Variability in the Prevalence of Microsatellite Instability in Colon Cancer

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ABSTRACT

BACKGROUND – Microsatellite instability is an important contributor to sporadic and familial colon cancer. Dysfunction of DNA mismatch-repair proteins by mutation or promoter methylation is a well-recognized cause of microsatellite instability. We conducted a study to identify variations in the prevalence of mismatch-repair protein deficiency in colon cancer and analyze clinical characteristics that may contribute to differences among ethnic subgroups of a community hospital-based patient population.

METHOD – A retrospective analysis of 272 cases was performed.

RESULTS – Reduced prevalence of mismatch-repair protein dysfunction was found in all non-Latino ethnicities (3.6%) compared to the Latino (13.1%) portion of our patient population (p=0.007). Mismatch repair protein deficient colon cancer was found to show significant correlation with right side location (p=0.017), young age in non-Latino ethnicities (p=0.030), tumor stage IIIC or greater (p=0.006), and high-grade histology (p=0.003). Colon cancer patients of Latino descent appear to have an increased rate of germline mutations within mismatch repair genes.

CONCLUSION – The prevalence of mismatch repair protein dysfunction in the non-Latino ethnicities of our patient population are less than reported in literature, while the rate of germline mutations within mismatch repair genes appears increased in those of Latino descent.

