

# Higher frequency of isolated PMS2 loss in colorectal tumors in Colombian population: preliminary results

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**Abstract:** Colorectal cancer (CRC) is the third most common cancer and the fourth most common cause of death worldwide. It accounts for >9% of all cancers. One of the pathogenic factors of CRC is germline mutation, leading to alteration and inactivation in the mismatch repair (*MMR*) genes. The aim of the study is to compare the frequency of alterations in *MMR* protein expression in Caucasian CRC patients with Colombian CRC patients.

A total of 45 Colombians and 48 Caucasians with CRC were studied. The microsatellite instability status of tumors was determined in primary CRC by immunohistochemistry using the automated Ventana Ultra. The combined loss of *MLH1* and *PMS2* was the most common alteration in both Colombian (11%, five out of 45) and Caucasian (12%, six out of 48) CRC patients. Interestingly, the loss of *PMS2* expression in the presence of intact *MLH1* was the second most common alteration in Colombians (8%, four out of 45), which was never seen in the Caucasian cohort ( $P=0.05$ ). The loss of *MLH1* alone and the combined loss of *MSH6* and *PMS2* expression were only observed in one out of 45 (2%) Colombians but not in Caucasians. The combined loss of *MSH2* and *MSH6* was not observed in any of the patients studied. The preliminary findings support a significant difference in alterations of *MMR* protein expression in Colombian CRC patients compared with Caucasian CRC patients. These findings are novel and warrant further studies in larger cohorts.

**Keywords:** colon cancer, MSI, MMR, immunohistochemistry

## Introduction

Colorectal cancer (CRC) is a leading cause of cancer death in both males and females in the US.<sup>1-3</sup> The incidence and mortality rates of CRC vary among major racial/ethnic groups. The African Americans have the highest incidence of CRC (males: 63.8/100,000; females: 47.6/100,000) and highest mortality (males: 29.4/100,000; females: 19.4/100,000).<sup>4</sup> The non-Hispanic Whites have the lowest incidence of CRC (males: 50.9/100,000 and females 38.6/100,000) and lowest mortality (males: 19.2/100,000; females: 13.6/100,000).<sup>4</sup> Compared to the African Americans and the Non-Hispanic Whites, the Hispanic/Latino population tends to have the lowest incidence of CRC (males: 47.3/100,000; females: 32.6/100,000) and lowest mortality (males: 16.1/100,000; females: 10.2/100,000).<sup>4</sup>

A subset of the CRC is due to the alteration in the mismatch repair (*MMR*) genes. These tumors are characterized by molecular alteration in the “serrated pathway”, including mutation of the proto-oncogene *BRAF* in sporadic microsatellite instability (MSI) CRC. Conversely, familial MSI CRC is associated with *KRAS* mutation.<sup>5</sup>

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The MMR system is responsible for correcting mismatches generated during DNA replication.<sup>6</sup> Mutations in one of the *MMR* genes (*MLH1*, *MSH2*, *MSH6*, and *PMS1*) are referred to as MSI, which in turn results in failure to repair errors that occur during DNA replication.<sup>5</sup> *MSH3* and *PMS1* genes are not found to be mutated in CRC with MSI.

MSI is the hallmark of hereditary nonpolyposis colorectal cancer, a condition responsible for 10%–15% of sporadic colon, gastric, and endometrial cancers. In sporadic primary CRC with MSI, hypermethylation of the 5' CpG of the *MLH1* gene was the most observed genetic alteration, which was often associated with the loss of hMLH1 protein expression.<sup>7</sup>

Comparison between populations with different ethnic or cultural background is a common strategy in cancer research to detect epidemiologic variables that can be traced back to molecular basics.

Studies have suggested a low prevalence of sporadic MSI CRC among Hispanics (individuals whose primary language is Spanish or whose family is from Mexico, Central America, South America, or the Caribbean where the primary language is Spanish) and that the molecular aberration associated with MSI CRC was most likely due to Lynch syndrome.<sup>8</sup>

The aim of this study is to compare the frequency of alterations in MMR protein expression in primary, sporadic CRC in Caucasian patients with that of Colombian patients.

