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ORIGINAL RESEARCH

Screening for Muir-Torre Syndrome Using Mismatch Repair Protein Immunohistochemistry of Sebaceous Neoplasms

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Abstract Screening for the Muir-Torre variant of Lynch Syndrome (LS) using Mismatch Repair (MMR) gene immunohistochemistry (IHC) on sebaceous neoplasms (SNs) is technically feasible. To date, research into the clinical utility of MMR IHC for this indication is limited. We conducted a retrospective chart review of 90 patients with MMR IHC completed on at least one SN from January 2005 to May 2010. SNs included were adenomas, epitheliomas, carcinomas and basal and squamous cell carcinomas with sebaceous differentiation. Of the 90 patients, 13 (14 %) had genetically confirmed or fulfilled clinical criteria for a diagnosis of MTS and 51 patients (57 %) presented with an abnormal MMR IHC result (loss of one or more MMR proteins) on at least one SN. Abnormal IHC had a sensitivity of 85 %, specificity of 48 %, positive predictive value (PPV) of 22 % and negative predictive value (NPV) of 95 % when evaluating for MTS. When personal or family history of colorectal cancer (≥2 family members with a history of colorectal cancer) was taken into consideration, ignoring IHC results, sensitivity was 92 %, specificity was 99 %,

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PPV was 92 % and NPV was 99 %. MMR IHC on SNs when used to screen for MTS has poor diagnostic utility. We recommend that MMR IHC not be performed routinely on SNs when the patient does not have either personal or family history of colorectal cancer.

Keywords Lynch syndrome · Muir-Torre syndrome · Mismatch Repair Genes · Immunohistochemistry · Sebaceous neoplasms

Introduction

Muir-Torre syndrome (MTS), a variant of Lynch syndrome (LS), is characterized by the occurrence of sebaceous neoplasms (SNs), including adenomas, carcinomas, epitheliomas (sebaceomas), and keratoacanthomas in association with colon cancer and other cancers seen in Lynch syndrome (colon, endometrial, duodenal, ovarian, urinary tract, hepatobiliary tract, small bowel and brain) (Lazar et al. 2007; Lynch et al. 1985). The term Muir-Torre syndrome was derived in honor of Dr. E.G. Muir and Dr. Douglas Torre who independently described two patients with both sebaceous neoplasms and visceral malignancies in 1967 and 1968 (Muir et al. 1967; Torre 1968). It was in 1980 and 1981 when Dr. Ramon Fusaro and Dr. Henry Lynch suggested that Torre's syndrome may be a result of the same underlying genetic predisposition responsible for the "cancer family syndrome" which is now referred to as Lynch syndrome (Fusaro et al. 1980; Lynch et al. 1981). MTS and LS are caused by deleterious germline mutations in the DNA mismatch repair (MMR) genes *hMLH1*, *hMSH2*, *hMSH6*, and *PMS2*. In one study, 9.2 % of identified patients with Lynch syndrome were diagnosed with Muir-Torre syndrome based on the presence of a sebaceous neoplasm (SN) (South et al. 2008). Some studies have shown that patients with Muir-Torre syndrome are more likely to have germline mutations in *hMSH2* than in the other MMR genes (South et al. 2008).

Almost all Lynch syndrome-related cancers demonstrate microsatellite instability (MSI) and absence of MMR protein expression that can be identified using immunohistochemistry (IHC) (South et al. 2008). In the past, MSI was used more commonly when screening colorectal cancer for Lynch syndrome. Sensitivity and specificity for MSI is reported as 77-91 % (depending on the location of the germline mutation) and 90.2 % respectively, when completed on colorectal tumors (de la Chapelle and Hampel 2010). The sensitivity of MMR IHC for LS in colorectal cancers has been reported as 92-94 % while specificity is reported as 88-100 % (Hampel et al. 2008; Hampel et al. 2005; Lindor et al. 2002). More recently IHC is replacing MSI as sensitivity and specificity is similar between the two tests, and IHC in general is more convenient and cost-effective (de la Chapelle and Hampel 2010). Additionally, MMR IHC is helpful in identifying the specific gene/s involved when ordering germline testing. Screening protocols for LS using MSI and IHC have been established for colon cancer, and screening of all colon cancers using MMR IHC is emerging and practiced in many medical centers across the United States (Mvundura et al. 2010).

The utility of MMR IHC on colon carcinomas as a test to identify LS is well-established. In this study our aim was to investigate whether MMR IHC could be used similarly to identify MTS patients by testing SNs, which are characteristic of the MTS variant. There have been several reports of MMR IHC on SNs, which have been limited by small sample size and lack of clinical data, including a detailed family history and genetic test results (Abbas and Mahalingam 2009; Morales-Burgos et al. 2008; Popnikolov et al. 2003). The focus of these reports was on the MMR IHC staining pattern, and the clinical utility of this testing for diagnosing MTS has not been previously described. Other studies focus on MMR IHC on SNs in known MTS patients and do not compare their findings to MMR IHC on sporadic SNs (Machin et al. 2002; Mathiak et al. 2002; Ponti et al. 2005). Interpretation of MMR IHC is considered to be straightforward; the tumor cells are histopathologically evaluated for retention or loss of MLH1, MSH2, MSH6 and PMS2 proteins. Impeding clinical utility is the lack of complete understanding of the underlying molecular mechanisms for each observed staining pattern in SNs. The SN MMR IHC staining patterns observed in this study, high rate of abnormal MMR IHC (57 %) and predominant staining pattern being loss of MSH2 and MSH6, appear to be unique compared to our previous understanding based on colon and endometrial cancer research (de la Chapelle and Hampel 2010). During our chart review an abundance of solid organ transplant recipients was noted. A subsequent literature review showed that sebaceous neoplasms were significantly overrepresented in a group of renal transplants recipients compared to immunocompetent patients (30 % vs. 6 %). This study only looked at renal transplant patients who were all receiving immunosuppressive therapy with azathioprine, prednisolone, and cyclosporine or azathioprine and prenisolone only. The time between tumor presentation and transplantation was 9.8 years (1–28 years). Two patients on this study were noted as having MTS; both presented with "multiple sebaceous adenomas" (Harwood et al. 2003). Based on this previously completed research an emphasis on solid organ transplant recipients was made.