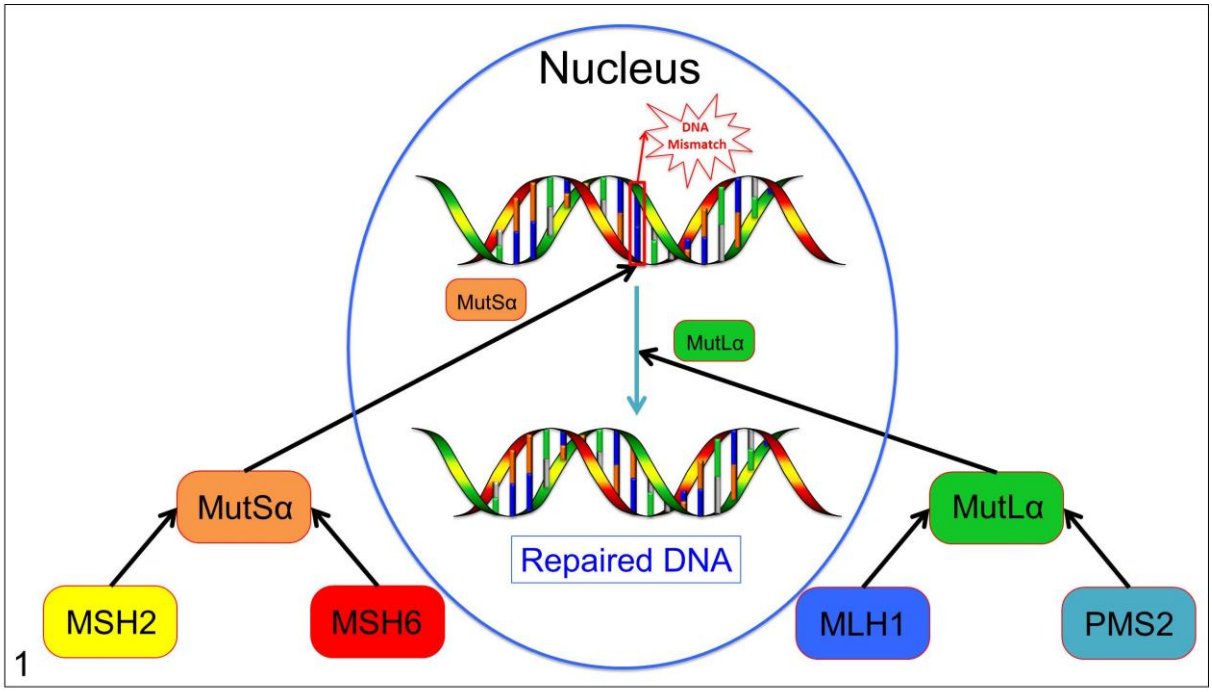
1. INTRODUCTION

Dysfunction of DNA mismatch repair (MMR) proteins is a common predisposition to the development of colorectal cancer (CRC) with a reported prevalence of 10-15%. [1-2] MMR complexes are responsible for base-base mismatch and deletion repair in response to DNA replication errors. These errors are believed to occur as a result of DNA polymerase slippage during the process of replication, which leads to insertions or deletions within nucleotide repeat sequences. [3] Dysfunction of the mismatch repair proteins leads to an accumulation of DNA mutations that have been linked to both sporadic and familial (Lynch Syndrome) adenocarcinoma of the colon. The increased mutation rate in regions of single and double nucleotide repeat sequences is referred to as microsatellite instability (MSI).

The DNA MMR protein genes include mutS homolog 2 (MSH2), mutS homolog 6 (MSH6), mutL homolog 1 (MLH1), and post-meiotic segregation increased 2 (PMS2). [3-5] The MSH2 protein combines with MSH6 to form the MutSα heterodimer MMR complex. MutSα is responsible for recognizing base mispairs and small insertions or deletions of post-replicated DNA. [3] MLH1 combines with PMS2 to form the MutLα MMR heterodimer. After regions of DNA requiring repair are identified by MutSα, MutLα is recruited to initiate the process of base-excision repair (Figure 1). Mutations of MSH2, MSH6, MLH1, and PMS2 have all been shown to be involved in the development of colon cancer. The remaining MMR genes, including MSH3, PMS1 and MLH3 have not been found to be important in the evolution of colon cancer. [3, 6]

Figure 1. MMR response to post-replication DNA base-base mismatch.

MSH2 and MSH6 combine in the cytoplasm to form MutSα; MLH1 and PMS2 form MutLα. MutSα migrates to the nucleus and identifies base-base mismatched nucleotides. After binding to DNA requiring repair, MutLα is recruited to initiate the process of base-excision repair.



Abbreviations: MMR, mismatch repair; MSH2, mutS homolog 2; MSH6, mutS homolog 6; MLH1, mutL homolog 1; PMS2, post-meiotic segregation increased 2; dMMR, deficient mismatch repair.

It is now common practice to detect the presence of MMR gene dysfunction in tumor tissue by immunohistochemistry, which is currently recognized to be as sensitive as molecular-based methods for detecting MSI when analyzing colon cancer cases. [7] Up to a third of MMR gene deficient colon cancer is attributable to germline mutations of MMR genes, while the remainder has been shown to be sporadic. [8, 9] The mechanism of sporadic MMR dysfunction is primarily the result of cytosine-phosphate-guanine (CpG) island hypermethylation of the MLH1 promoter region. [3, 8, 9]

Due to conflicting reports in literature concerning the prevalence of MMR dysfunction in colon cancer among various ethnic groups [10-17], we designed a study to investigate the rate of dysfunctional MMR protein expression within colon cancer cases from our patient population. Our primary aim was to study the prevalence and clinical presentation of MMR deficient CRC, while also evaluating for differences between various ethnicities.