## Material and methods

Forty-five Colombian CRC patients (21 females:24 males; age: 27–91 years; median: 63 years) who visited Instituto de Patología Mejía Jimenez (Cali, Colombia) and 48 Caucasian CRC patients (21 females:27 males; age: 35–92 years; median: 65 years) who visited Moffitt Cancer Center (Tampa, FL, USA) were studied retrospectively. Individuals were not prescreened by laboratory methods to identify hereditary CRC, but the selection process was based on the evaluation of familial clinical history. Individuals with hereditary CRC were not included in this study. The tumor tissues were formalin fixed, paraffin embedded, cut into slices of 4 µm thickness, and stained with hematoxylin and eosin for evaluation. The hematoxylin and eosin-stained tumor tissues were examined by gastrointestinal pathologists (JM and DC) at both institutions to assess adequacy and confirm the diagnosis. The tumor tissues were further examined for the MMR protein expression MLH1 (Clone ES05; Dako Denmark A/S, Glostrup, Denmark), MSH2 (Clone FE11; Dako Denmark A/S), MSH6 (Clone EP49; Dako Denmark A/S), and PMS2 (Clone EP51; Dako Denmark A/S) by immunohistochemistry using the automated Ventana Ultra. The staining was evaluated using the Allred score system.<sup>9</sup>

This study was approved by the Moffitt Cancer Center Institutional Review Board. All patients provided written informed consent to participate in this study.

## Statistical analysis

A paired *t*-test was used to evaluate the difference in the expressions of MLH1, MSH2, MSH6, and PMS2 in Colombians and Caucasians. A *P*-value of <0.05 was considered to be statistically significant.

## Results

This study showed higher frequency of MMR alterations in Colombian CRC patients (24%, eleven out of 45) compared with Caucasian CRC patients (12%, six out of 48) (Table 1). Age, sex, cancer location, tumor grade, and tumor stage in MSI colon cancer in Colombians and Caucasians are shown in Tables 2 and 3, respectively.

The combined loss of MLH1 and PMS2 was the most common alteration in both Colombian (11%, five out of 45) and Caucasian (12%, six out of 48) CRC patients (Figure 1). Interestingly, the loss of PMS2 expression in the presence of intact MLH1 was the second most common alteration in Colombians (8%, four out of 45; Figure 2), which was never seen in the Caucasian cohort (*P*=0.05). The clinical data for the four cases with isolated PMS2 loss are reviewed (Table 2). The loss of MLH1 alone and the combined loss of MSH6 and PMS2 expression were only observed in one out of 45 (2%) Colombians but not in Caucasians. The combined loss of MSH2 and MSH6 was not observed in any of the patients studied. These findings in this small cohort highlight the significant difference in alterations in the expression of MMR protein in CRC patients from Colombia compared with Caucasians.

## Discussion

In this study, we compared the frequency of MSI in CRC patients from Colombian and Caucasian CRC patients.

**Table 1** MMR Alterations in Colombian patients compared to Caucasians

|                                   | Colombian patients<br>(Instituto de Patología<br>Mejía Jimenez, Cali,<br>Colombia) n=45 (%) | Caucasian patients<br>(Moffitt Cancer<br>Center, Tampa, FL)<br>n=48 (%) |
|-----------------------------------|---------------------------------------------------------------------------------------------|-------------------------------------------------------------------------|
| MSI                               | n=11 (24)                                                                                   | n=6 (12)                                                                |
| Combined MLH1<br>and PMS2         | n=5 (11)                                                                                    | n=6 (12)                                                                |
| Isolated loss of PMS2             | n=4 (8)*                                                                                    | n=0 (0)                                                                 |
| Isolated loss of MLH1             | n=1 (2)                                                                                     | n=0 (0)                                                                 |
| Combined loss of<br>MSH6 and PMS2 | n=1 (2)                                                                                     | n=0 (0)                                                                 |

**Note:** \**P*=0.05.

**Abbreviation:** MSI, microsatellite instability.

**Table 2** Age, sex, cancer location, tumor grade, and tumor stage in MSI colon cancer in Colombians

| Age, years | Sex | Tumor site                        | Tumor grade | Tumor stage | LN      | MLH1 | MSH2 | MSH6 | PMS2 |
|------------|-----|-----------------------------------|-------------|-------------|---------|------|------|------|------|
| 37         | M   | Left colon, sigmoid               | Mucinous    | pT3         | pN2a    | –    | +    | +    | –    |
| 69         | F   | Right colon, ascending            | Low grade   | pT2         | pN0     | –    | +    | +    | –    |
| 38         | M   | Transverse colon, splenic flexure | Mucinous    | pT4b        | pN0     | +    | +    | +    | –*   |
| 54         | F   | Right colon                       | High grade  | pT2         | pN0     | –    | +    | +    | –    |
| 50         | F   | Right colon                       | Low grade   | pT2         | pN0     | –    | +    | +    | –    |
| 57         | F   | Right colon                       | Low grade   | pT2         | pN0     | +    | +    | +    | –*   |
| 83         | M   | Left colon, sigmoid               | Low grade   | pT3         | pN1b    | +    | +    | +    | –*   |
| 29         | M   | Left colon, rectum                | Low grade   | pT3         | pN0     | +    | +    | +    | –*   |
| 89         | M   | Right colon, cecum                | Low grade   | pT2         | pN0     | –    | +    | +    | –    |
| 62         | F   | Right colon, ascending            | Low grade   | pT3         | pN2a    | –    | +    | +    | +    |
| 76         | M   | Left colon, rectum                | Low grade   | Unknown     | Unknown | +    | +    | –    | –    |