Materials & Methods

Data Collection

All patients with SNs that underwent MMR IHC analyses at Mayo Clinic in Rochester, Minnesota, Scottsdale, Arizona,

and Jacksonville, Florida between January 2005 and May 2010 were included. SNs included in this study were sebaceous adenomas, sebaceous epitheliomas, sebaceous carcinomas, and basal or squamous cell carcinomas with sebaceous differentiation. Patients with only sebaceous hyperplasia were excluded. While keratoacanthomas are associated with MTS, they were not included in our data.

An abnormal IHC test result was considered a loss of expression of one or more of the MMR gene protein products (MLH1, MSH2, MSH6, or PMS2). A patient was considered to have a confirmed diagnosis of MTS if genetic testing found a deleterious germline mutation in one or more of the MMR genes. Patients who did not have germline genetic testing were suspected of having MTS if they had all of the following: a visceral malignancy known to be associated with LS, a personal history of at least one SN, at least one relative with a Lynch-related cancer, and no history of another hereditary gastrointestinal cancer syndrome, i.e. *MUTYH* Associated Polyposis (MAP) or Familial Adenomatous Polyposis (FAP). This definition is based on the classic definition of MTS (the presence of a sebaceous neoplasm plus visceral malignancy) and Revised Bethesda Guidelines, which are used to identify individuals at risk for Lynch syndrome and appropriate candidates for microsatellite instability (MSI) tumor testing (Umar et al. 2004). This study was submitted to the Mayo Clinic IRB and was subsequently deemed minimal risk on August 17, 2010.

Statistical Analysis

Patient and lesion characteristics were summarized using sample median, minimum, and maximum for numerical variables and number and percent for categorical variables. In examining the diagnostic utility of IHC for known or suspected MTS, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were estimated along with exact binomial 95 % confidence intervals (CIs). Associations of individual patient characteristics with known or suspected MTS were explored using Fisher's exact test for categorical variables and a Wilcoxon rank sum test for numerical variables. Patient characteristics significantly associated with MTS with a p-value of 0.05 or less were evaluated further for their diagnostic utility. In examining the diagnostic utility of patient characteristics (individually and in combination) for MTS, ssensitivity, specificity, PPV, and NPV were estimated along with 95 % CIs. P-values$\leq$0.05 were considered statistically significant and no adjustment for multiple testing was made due to the exploratory nature of the study. All analyses were performed using SAS (version 9.2; SAS Institute Inc.; Cary, NC).

Results

Patient characteristics are summarized in Table 1 (n=90) by comparing known and suspected MTS patients to non-MTS patients. Germline genetic testing of the MMR genes to evaluate for MTS was performed in 14 patients, of which 8 patients tested positive for a germline MMR gene mutation giving them the diagnosis of MTS. None of the 6 patients with negative germline test results met our "suspected MTS" criteria; only 1 had a family history of CRC (1 paternal 2nd degree relative and 1 maternal second degree relative both diagnosed with CRC >60) and 0 had a personal history of CRC. All 6 patients had abnormal MMR IHC on a SN that led to germline testing. Of the 76 patients that did not have genetic testing, 5 patients were suspected of having MTS based on established criteria discussed in the methods section. A summary of known MTS, non-MTS (MMR mutation negative) and suspected MTS patients personal and family history is provided in supplemental table 1. All but one patient with a proven deleterious MMR gene mutation would have met our "suspected MTS" criteria. The patient who did not meet the criteria had no personal or family history consistent with Lynch syndrome, but presented at the age of 71 with a sebaceous adenoma on the left lateral aspect of the neck; he was found to have a germline *hMSH6* mutation after MMR IHC showed loss of MSH6 only on his SN. Of note that this patient had a total of 3 sebaceous adenomas; MMR IHC was only completed on one. In total, 13 (14 %) patients were diagnosed with either known or suspected MTS.

MMR IHC, when performed on the patient's primary SN, resulted in an abnormal IHC test result in 51/90 (57 %) of patients. As shown in Table 2, 11 of the 13 (85 %) patients with known or suspected MTS had abnormal IHC on a SN. However, MMR IHC resulted in a likely false-positive finding (loss of one or more MMR proteins) for 40 of the 77 (52 %) patients without known or suspected MTS. Location of each patient's primary SN was noted as location (head and neck vs. non-head and neck) has previously been suggested to be a possible differentiator between MTS and non-MTS (Singh et al. 2008). After further review it was determined that non-head and neck SNs have a slightly higher rate of abnormal MMR IHC (8/11, 73 %) than head and neck SNs (47/79, 59 %) but this was not statistically significant (p=0.518). It is also important to note that only 1 (13 %) of our known MTS patients initially presented with a non-head SN, while the other 7 (88 %) presented with a head and neck SN.