2. MATERIALS AND METHODS

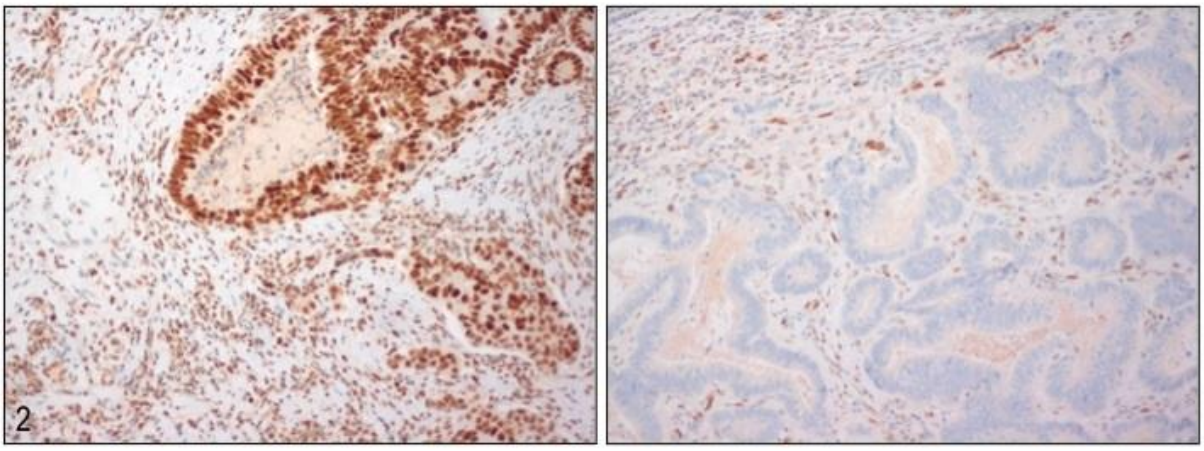
A retrospective analysis was performed including all colonic adenocarcinoma surgical specimens consecutively collected at our community hospital in the time period of 2009-2014. Clinical data, including gender, ethnicity, age, imaging findings, family history, germline testing, and tumor location (right defined as cecum, ascending, and transverse; left defined as descending, sigmoid, and rectum) were collected from electronic medical records. Over 5000 colon-related excisions and biopsies were screened for adenocarcinoma, resulting in the identification of 320 patients with adenocarcinoma. Exclusion criteria included specimens with rare tumor cells, absence of tumor-associated lymphocytes as internal control, primary adenocarcinoma outside the colon or rectum, neo-adjuvant treatment without available tissue prior to treatment, and unavailable medical record data. After review, 272 were screened for absence of MMR proteins by immunohistochemistry. All cases were reviewed and differentiation was designated as well, moderate, poor, or undifferentiated based on the degree of gland formation. [18] Well and moderate differentiated cases were considered low grade; while poor and undifferentiated were considered high grade due to proven prognostic value, relative simplicity, reproducibility[19] , and for statistical analysis.

Paraffin embedded tissue blocks for the 272 cases were collected. Four micron thick tissue sections were cut from each tissue block and applied to 4 positively charged glass slides. Antigen retrieval was performed using an alkaline Tris-based buffer and immunohistochemistry staining was performed with monoclonal MMR protein antibodies including MSH2, MSH6, MLH1, and PMS2. Antibody binding was detected by ultraView universal DAB detection kit and was carried out on a Ventana Benchmark XT autostainer. Validation of all antibodies was performed by manufacturer specifications. Positive MMR (pMMR) protein expression was interpreted as homogeneous chromatin staining of any tumor nuclei in the presence of positive nuclear staining of non-neoplastic tissue. Complete absence of nuclear staining in neoplastic cells, in the presence of appropriate controls, was defined as MMR deficient (dMMR) (Figure 2). Equivocal staining results were excluded after two attempts. When possible, colon tumors were staged according to the American Joint Committee on Cancer (AJCC) Staging Manual. [20] MSI-associated histologic features were identified for each dMMR case as follows: medullary growth pattern (involving >10% of examined tumor), Crohn's-like lymphoid reaction (two or more

lymphoid aggregates not associated with either mucosa or lymph node), mucinous or signet ring cell features (involving >10% of examined tumor), and intraepithelial lymphocytosis (>20 lymphocytes per high power field – 0.94 mm^2 in five high power fields). Statistical analysis was performed using Fishers' exact test and t-test for comparison of two means with a significance value set at $p<0.05$.

Figure 2. Representative sections stained with MMR antibodies.

Antibody stained sections showing nuclear positivity for the presence of MSH2 (left) and complete absence of nuclear staining for PMS2 (right).



Abbreviations: MMR, mismatch repair; MSH2, mutS homolog 2; PMS2, post-meiotic segregation increased 2.

3. RESULTS

A total of 275 colon cancer cases were identified, which met our inclusion criteria for the period of study. Three cases were excluded for either equivocal staining or limited tissue. The final total was 272 study cases.