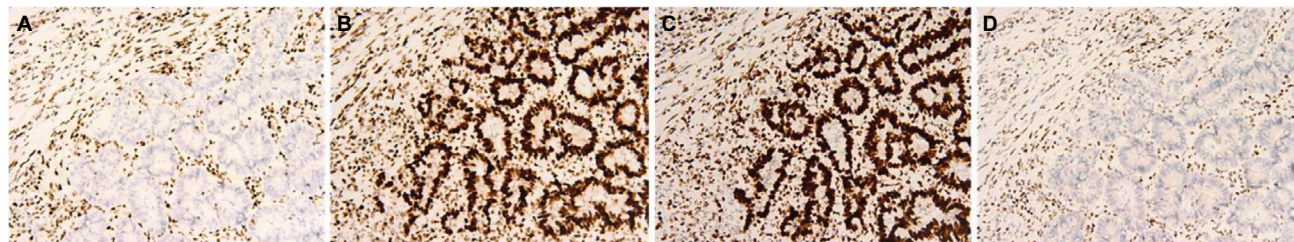
**Note:** \* refers to Isolated PMS2 loss.

**Abbreviations:** LN, lymph node; MSI, microsatellite instability; M, male; F, female.

**Table 3** Age, sex, cancer location, tumor grade, and tumor stage in MSI colon cancer in Caucasians

| Age, years | Sex | Tumor site               | Tumor grade | Tumor stage | LN       | MLH1 | MSH2 | MSH6 | PMS2 |
|------------|-----|--------------------------|-------------|-------------|----------|------|------|------|------|
| 71         | F   | Ascending colon          | Low grade   | PT2PNO      | Negative | –    | +    | +    | –    |
| 70         | M   | Ascending colon          | Low grade   | PT4bPN0     | Negative | –    | +    | +    | –    |
| 86         | M   | Ascending + transv colon | Low grade   | PT4aPN2b    | 7 LN     | –    | +    | +    | –    |
| 82         | F   | Ascending colon          | Low grade   | PT3PN0      | Negative | –    | +    | +    | –    |
| 66         | F   | Ascending colon          | Low grade   | PT2ON0      | Negative | –    | +    | +    | –    |
| 69         | F   | Ascending + transv colon | High grade  | PT4aPN0     | 1 LN     | –    | +    | +    | –    |

**Abbreviations:** LN, lymph node; MSI, microsatellite instability; F, female; transv, transverse; M, male.



**Figure 1** Colorectal adenocarcinoma showing loss of MLH1 (A) and PMS2 (D). The MLH1 and PMS2 proteins are expressed in the proliferating stromal cells. The MSH2 (B) and MSH6 (C) are expressed in the tumor cells and the stromal cells. Cases from Moffitt Cancer Center, Tampa, FL, USA.

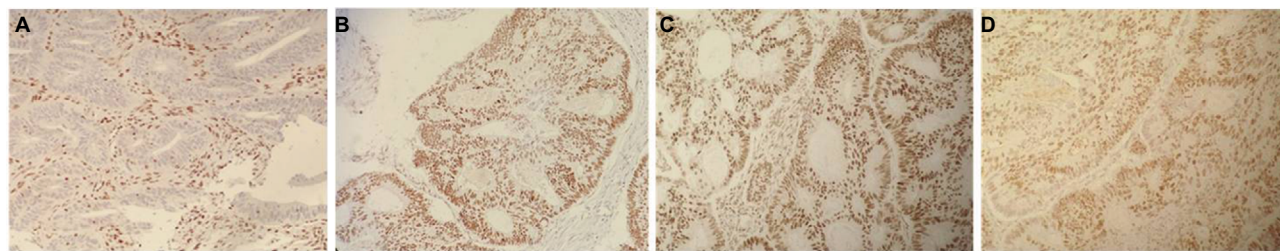
The Colombian CRC patients showed a higher frequency of MMR alterations (24%) when compared with Caucasian CRC patients (12%). The combined loss of MLH1 and PMS2 was the most common alteration in both Colombian and Caucasian CRC patients. We found the frequency of such alteration to be almost identical in Colombian (11%) and Caucasian (12%) CRC patients. Interestingly, we observed the isolated loss of PMS2 protein expression with normal MLH1, MSH2, and MSH6 expression only in Colombian CRC patients and not in the Caucasian cohort.