Table 1 also shows comparisons of those variables that were not involved directly in the diagnosis of MTS, between patients with and without known or suspected MTS. Compared to patients without MTS, those patients with known or suspected MTS were more likely to have an

Table 1 Comparisons of patient characteristics and medical history between patients with known or suspected Muir-Torre syndrome and patients without Muir-Torre syndrome

Variable	Known/suspected MTS		P-value
	No (*N*=77)	Yes (*N*=13)	
Demographic information			
Age at SN presentation (years)	72 (32–93)	69 (42–83)	0.078
Gender (male)	59 (77 %)	12 (92 %)	0.29
Personal history			
Colon cancer	5 (6 %)	8 (62 %)	<0.001
Colon cancer before age 60	1 (1 %)	5 (38 %)	<0.001
Other Lynch-related cancer	3 (4 %)	6 (46 %)	<0.001
Colon polyps	44 (56 %)	10 (77 %)	0.23
Family history			
Colon cancer	11 (14 %)	12 (92 %)	<0.001
Colon cancer before age 60	1 (1 %)	7 (54 %)	<0.001
Two or more relatives with colon cancer	1 (1 %)	7 (54 %)	<0.001
Endometrial cancer	1 (1 %)	5 (38 %)	<0.001
Other Lynch-related cancer	16 (21 %)	6 (46 %)	0.077
Any Lynch-related cancer	21 (27 %)	12 (92 %)	<0.001
Personal or family history			
Colon cancer	16 (21 %)	12 (92 %)	<0.001
Colon cancer before age 60	2 (3 %)	10 (77 %)	<0.001
Two or more relatives with colon cancer	1 (1 %)	12 (92 %)	<0.001
Other Lynch-related cancer	18 (23 %)	9 (69 %)	0.002
Significant pathological findings			
Keratoacanthoma[a]	8 (10 %)	5 (38 %)	0.019
Two or more SNs	9 (12 %)	10 (77 %)	<0.001
Three or more SNs	3 (4 %)	8 (62 %)	<0.001
Abnormal MMR IHC results of primary SN	40 (52 %)	11 (85 %)	0.037

Sample median (minimum, maximum) is given for numerical variables, while n(%) is given for categorical variables. P-values result from Wilcoxon rank sum test for numerical variables and Fisher's exact test for categorical variables. [a]Keratoacanthoma includes squamous cell carcinoma with features of keratoacanthoma

abnormal MMR IHC result on a SN (p=0.037), or a personal history of colon cancer (p=<0.001), colon cancer before age 60 (p=<0.001), a Lynch-related cancer other than colon or endometrial cancer (p=<0.001), pathology findings with features of keratoacanthoma (p=0.019), 2 or more SNs (p=<0.001). Similarly, known or suspected MTS patients were more likely to have a family history of colon cancer (p=<0.001), colon cancer before age 60 (p=<0.001), two or

Table 2 Comparison of mismatch repair immunohistochemistry staining patterns between the total study participants, known/suspected Muir-Torre syndrome and non Muir-Torre syndrome patients

IHC staining pattern	Patient type		
	Summary (*N*=90)	Known/ Suspected MTS (*N*=13)	Non-MTS Patients (*N*=77)
No loss of expression of MMR proteins	39 (43 %)	2 (15 %)[a]	37 (48 %)
Loss of expression of one or more MMR proteins	51 (57 %)	11 (85 %)	40 (52 %)
MSH2 and MSH6	24/51 (47 %)	5/11(45 %)	19/40 (48 %)
MSH6 alone	15/51 (29 %)	3/11 (27 %)	12/40 (30 %)
MLH1 and PMS2	6/51 (12 %)	1/11 (9 %)	5/40 (13 %)
MSH2 alone	3/51 (6 %)	2/11 (18 %)	1/40 (3 %)
PMS2 alone	1/51 (2 %)	0/11 (0 %)	1/40 (3 %)
MLH1, MSH6, and PMS2	2/51 (4 %)	0/11 (0 %)	2/40 (5 %)

[a] Two patients who are classified as known or suspected MTS presented with retention of all four MMR gene protein products. One is a known MTS patient who tested positive for a known familial MSH2 mutation. The other is classified as suspected-MTS based on a personal history of colon cancer (dx age 58) and pancreatic cancer (dx 85) as well as his father's history of colon cancer (dx 63)

more relatives with colon cancer (p=<0.001), endometrial cancer (p=<0.001), or any Lynch-related cancer (p=0.084) compared to patients without MTS.

Table 3 demonstrates the ability of these individual statistically significant variables to classify patients as MTS or non-MTS, without consideration of the MMR IHC results. Here, estimates of sensitivity, specificity, positive predictive value, and negative predictive value are given. The variable that was best able to differentiate between MTS and non-MTS patients was having two or more relatives, which could include the patient, with a history of colon cancer. Of the 13 patients with known or suspected MTS, 12 (92 %) had two or more relatives with a history of colon cancer (sensitivity 95 % CI: 64 %-100 %). Of the 77 patients without known or suspected MTS, 76 (99 %) had fewer than two relatives with a history of colon cancer (specificity 95 % CI: 93 %-100 %). Likewise, when there is a personal or family history of two or more relatives with a history of colon cancer, the positive predictive value is 92 % (CI: 64-100 %) and the negative predictive value is 99 % (CI: 93-100 %).

In an exploratory analysis, we examined the utility of these individual variables in combination with SN MMR IHC results in diagnosing MTS; these results are shown in Table 4. When considering the utility of abnormal MMR IHC test results in the diagnosis of MTS, sensitivity was 85 % (11/13, 95 % CI: 54 %-98 %), while specificity was only 48 % (37/77, 95 % CI: 37 %-60 %). When abnormal IHC results are combined with selected individual variables, specificity ranges from 83 % to 100 %; however, sensitivity drops to values between 31 % and 77 %. This indicates that abnormal IHC results independently or in combination with other variables, are not as useful as personal and family history in the diagnosis of MTS when a patient presents with a SN.