The ages for our patient population ranged from 22-89 years with a mean of 56.4 years. Subdividing the population by ethnicity, there were 107 (39%) Latino, 80 (29%) African-American, 48 (18%) Caucasian, 32 (12%) Asian, and 5 (2%) Middle Eastern. Mean age at diagnosis was significantly different when comparing Latino with non-Latino populations (Table 1).

Based on the findings of Yearsly and group (2006) including the observation of young age at diagnosis as being the only significant variable associated with germline mutations of MMR genes, [21] we divided our patient population into two groups by age (less than 50 years and greater than or equal to 50 years). 56 (21%) were diagnosed at less than 50 years of age. Similar to mean ages by ethnicity, young age at diagnosis was most common within the Latino subpopulation (32%), followed by African-American (18%), Asian (9%), and Caucasian (8%). There was no mean age difference when dividing by gender (Table 1).

Table 1. Manufacturers and details for assay components.

Component	Manufacturer and Details
Glass Slides	Leica Biosystems, Canada
Antigen Retrieval	CC1: Ventana Medical Systems Inc., Tucson, Arizona
MSH2	Clone G219-1129, Ventana Medical Systems Inc., Tucson, Arizona
MSH6	Clone 44, Ventana Medical Systems Inc., Tucson, Arizona
MLH1	Clone M1, Ventana Medical Systems Inc., Tucson, Arizona
PMS2	Clone EPR3947, Ventana Medical Systems Inc., Tucson, Arizona
Antibody Detection	Ventana Medical Systems Inc., Tucson, Arizona
Autostainer	Ventana Medical Systems Inc., Tucson, Arizona

Abbreviations: MSH2, mutS homolog 2; MSH6, mutS homolog 6; MLH1, mutL homolog 1; PMS2, post-meiotic segregation increased 2; dMMR, deficient mismatch repair.

In total, there were 165 men and 107 women (M:F=1.5:1). Colon cancer patients of Asian origin showed a decreased proportion of men to women (M:F = 0.7:1) when compared to the non-Asian population. Patients of Caucasian descent showed a relative male predominance (M:F = 2.7:1) compared to other ethnicities (Table 2).

Concerning cancer location in the colon, 179 (67%) of the tumors were found on the left (distal to splenic flexure) and 88 (33%) were located on the right (proximal to splenic flexure), excluding 5 cases with synchronous, multifocal tumors. Women showed a slightly higher prevalence of right-sided cancer compared to men; however, the difference was not clinically significant (p=0.183, data not shown). When subdividing by ethnicity and gender, left-sided cancer was always found to be more common than cancer arising in the right colon (data not shown).

Immunohistochemistry for MMR proteins were interpreted by an experienced pathologist and showed dMMR in 20 of 272 (7.4%) colon cancer cases. The Latino portion of our patient population showed the greatest prevalence of dMMR tumors (13.1%). Low prevalence of dMMR colon cancer achieved statistical significance when comparing the Latino population with combined non-Latino ethnicities, African-Americans, and Asians (Table 3). The Middle Eastern subgroup (5 cases) showed a prevalence of 20%, which was insignificant due to low sample size. The age range for dMMR cases was 22-76 years old with a mean age of 52. Combining all non- Latino ethnicities, the mean age at diagnosis for dMMR tumors was significantly younger than the observed mean for pMMR tumors. Within the Latino population, mean age of diagnosis was very similar for both dMMR and pMMR cases (Table 3).

Table 2. Mean age at diagnosis of colon cancer for Latino ethnicity compared with non-Latino ethnicities individually and combined, and combined totals for gender.

		Total (%, *n*=263)	Mean Age	p-value
Ethnicity	Latino	102 (39)	53.9	
	All non-Latino	161 (61)	58.0	0.003
	Asian	31 (12)	59.7	0.021
	African American	77 (29)	57.3	0.045
	Caucasian	48 (18)	58.2	0.034
	Middle Eastern	5 (2)	54.4	0.930
Gender	Male	160 (61)	56.5	
	Female	103 (39)	56.3	0.996

Table 3. Association of colonic adenocarcinoma with gender and ethnicity.