The function of *MMR* genes is to correct mismatches during DNA replication, thus maintaining genetic stability. Many tumors such as gastric cancer, ovarian cancer, bladder

cancer, endometrial cancer, and CRC harbor mutation in the MMR, resulting in what is called MSI tumor.<sup>10–15</sup>

MSI CRCs were first described by Thibodeau et al.<sup>16</sup> They showed that MSI colon cancers usually occur in the proximal colon with dense lymphocytic infiltrate. They are poorly differentiated with mucinous or signet ring appearance. MSI colon cancer tends to have increased survival and better response to chemotherapy when compared with microsatellite stable colon cancer.<sup>16–18</sup> Thus, the microsatellite status of colon cancer is essential for proper clinical management.

As a result, the National Cancer Institute proposed international guidelines for mutation analyses of the MMR to determine the appropriate clinical management of MSI tumors.<sup>17,18</sup>



**Figure 2** Colorectal adenocarcinoma showing loss of PMS2 (A). The PMS2 proteins are expressed in the proliferating stromal cells. The MLH1 (B), MSH2 (C), and MSH6 (D) are expressed in the tumor cells and the stromal cells. Cases from Instituto de Patología Mejía Jimenez, Cali, Colombia.

The level of MSI was determined as 1) MSI-high (MSI-H, >30% of tested loci are unstable), 2) MSI-low (MSI-L, <30% of tested loci are unstable), and 3) microsatellite stability (unstable loci are absent).

MMR genes include *MLH1*, *MSH2*, *MSH6*, and *PMS2*. Studies have shown that the majority of colorectal tumors with MSI have combined loss of MLH1 and PMS2 protein expression.<sup>17,18</sup> These findings were similar to the findings of this study.

Similarly, Gill et al<sup>19</sup> showed that the majority of MSI-H colon cancers (499 of 535, 93%) had loss of expression for at least one of the proteins for MLH1, MSH2, and/or MSH6. However, the selective loss of PMS2 (with normal expression of MLH1, MSH2, and/or MSH6) was detected in 4.3% (23 of 529) of all MSI-H colon cancer.<sup>19</sup>

According to Gill et al<sup>19</sup>, PMS2 negative cancer occurs mostly in males (64%) aged 28–67 years and is more common in the right colon (52%). In our study, PMS2 negative colon cancers were more common in males (75%) aged 29–83 years as well. However, PMS2 negative colon cancers were diagnosed in the left colon (50%), transverse colon (25%), and the right colon (25%).

Another study by Nakagawa et al was conducted on 103 patients with MSI CRC. The combined loss of MLH1 and PMS2 was seen in 19% of the MSI CRC (20 of 103). The isolated PMS2 loss, with intact MLH1, was seen in 6% of the MSI CRC (seven out of 103). The isolated PMS2 loss in these seven patients was due to either frameshift mutation or deletion of the *PMS2* gene. Other types of germline mutation or somatic mutation could not be ruled out.<sup>20</sup>

In another study, MMR loss was detected in 13.2% (139 out of 1,048 patients) of unselected CRC cases.<sup>21</sup> The combined loss of MLH1 and PMS2 was the most frequent mutation and was seen in 10% (109 out of 1,048) of all CRC cases. Isolated PMS2 loss was detected in 1.5% of all CRC cases.<sup>21</sup> They reported that these patients with isolated *PMS2* loss were in the fifth and sixth decades of life and showed several extracolonic PMS2-deficient tumors. Their data excluded the methylation of the *PMS2* promoter, which is frequently

associated with sporadic CRC and rather suggested germline mutation of the *PMS2*.<sup>21</sup>

## Conclusion

Our preliminary findings on PMS2 in Colombian CRC patients suggest PMS2 inactivation in the development of MSI-H CRCs in Colombia. PMS2-deficient CRCs may be associated with germline alterations. Molecular evaluation is warranted to detect specific of somatic and/or germline mutation of the *PMS2* gene in the presence of wild-type *MLH1*.

## Disclosure

The authors report no conflicts of interest in this work.

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