Sebaceous Neoplasm IHC Staining Patterns

In both the known and suspected MTS group and the non-MTS group, loss of MSH2 and MSH6 was the most common IHC staining pattern (5/11 [45 %] vs. 19/40 [48 %]). Table 2 shows the similarity in staining patterns between the known and suspected MTS patients (n=13) in comparison to the non-MTS patients (n=77). When IHC was completed on the patients' primary SN, loss of expression of at least one MMR gene protein product was seen in 11/13 (85 %) of our known and suspected MTS patients and 40/77 (52 %) of our non-MTS patients. Four out of our 8 (50 %) known MTS patients had MMR IHC completed on more than one SN. A summary of their MMR IHC staining patterns, SN locations and genetic test results can be found in supplemental table 2.

Discordant MMR IHC Results

Of 90 patients, 8 (9 %) had MMR IHC completed on two or more SNs. In 6 of these 8 (75 %) patients, there were discordant MMR IHC results among their different SNs. (Supplemental Table 3) In 5 of the 6 (83 %) cases, the germline genetic testing algorithm would have been affected by the discordant MMR IHC results.

Patients that Underwent Germline Genetic Testing (Genotype-Phenotype Correlation)

A total of 14 patients completed germline testing after an abnormal MMR IHC result on a SN. Eight of these 14 (57 %) patients tested positive for a germline MMR gene mutation. Six of these 8 (75 %) were found to harbor a deleterious *hMSH2* mutation, while the other 2 (25 %) were found to harbor a deleterious *hMSH6* mutation; there were no *hMLH1* or *PMS2* mutations identified. All *hMSH2* mutations in the 6 carriers tested involved exons 5 and 6; this is summarized in supplemental table 4.

MTS Diagnosis Given to One Patient and Previously Incorrectly Given to Two Patients

One patient was identified as most likely having MTS due to the presence of abnormal MMR IHC on a sebaceous adenoma 5 years prior. No formal workup was completed prior to identifying him through this study (Fig. 1). Identification of this patient and subsequently diagnosing him with MTS resulted in clinically significant findings, including the discovery of a large ampullary adenoma that required ampullectomy.

We found two patients with a history of one SN that were given the diagnosis of MTS based on the cutaneous pathological findings alone by an outside physician prior to consultation with Medical Genetics; both of these diagnoses were removed after subsequent review of each case being there was not enough evidence to support giving a clinical diagnosis. To have a clinical diagnosis of Lynch syndrome one must meet either Amsterdam I or II criteria. Neither of these patients met these criteria. One patient was a 51 year old man, with no personal or family history of CRC, who presented with loss of MSH2 and MSH6 on a sebaceous adenoma. He underwent germline testing which included sequencing of *hMSH2*, deletion/duplication analysis of *hMSH2* and the 3' region of EPCAM. All genetic testing was negative. While this patient does not appear to have MTS based on personal and family history the possibility of Lynch syndrome can not be completely excluded (germline *hMSH6* genetic testing was not completed). Our other patient was a 72 year old female, only with a single

Table 3 Utility of patient/family history in diagnosing Muir-Torre syndrome

Variable (does NOT include MMR IHC results)	Sensitivity		Specificity		PPV		NPV	
	Fraction (%)	95 % CI	Fraction (%)	95 % CI	Fraction (%)	95 % CI	Fraction (%)	95 % CI
Personal history								
Colon cancer	8/13 (62 %)	32 %-86 %	72/77 (94 %)	86 %-98 %	8/13 (62 %)	32 %-86 %	72/77 (94 %)	85 %-98 %
Colon cancer before age 60	5/13 (38 %)	14 %-68 %	76/77 (99 %)	93 %-100 %	5/6 (83 %)	36 %-100 %	76/84 (90 %)	82 %-96 %
Other Lynch-related cancer	6/13 (46 %)	19 %-75 %	74/77 (96 %)	89 %-99 %	6/9 (67 %)	30 %-93 %	74/81 (91 %)	83 %-96 %
Family history								
Colon cancer	12/13 (92 %)	64 %-100 %	66/77 (86 %)	76 %-93 %	12/23 (52 %)	31 %-73 %	66/67 (99 %)	92 %-100 %
Colon cancer before age 60	7/13 (54 %)	25 %-81 %	76/77 (99 %)	93 %-100 %	7/8 (88 %)	47 %-100 %	76/82 (93 %)	85 %-97 %
2 or more with history of colon cancer	7/13 (54 %)	25 %-81 %	76/77 (99 %)	93 %-100 %	7/8 (88 %)	47 %-100 %	76/82 (93 %)	85 %-97 %
Endometrial cancer	5/13 (38 %)	14 %-68 %	76/77 (99 %)	93 %-100 %	5/6 (83 %)	36 %-100 %	76/84 (90 %)	82 %-96 %
Any Lynch-related cancer	12/13 (92 %)	64 %-100 %	56/77 (73 %)	61 %-82 %	12/33 (36 %)	20 %-55 %	56/57 (98 %)	91 %-100 %
Personal or family history								
Colon cancer	12/13 (92 %)	64 %-100 %	61/77 (79 %)	68 %-88 %	12/28 (43 %)	24 %-63 %	61/62 (98 %)	91 %-100 %
Colon cancer before age 60	10/13 (77 %)	46 %-95 %	75/77 (97 %)	91 %-100 %	10/12 (83 %)	52 %-98 %	75/78 (96 %)	89 %-99 %
2 or more with history of colon cancer	12/13 (92 %)	64 %-100 %	76/77 (99 %)	93 %-100 %	12/13 (92 %)	64 %-100 %	76/77 (99 %)	93 %-100 %
Other Lynch-related cancer	9/13 (69 %)	39 %-91 %	59/77 (77 %)	66 %-86 %	9/27 (33 %)	17 %-54 %	59/63 (94 %)	85 %-98 %
Skin lesion characteristics								
Keratoacanthoma[a]	5/13 (38 %)	14 %-68 %	69/77 (90 %)	81 %-95 %	5/13 (38 %)	14 %-68 %	69/77 (90 %)	81 %-95 %
2 or more SNs	10/13 (77 %)	46 %-95 %	68/77 (88 %)	79 %-95 %	10/19 (53 %)	29 %-76 %	68/71 (96 %)	88 %-99 %
3 or more SNs	8/13 (62 %)	32 %-86 %	74/77 (96 %)	89 %-99 %	8/11 (73 %)	39 %-94 %	74/79 (94 %)	86 %-98 %