Ethnicity (*n*)	Male (%)	Female (%)	p-value
Asian (*31*)	13 (42)	18 (58)	
Non-Asian (*232*)	147 (63)	85 (37)	0.030
Caucasian (*48*)	35 (73)	13 (27)	
Non-Caucasian (*215*)	125 (58)	90 (42)	0.072
Latino (*102*)	60 (59)	42 (41)	
Non-Latino (*161*)	100 (62)	61 (38)	0.610
African-American (*77*)	48 (65)	29 (35)	
Non-African-American (*186*)	112 (60)	74 (40)	0.783
Middle Eastern (*5*)	4 (80)	1 (20)	
Non-Middle Eastern (*258*)	156 (60)	102 (40)	0.651

Right-sided tumors were found to be significantly more common in dMMR patients when compared with pMMR (Table 3). Two dMMR and three pMMR cases were excluded from this portion of analysis due to multifocal tumor location. The dMMR cases included 12 men and 8 women (M:F = 1.5:1) with no correlation between gender and MMR protein status (Table 3). Seven patients were identified under the age of 50 years, while the remaining patients either fell between the ages of 50 – 60 years (7) or 60 – 70 years (5), with only 1 patient greater than 70 years. High-grade histology showed significant correlation with dMMR tumor status (Table 4).

Table 4. Association of colon cancer mismatch repair protein status with ethnicity, mean age, location of tumor, gender, and histologic grade.

		pMMR (%, *n*=243)	dMMR (%, *n*=20)	p-value
Ethnicity (*n*)	Latino (*102*)	88 (36)	14 (70)	
	Non-Latino (*161*)	155 (64)	6 (30)	0.004
	African American (*77*)	74 (30)	3 (15)	0.037
	Asian (*31*)	31 (13)	0 (0)	0.039
	Caucasian (*48*)	46 (19)	2 (10)	0.093
Non-Latino	Mean Age	58.3	49.0	
	Total	155 (64)	6 (30)	0.023
Latino Age	Mean Age	53.5	51.9	
	Total	88 (36)	14 (70)	0.638
Location* (*n*)	Right (*87*)	76 (32)	11 (61)	
	Left (*171*)	164 (68)	7 (39)	0.018
Gender (*n*)	Male (*160*)	148 (61)	12 (60)	
	Female (*103*)	95 (39)	8 (40)	1.000
Grade (*n*)	Low Grade (*232*)	219 (90)	13 (65)	
	High Grade (*31*)	24 (10)	7 (35)	0.004

* Five cases excluded due to multifocal tumor location.

Abbreviations: pMMR, mismatch repair proteins present; dMMR, deficient mismatch repair.

The pattern of MMR proteins found to be deficient included the combination of MLH1 and PMS2 (9, 45%) or PMS2 alone (6, 30%), followed by the combination of MSH2 and MSH6 (3, 15%), while the remaining 2 (10%) patients showed loss of MSH6 alone. PMS2 was the most commonly lost protein (15, 75% of cases) followed by MLH1 (8, 40% of cases), MSH6 (5, 25% of cases), and MSH2 (3, 15% of cases). Only PMS2 and MSH6 showed cases of single protein expression loss, while both MLH1 and MSH2 expression was only lost in combination with their functional partners (PMS2 and MSH6 respectively). There was no significant difference between the combinations of MMR protein loss when patients were divided by gender, age, tumor differentiation, region of colon, or ethnicity. Sixteen of the twenty patients underwent colectomies with a spectrum of pathologic staging that ranged from stage I to stage IIIC. Four of the twenty patients had documented metastasis by CT imaging at presentation and underwent biopsy without associated resection. AJCC staging was possible in a total of 193 cases (20 dMMR and 173 pMMR). Combinations of staging groups were analyzed for differences between dMMR and pMMR patients (Table 4). Association between dMMR and advanced tumor stage was only found when comparing stages 0 - IIIB and IIIC - IVB (p=0.006).

Table 5. Association of dMMR and pMMR colonic adenocarcinoma with AJCC stage.

