20% of these were in hotspots that encoded G12D or G13D. These hotspot variants occurred more frequently in CRCs associated with variants in *MLH1* (37.5%; $P = .44$) and *MSH2* (71.4%; $P = .035$) than in those associated with variants in *PMS2*. **CONCLUSIONS:** In a genetic analysis of 84 colorectal tumors, we found tumors from patients with *PMS2*-associated Lynch syndrome to be distinct from colorectal tumors associated with defects in other mismatch repair genes. This might account for differences in development and less frequent occurrence.

Keywords: Mismatch Repair; Colon Cancer; Wnt Signaling; Genetics.

Patients carrying a germline variant in one of the mismatch repair (MMR) genes *MLH1*, *MSH2* (*EPCAM*), *MSH6*, or *PMS2* have Lynch syndrome. These patients have a strongly increased risk of developing colorectal cancer (CRC) and endometrial cancer. In contrast to sporadic tumors, which usually show chromosomal instability, MMR-deficient (dMMR) tumors are characterized by microsatellite instability (MSI) and a high mutational burden. The dMMR tumors show different treatment responses and often better patient survival.[1–3] Previous studies have found notable differences in the mutational spectrum between sporadic and Lynch-associated MSI-H tumors, exemplified by a higher degree of activation of the Wnt signaling pathway.[4]

Studies of Lynch-related tumors to date have mainly focused on *MLH1*-, *MSH2*-, and *MSH6*-deficient tumors. Data on tumors associated with *PMS2* variants (*PMS2*-associated tumors) are still relatively sparse, probably because of challenging *PMS2* variant detection.[5] The recognition of *PMS2*-related families also can be challenging, because *PMS2* carriers develop CRCs less frequently and at an older age compared with other MMR variant carriers.[6,7] One explanation for lower penetrance in *PMS2*-associated Lynch syndrome is that the functional binding partner of *PMS2*, *MLH1*, can form an alternative heterodimer with *MLH3* or *PMS1*. This alternative heterodimer could in part rescue MMR function in the absence of *PMS2*. This redundancy might not only manifest in the clinical phenotype but also result in distinct features of these carcinomas. Therefore, our goal was to define the molecular hallmarks of *PMS2*-associated CRCs compared with other Lynch-associated and sporadic carcinomas.

Methods

Patients and Samples

The cohort consisted of 24 CRCs from Dutch confirmed heterozygous pathogenic *PMS2* variant carriers who had given informed consent for use of clinical data and tissue samples. Tissue blocks were retrieved from Dutch pathology centers in

[§]Authors share co-senior authorship. Q2

Abbreviations used in this paper: CRC, colorectal cancer; dMMR, mismatch repair deficient; MMR, mismatch repair; MSI, microsatellite instability; MSI-H, ■■■. Q3

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WHAT YOU NEED TO KNOW

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2017. We also collected tissue blocks from *MLH1*- and *MSH2*-associated CRCs from confirmed *MLH1* and *MSH2* carriers that had been previously analyzed for MMR deficiency status at the Leiden University Medical Center (Leiden, Netherlands; n = 24 and n = 18, respectively). In addition, we retrieved somatic variant data from a cohort of sporadic CRCs without suspected Lynch syndrome (n = 22) that were analyzed in the context of routine diagnostics (mainly to assess therapy options) using the same panel as this study, also in the Leiden University Medical Center.

Sample Preparation

Tissue samples were extracted from tissue blocks by taking a minimum of 3 tissue cores of variable length (0.3 mm in diameter or 0.7 mm in case of tissue with a low cell count). Tumor DNA was isolated using the Tissue Preparation System with VERSANT Tissue Preparation Reagents (Siemens Healthcare Diagnostics, Tarrytown, NY), as previously described.[8] Briefly, nucleic acid was extracted from paraffin-embedded tissue using heat lysis in FFPE buffer and subsequent enzymatic lysis with proteinase K. The lysate was incubated with silica-coated iron oxide beads, which bind nucleic acid, and subjected to magnetic separation for purification. After being transferred to a new tube, the lysate was again incubated with beads, washed 3 times, and the nucleic acid was eluted in elution buffer.

Histologic and Molecular Evaluation

Histologic hallmarks were scored using the original report, with missing data on specific Lynch-associated hallmarks supplemented by an experienced pathologist when possible. Somatic variant analysis was performed using custom-designed AmpliSeq sequencing panels (ThermoFisher Scientific, Waltham, MA). Sequencing libraries were prepared according to the manufacturer's recommendations and sequenced on the Ion Torrent Proton Platform (ThermoFisher Scientific). The used cancer hotspot panel is adapted from Ion AmpliSeq Cancer Hotspot Panel 2 (composition on request; ThermoFisher Scientific) and is designed to detect variations in 207 amplicons covering 21,820 bases of 50 genes with known cancer associations (~3,000 COSMIC variants), including well-known somatically mutated genes such as *KRAS*, *APC*, and *TP53*. Samples from the *PMS2* cohort also were sequenced with a second AmpliSeq panel (custom) covering the coding regions of *MLH1*, *MSH2*, *MSH6*, *PMS2*, *MUTYH* and the exonuclease domain of *POLD1* and *POLE*. In total, 19,904 bases were covered and spread over 202 amplicons (composition on request). This second panel also was used to detect a second hit (somatic point variants or loss of heterozygosity in these tumors). Results for this panel are presented in Table 1 (second hits in *PMS2*) and Supplementary Figure 3 (analysis of *POLE* variants in 3 *KRAS*-mutated CRCs).

The unaligned bam file generated by the proton sequencer was mapped against the human reference genome (GRCh37/hg19) using TMAP 5.0.7 software with default parameters (https://github.com/iontorrent/TS). Subsequently, variant calling was done using the Ion Torrent specific caller, Torrent Variant Caller 5.0.2, using the recommended Variant Caller Parameter for Cancer Hotspot Panel 2. Variant interpretation was done using Genetic Assistant, which assigns functional prediction, conservation scores, and disease-associated information to each variant (http://softgenetics.com/GeneticistAssistant_2.php). Variants were called using a pipeline developed in house and analyzed using the Geneticist Assistant NGS Interpretative Workbench 1.1.8 (SoftGenetics, State College, PA). All variants were manually curated and visualized in the Integrative Genomics Viewer.[9,10] Variants with low coverage (<100 reads) and/or with an allele frequency lower than 10% were not considered. Although the latter approach is conservative, it was adopted because of the substantial background noise from FFPE treatment of the tissue samples, as evinced by a high transition-to-transversion ratio. Four samples of *PMS2*-associated CRCs showed a large number of variants and a high transition-to-transversion ratio and were excluded from the final analysis. The ratio of these samples was considered too high for meaningful interpretation, regardless of the known association dMMR cancer and C>T transitions (mutational signature 6).[11]

Proportions were compared using the Fisher exact test in STATA 14 (StataCorp, College Station, TX). Reported *P* values are 2-tailed.

Results

Cohort Description

A description of the cohort is presented in Table 1. The analyzed cohorts were comparable in index status, sex, and age at CRC diagnosis. A full description of all available histologic and molecular characteristics (including germline MMR and somatic variants) for each analyzed CRC is presented in Supplementary Table 1 and Supplementary Figure 1.

Most Frequently Mutated Genes

The most frequently mutated genes were *TP53*, *KRAS*, *FBXW7*, *CTNNB1*, *APC*, and *PIK3CA* (Table 2). The relative