- Abnormal IHC is the loss of expression of one or more MMR proteins as detected via immunohistochemistry (IHC) analysis. Only variables that differed significantly between known or suspected MTS patients and patients without MTS were considered for diagnostic evaluation. [a] Keratoacanthoma includes squamous cell carcinoma with features of keratoacanthoma. *PPV* positive predictive value, *NPV* negative predictive value

Table 4 Utility of abnormal mismatch repair immunohistochemistry and patient/family history in diagnosing Muir-Torre syndrome

Variable (DOES include MMR IHC results)	Sensitivity		Specificity		PPV		NPV	
	Fraction (%)	95 % CI	Fraction (%)	95 % CI	Fraction (%)	95 % CI	Fraction (%)	95 % CI
Abnormal IHC	11/13 (85 %)	54 %-98 %	37/77 (48 %)	37 %-60 %	11/51 (22 %)	11 %-35 %	37/39 (95 %)	83 %-99 %
Personal history and abnormal IHC								
Colon cancer	7/13 (54 %)	25 %-81 %	75/77 (97 %)	91 %-100 %	7/9 (78 %)	10 %-97 %	75/81 (93 %)	85 %-97 %
Colon cancer before age 60	4/13 (31 %)	9 %-61 %	76/77 (99 %)	93 %-100 %	4/5 (80 %)	28 %-99 %	76/85 (89 %)	81 %-95 %
Other Lynch-related cancer	5/13 (38 %)	14 %-68 %	74/77 (96 %)	89 %-99 %	5/8 (63 %)	24 %-91 %	74/82 (90 %)	82 %-96 %
Family history and abnormal IHC								
Colon cancer	10/13 (77 %)	46 %-95 %	71/77 (92 %)	84 %-97 %	10/16 (63 %)	35 %-85 %	71/74 (96 %)	89 %-99 %
Colon cancer before age 60	6/13 (46 %)	19 %-75 %	77/77 (100 %)	95 %-100 %	6/6 (100 %)	54 %-100 %	77/84 (92 %)	84 %-97 %
2 or more with history of colon cancer	6/13 (46 %)	19 %-75 %	76/77 (99 %)	93 %-100 %	6/7 (86 %)	42 %-100 %	76/83 (92 %)	83 %-97 %
Endometrial cancer	4/13 (31 %)	9 %-61 %	76/77 (99 %)	93 %-100 %	4/5 (80 %)	28 %-99 %	76/86 (90 %)	81 %-95 %
Any Lynch-related cancer	10/13 (77 %)	46 %-95 %	63/77 (82 %)	77 %-90 %	10/24 (42 %)	22 %-63 %	63/66 (95 %)	87 %-99 %
Personal or family history and abnormal IHC								
Colon cancer	10/13 (77 %)	46 %-95 %	69/77 (90 %)	81 %-95 %	10/18 (56 %)	31 %-78 %	69/72 (96 %)	88 %-99 %
Colon cancer before age 60	8/13 (62 %)	32 %-86 %	76/77 (99 %)	93 %-100 %	8/9 (89 %)	52 %-100 %	76/81 (94 %)	86 %-98 %
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Other Lynch-related cancer	7/13 (54 %)	25 %-81 %	64/77 (83 %)	73 %-91 %	7/20 (35 %)	15 %-59 %	64/70 (91 %)	82 %-97 %
Skin lesion characteristics								
Keratoacanthoma[a]	5/13 (38 %)	14 %-68 %	73/77 (95 %)	87 %-99 %	5/9 (56 %)	21 %-86 %	73/81 (90 %)	82 %-96 %
2 or more SNs	10/13 (77 %)	46 %-95 %	73/77 (95 %)	87 %-99 %	10/14 (71 %)	42 %-92 %	73/76 (96 %)	89 %-99 %
3 or more SNs	8/13 (62 %)	32 %-86 %	75/77 (97 %)	91 %-100 %	8/10 (80 %)	44 %-97 %	75/80 (94 %)	86 %-98 %

- Abnormal IHC is the loss of expression of one or more MMR proteins as detected via immunohistochemistry (IHC) analysis. Only variables that differed significantly between known or suspected MTS patients and patients without MTS were considered for diagnostic evaluation. [a] Keratoacanthoma includes squamous cell carcinoma with features of keratoacanthoma. *PPV* positive predictive value, *NPV* negative predictive value. Estimates of PPV and NPV are presented in Tables 3 and 4 with the assumption that the prevalence of MTS among patients with SNs seen in clinical practice is the same as was reported in the current study [14 %]. The estimates of PPV and NPV should be interpreted with this in mind