Stage (*n*)	dMMR (%, *n*=20)	pMMR (%, *n*=164)	p-value
0 – IIIB (*153*)	12 (60)	141 (86)	
IIIC – IVB (*31*)	8 (40)	23 (14)	0.008
0 – IIIA (*105*)	8 (40)	97 (59)	
IIIB – IVB (*79*)	12 (60)	67 (41)	0.150
0 – IIC (*97*)	7 (35)	90 (55)	
IIIA – IVB (*87*)	13 (65)	74 (45)	0.103
0 – IIIC (*161*)	16 (80)	145 (88)	
IVA – IVB (*23*)	4 (20)	19 (12)	0.285
0 – I (*30*)	2 (10)	28 (17)	
IIA – IVB (*154*)	18 (90)	136 (83)	0.538

Abbreviations: pMMR, mismatch repair proteins present; dMMR, deficient mismatch repair; AJCC, American Joint Committee on Cancer.

Histologic characteristics of MSI-high tumors (medullary growth pattern, Crohn's-like lymphoid reaction, mucinous/signet ring cell features, and intraepithelial lymphocytosis) were also evaluated for the 16 cases in which colectomies were performed. All 16 cases included intraepithelial lymphocytosis and all but 1 case (94%) had at least focal mucinous differentiation. Medullary growth pattern was identified in 4 of the cases (25%) and a Crohn's-like lymphoid reaction was seen in 8 cases (50%).

KRAS mutation analysis was performed on 10 of the 20 dMMR cases with mutations identified in 4 of the 10 cases (3 - Gly12Asp and 1 - Gly12Ser). BRAF testing was only performed on 2 of the 20 cases and was found to be negative for mutations in both.

Review of electronic medical records for the 20 dMMR patients revealed a positive family history of cancer in 13 cases. All dMMR patients were offered follow-up counseling and germline testing; however, only 10 of the 20 patients returned for these consultations (all were of Latino-descent). These patients received germline testing directed by the results of their MMR immunohistochemistry. Germline mutations in MMR genes were identified in 8 of the 10 tested patients including 1 MSH-2 deletion, 1 MSH-6 deletion, 1 MLH-1 deletion, 3 MLH-1 point mutations, and 2 PMS-2 point mutations.

No significant correlations were identified when comparing histologic features with ethnicity, germline mutation status, or BRAF and KRAS mutation status.

4. DISCUSSION

Colorectal cancer remains the third most common cancer and the third leading cause of cancer death among men and women in the United States. [22] MSI due to dMMR has been shown to carry a more favorable prognosis [23-25], may suggest relative resistance to specific chemotherapy agents[23] , and can help direct early screening in families found to harbor germline mutations of MMR genes. [26] Due to the potential implications of dMMR on the management of CRC, it is now suggested that all cases of colonic adenocarcinoma be tested by immunohistochemistry, MSI analysis, or germline testing with polymerase chain reaction (PCR). [27] When compared to the more complicated and expensive molecular methods of detection, such as PCR-based assays including MSI and germline testing, immunohistochemistry has similar sensitivity and specificity with substantially lower cost. [28-29]

Germline mutations in MMR genes are reported as most commonly found in MLH1 (40-50%) followed by MSH2 (40%), MSH6 (10%) and PMS2 (5%).[3, 6] The vast majority of MMR-related sporadic adenocarcinoma of the colon is reported to be related to loss of MLH1 through promoter methylation. [1] Based on these observations, one would expect to find MLH1 to be the most commonly dysfunctional MMR protein in a randomly selected population of dMMR colon cancer patients. Although our sample size (20 dMMR patients) was low, our results appear to support the reported prevalence of MLH1 germline mutations in dMMR colon cancer. The 10 Latino patients that pursued follow-up counseling with genetics showed a significantly high rate of germline mutations in MMR genes (80%): 4 involving MLH1, 2 mutations of PMS2, and 1 mutation each for MSH2 and MSH6. Admittedly, this is a small sample size for drawing significant conclusions; however, our results suggest an increased prevalence of MMR protein dysfunction related to germline mutations within our Latino cohort. Additional investigation including germline testing and improved successful follow-up is necessary to confirm these data.