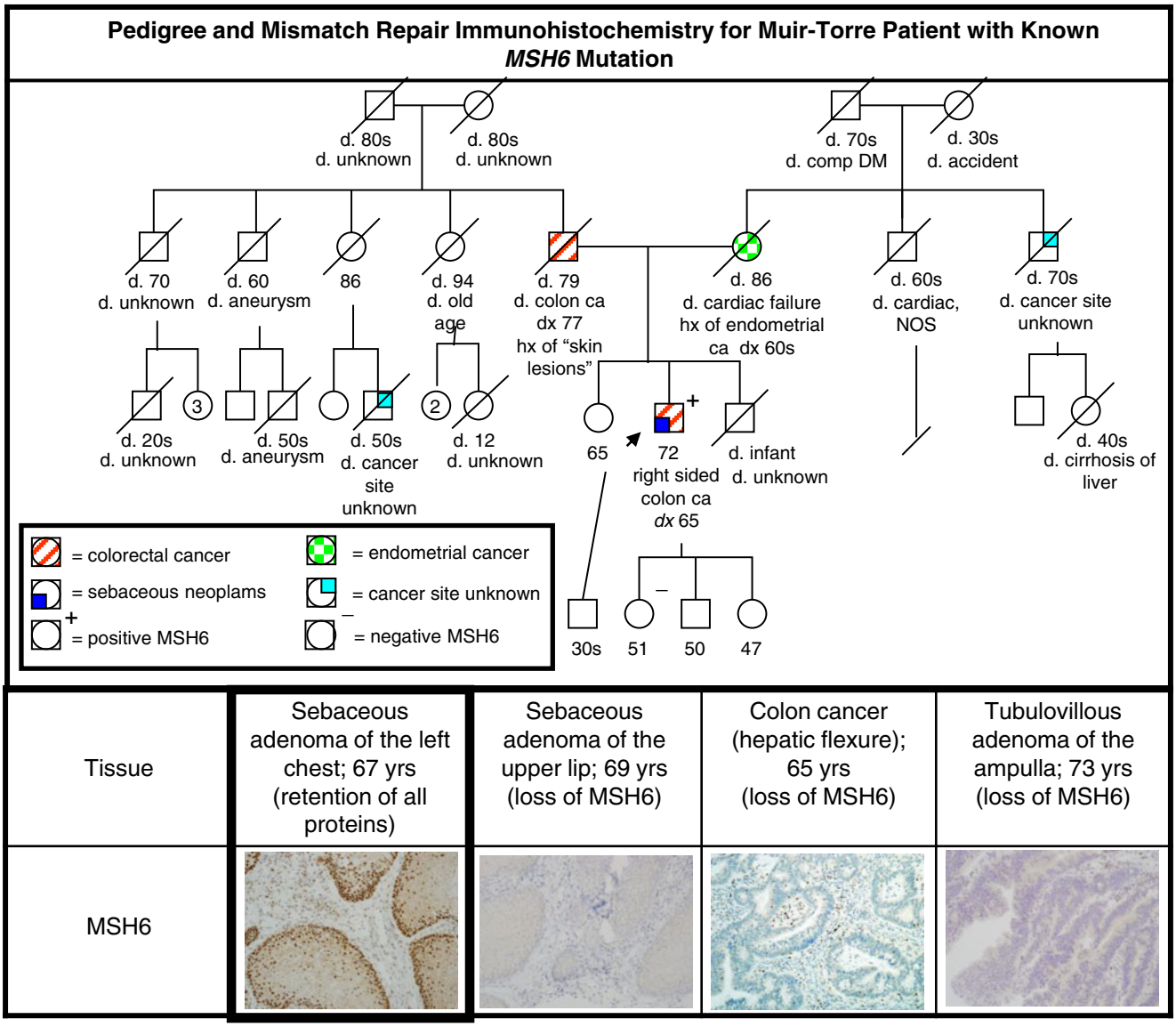

Fig. 1 The patient is designated by the arrow. This is the pedigree of a 72 year old man that was diagnosed with MTS as a result of this study. This past medical history consisted of a right sided colon cancer at the age of 65, a sebaceous adenoma of the left chest at 67 that showed retention of all four (MLH1, MSH2, MSH6 and PMS2) proteins via MMR IHC and a sebaceous adenoma of the upper lip at 69 that showed loss of MSH6 only on MMR IHC. Due to the questions regarding utility of MMR IHC on sebaceous neoplasms and the discordant MMR IHC results between his two sebaceous adenomas it was recommended that this patient have MMR IHC completed on his colon tumor; his colon tumor subsequently showed loss of MSH6. From this point germline line *hMSH6* genetic testing was initiated. He was found to harbor a deleterious *hMSH6* mutation designated as 718 C>T (R240X). Colonoscopy and upper endoscopy were recommended at this point. Colonoscopy showed 8 polyps (both adenomatous and hyperplastic) while upper endoscopy revealed two duodenal polyps; one on the ampulla and 1.5 cm in size and the other distal to the ampulla and 0.5 cm in size. The ampullary polyp was later proven to be a tubulovillous adenoma and required ampullectomy. His sebaceous adenoma of the left chest was discordant with retention of all four MMR proteins suggesting that the patient does not have Lynch syndrome. If the only MMR IHC available would have been from this sebaceous adenoma of the left chest we would have most likely not offered germline testing

second degree relative with CRC at 85, who presented with normal MMR IHC staining (retention of all four proteins) on a sebaceous epithelioma. Genetic testing was not pursued since this patient did not meet insurance criteria for germline testing and the previously given diagnosis of MTS was removed based on the normal MMR IHC and lack of supporting family history. Both patients were previously undergoing annual colonoscopy based on the presence of a sebaceous neoplasm assuming that this was a clear indication for MTS; we advised both patients that colorectal cancer screening should be based on personal and family history.

Staining Patterns in Solid Organ Transplant Patients

The MMR IHC staining patterns in transplant patients were similar to staining patterns of the remaining cohort of patients. These 16 transplant patients comprised 18 % (16/90) of all patients, and 12 (75 %) had abnormal IHC result. Of all patients with abnormal IHC results, the transplant patients comprised 24 % (12/51). None of the 16 transplant patients had a documented MMR gene mutation in their chart and none met our "suspected MTS" criteria. Due to the high percentage of abnormal MMR IHC in the transplant group we removed them from the data to see if it would greatly impact the sensitivity and specificity of MMR IHC. Sensitivity remained the same at 85 % while specificity did rise, it was only from 48 % to 54 %.

In transplant patients, the most common MMR IHC staining pattern was loss of MSH2 and MSH6 (7/12, 58 %). Loss of MSH2 and MSH6 was also the most common pattern seen in the known and suspected MTS and in the non-MTS groups, supplemental table 5 compares staining patterns between known and suspected MTS, non-MTS, and transplant patients.

The median time between transplant and SN diagnosis was 8 years (range: 0.6-18 years). The patients had a variety of solid organ transplants (kidney, liver, lung and heart) and were treated with different immunosuppressive regimens.

SNs in Patients with *MUTYH* Associated Polyposis (MAP)

We identified two patients with history of at least one SN and the diagnosis of MAP, another hereditary predisposition to colorectal cancer. Both patients were found to harbor two copies (homozygous) of the common *MUTYH* mutation Y165C. Nine other patients with MAP and a history of SNs were identified as result of a literature search. (Supplemental Figure 1 and Supplemental Table 6). Two *MUTYH* mutations, Y165C and G382D, account for approximately 70 % of all identified germline mutations in the *MUTYH* gene (Molatore et al. 2010). Of the total 11 patients identified to have MAP and a history of SNs, 7 (64 %) were found to be either homozygotes or compound heterozygotes for the two common *MUTYH* mutations.

Discussion

Strengths of the study include the large number of samples tested; this is the largest study to date that investigated the utility of MMR IHC of SNs for the diagnosis of MTS. This study is limited by its retrospective design and by the relatively small percentage of patients (16 %) that had germline genetic testing for germline MMR gene mutations. Germline genetic testing is the standard for diagnosing MTS. Many patients elected not to proceed with germline testing due to insurance restrictions and associated costs. Due to these limitations, additional studies would be helpful to validate findings. We acknowledge that there could be factors that are not currently understood, such as lower penetrance genes, modifiers, or somatic events responsible for some patients' phenotypes. Additional molecular studies are still needed to understand the molecular mechanisms behind the loss of expression of MMR gene protein products in sebaceous neoplasms. Based on previously completed MMR IHC studies in CRC, one could postulate that the same MLH1 hypermethylation phenomenon may also be present in sebaceous lesions with loss of MLH1 and PMS2. If this is the case, reflexing to hypermethylation may be necessary when loss of MLH1 and PMS2 is observed. If hypermethylation was present, no further testing would be needed, eliminating unnecessary subsequent genetic testing. However, MLH1 hypermethylation would potentially only explain a small proportion of abnormal MMR IHC, as loss of MLH1 and PMS2 were seen in only 12 % of our cohort.

The loss of expression of one or more MMR gene protein product as detected by IHC analysis had reasonable sensitivity, but poor PPV in the diagnosis of MTS, when personal and family history is not taken into consideration. The poor PPV contrasts with the findings of a previous study, which reported PPVs of 55 % to 100 %, associated with various MMR IHC staining patterns (Chhibber et al. 2008). This previous study did identify a high rate of abnormal MMR IHC similar to what was found in our study (24/41, 59 % vs. 51/90, 57 %). However, we would argue that the previously reported PPVs are incorrectly high due to less stringent clinical criteria used to diagnose MTS, which was defined as the presence of a SN plus of any one of the following: keratoacanthoma, non-cutacneous malignancy (not limited to Lynch related cancers) or the presence of an adenomatous polyp. In contrast to the above study, our data includes confirmatory germline testing for 9 % (8/90) of our patients. Our study also utilizes more stringent diagnostic criteria based upon the classic MTS definition in combination with the revised Bethesda criteria.

In the largest and most recent study of SN MMR IHC, other than this review, 79 SNs were evaluated in 70 different patients using MMR IHC (Cesinaro et al. 2007). This study showed a predominant loss of MSH2 on MMR IHC, similar to our findings. However, this study's overall rate of abnormal MMR IHC was lower than our findings. Of the 70 patients reported in this study, 18 (25.7 %) showed loss of at least one MMR protein via IHC on at least one SN. There are several differences in methodology between this report and ours. The previous study evaluated MMR IHC for only MSH2 and MLH1; MSH6 and PMS2 were not evaluated as they were in our study. The previous study also included

sebaceous hyperplasia, which our review did not include. Previous publications have noted that sebaceous hyperplasia does not appear to be a clinical indicator of MTS (Lazar et al. 2007). These differences in methodology may explain, at least partly, the differences in the rate of abnormal MMR IHC.

Without a clinical phenotype that meets criteria for MTS, MMR IHC has a low predictive value for the identification of germline mutations based on current methods. To improve the positive predictive value of MMR IHC for detection of a germline mutation and to avoid unnecessary subsequent genetic testing, we suggest a thorough review of the patient's personal and family history prior to requesting MMR IHC. In our study we found that the most predictive clinical indicator for MTS was the presence of two or more family members with a history of colorectal cancer.

The abnormal MMR IHC pattern observed most frequently in this study, loss of MSH2 and MSH6, traditionally has been consistent with a diagnosis of Lynch syndrome when observed in colon or endometrial cancers. The abundance of this staining pattern, in addition to the low positive predictive value, suggests that we cannot rely on previously completed MMR IHC results when trying to diagnosis MTS in patients who present with SNs. Our findings further challenge clinical interpretation and suggest that more research on this topic is needed.