Variability in the rate of dMMR colon cancer has also been reported among different ethnicities. Between the years of 2003 and 2014, numerous conflicting articles have been published concerning the prevalence of MSI and MMR deficiency among different ethnicities with colon cancer.[10-17] Ashktorab and colleagues (2003 and 2005) showed greater than 40% prevalence of MSI-high colon cancer in African-Americans from Washington DC.[13,17] However, a study performed by Hatch and colleagues (2005) in North Carolina showed the frequency of MSI-H colon cancer among Caucasians and African-Americans was very similar at 10%.[14] In 2008, Jin and collaborators found 13% MSI-H among Chinese colon cancer patients at Nanjing University in China.[12] In contrast, a recent article published by Cheah and colleagues (2014) identified only 6.2% dMMR colon cancer in the Chinese population of Malaysia compared to a combined 14.8% in Malays and Indians of Malaysia.[15] Finally, conflicting reports have also been published concerning the prevalence of dMMR colon cancer in the Latino population. Jesus-Monge and group (2010) showed only 4.3% of colon cancer to be MMR deficient in the Latino population of Puerto Rico; however, only MLH1 and MSH2 antibodies were used to perform this study.[10] In contrast, Gupta and collaborators (2010) found 14.6% dMMR in the Latino population with colon cancer in Dallas, Texas using 4-marker MMR immunohistochemistry.[16]

Similar to previous studies, we found statistically significant correlations with dMMR including high-grade histology (p=0.003) and right colon location (p=0.017).[1,15,21] In contrast to previous population studies concerning colonic adenocarcinoma, we found a significant correlation between advanced stage colon cancer and dMMR (p=0.006).[10,15] We also identified an overall reduced prevalence of dMMR in our non-Latino patient population (3.6%), which accounts for approximately one quarter of the expected prevalence reported in the general US population (10-15%).[1, 2] The Latino population was found to be within the expected prevalence range for dMMR. Compared with the Latino population, the non-Latino ethnicities showed a significantly reduced rate of dMMR colon cancer (p=0.007). The low prevalence found in the African-American and Asian subpopulations was additionally found to have independent statistical significance when compared to the Latino population; however, the population size for these subgroups was smaller in our cohort.

Potential etiologies for the decreased prevalence of MMR deficient colon cancer in the non-Latino portion of our patient population are currently unclear. Potential factors contributing to this finding may include differences in diet, socioeconomic class, lifestyle choices, or ethnicity-related, region-specific genetic polymorphisms. The younger mean age at diagnosis of our non-Latino dMMR patients (49 years) may indicate an increased prevalence of germline mutations of MMR proteins and potentially decreased rate of sporadic mutation due to MLH1 methylation. However, germline testing was not pursued by the six non-Latino patients identified in our study, preventing confirmation of this theory. In contrast, the germline test results of the Latino patient population may suggest a higher propensity for germline mutations in this group, which may contribute to the overall younger age at colon cancer diagnosis in this population. Although, the similar mean age at diagnosis for both pMMR and dMMR colon cancers among Latinos in our patient population remains unexplained.

Institutional bias is the main limitation of our study; which is derived from our patient population (Latino predominant).

Little is known of the prevalence and significance of dMMR in patients of Latino descent. Further investigation is needed to identify possible causes for the observed variability of dMMR colon cancer prevalence among our patients. To our knowledge, no other studies have reported instances of increased prevalence for germline mutations among those of Latino descent, or decreased rates of dMMR in Caucasian, African-American, and Asian ethnicities in the United States.

In conclusion, we have confirmed the well-known associations of dMMR colon cancer with right-sided location and high-grade histology. We also identified a significant association between dMMR colon cancer and advanced stage. We have demonstrated that the overall prevalence of dMMR colon cancer within our non-Latino population is much lower than reported in literature, while germline mutations in MMR genes of our Latino population appear more frequent than what would be expected for the general population. Further investigation concerning possible etiologies for the patterns of MMR dysfunction among specific ethnicities in our patient population is ongoing. Through the observations reported here, we hope to offer additional clues in the efforts to reveal the connection between apparent regional and ethnic differences in the prevalence of dMMR colon cancer.

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