Discordance of SN MMR IHC Between Multiple Sebaceous Neoplasms

The discordance of MMR IHC results between different SNs in the same patient as seen in our study is important with respect to further recommendations for germline genetic testing. In 5 of the 6 cases with discordant SN MMR IHC results, the germline genetic testing algorithm would have been different depending on which SN was being referenced. This finding suggests the possibility of missed diagnoses due to both false-negative and false-positive SN MMR IHC results (supplemental table 3). The presence of discordant SN MMR IHC within multiple individuals is indicative of a sporadic mechanism that has yet to be identified and further suggests that clinicians should not heavily rely on MMR IHC when evaluating patients for MTS who present with a SN.

Significance of Germline *hMSH6* Mutations

Patients with germline *hMSH6* mutations will generally not have classic personal and family histories of young-onset colorectal cancer (CRC) that is commonly reported with Lynch syndrome and deleterious *hMLH1* and *hMSH2* mutations (Bonadona et al. 2011). Patients with deleterious *hMSH6* mutations have lower incidences of CRC and tend to present with disease at later ages compared to patients with germline *hMLH1* and *hMSH2* mutations (Cederquist et al. 2004). Patients with germline *hMSH6* mutations have higher incidences of endometrial cancer with later age of onset of the disease (Plaschke et al. 2004). We identified two patients with germline *hMSH6* mutations; one patient with no personal or family history of colon or endometrial cancer, and one patient with a family history of colon and endometrial cancer in family members over 60 years of age (Fig. 1). Therefore, family history may not aid in the identification of potential MTS patients who present with loss of MSH6 only on MMR IHC.

A Possible Genotype Phenotype Correlation

We postulate that mutations involving exons 5 and 6 in the *MSH2* gene may have a higher risk for the Muir-Torre variant of Lynch syndrome, and thus confer an increased risk for the development of SNs. Peltomaki et al. reported that exons in 3 and 12 in the *hMSH2* gene have been found to harbor mutations more frequently than others. *hMSH2* has 16 exons; mutations in exons 5 and 6 make up approximately 13 % of total mutations in the *hMSH2* gene. This study also reports a higher frequency of a splice site mutation, IVS5+3A>T, in exon 5 which is a known founder mutation in Newfoundland and Canada and was identified in 2 of our MTS patients.

Sebaceous Neoplasms in Solid Organ Transplant Patients

Harwood et al. evaluated the presence of sebaceous carcinoma in 9 patients and found that 5 (56 %) were renal transplant recipients. Similar to our findings, they reported that 3/5 (60 %) transplant patients had loss of at least one MMR gene protein on IHC; our study showed 12/16 (75 %) transplant patients had loss of at least one MMR gene protein on IHC (Harwood et al. 2001). A summary of their findings compared to our findings can be found in supplemental table 7.

Many publications, including the study by Harwood et al., have shown that renal transplant patients are at an elevated risk for sebaceous hyperplasia and carcinoma (Harwood et al. 2003; Pang and Chau 2005). In addition, a few reports describe SNs in non-renal solid organ transplant recipients. Our data strongly suggest that transplant patients have an increased incidence of not only sebaceous hyperplasia and sebaceous carcinoma, but also sebaceous adenoma and epithelioma with loss of MMR protein expression. The predominant abnormal staining pattern of loss of MSH2 and MSH6 in both the non-MTS and transplant patients suggests that sporadic SNs have a propensity to this staining pattern which is very different than the published MMR IHC data on colon or endometrial cancer.

Sebaceous Neoplasms in Patients with *MUTYH* Associated Polyposis (MAP)

MAP is an autosomal recessive colorectal cancer syndrome unrelated to Lynch Syndrome. Like Lynch Syndrome patients, MAP patients may present with SNs. Two MAP patients in this study presented with a MTS phenotype. MAP should be considered as a differential diagnosis when a patient presents with a SN and a personal and/or family history of CRC, if MTS has been ruled out. Clues to the diagnosis of MAP are multiple adenomatous colon polyps (generally 10–100); a family history that is negative for colon cancer or a family history of colon cancer only in siblings, but not in prior or subsequent generations (autosomal recessive inheritance pattern); and retention of all MMR protein products via MMR IHC (microsatellite stability) on the patient's colon tumor (Goodenberger and Lindor 2011). Vogt et al. reported a total of 276 cases of MAP from 181 unrelated families and found that 5 patients (5/276, 1.8 %) had a history of one or more SNs. They also reported that 4 out of 5 patients had a genotype known to be associated with MAP and had no family history suggestive of Lynch syndrome (Vogt et al. 2009).

Conclusion and Clinical Reporting Recommendations

We conclude that MMR IHC is less reliable when completed on SNs than on colon or endometrial tumors in regards to accurately diagnosing MTS and Lynch syndrome, respectively. Although germline testing is the standard for confirming the diagnosis of MTS, at this time personal and family history is the most valuable tool to evaluate patients who present with SNs for MTS albeit the inability of personal and family history to identify some cases of MTS, especially carriers of *hMSH6* mutations. Until more research is completed on this topic and the underlying sporadic mechanisms responsible for the abundance of abnormal MMR IHC is better understood, the utility of MMR IHC of SNs in diagnosing MTS is not clear. However, MMR IHC on selected SN samples may be useful and could be considered for those with a personal and/or family history of colon cancer. If a patient presents with a SN with concerns of MTS and colon or endometrial tumor exists we recommend that MMR IHC be completed on these tissue types before it is completed on a SN.

There are no accepted colon cancer screening recommendations for the large group of patients who present with a sebaceous neoplasm, abnormal MMR IHC and subsequent negative germline genetic testing. At this time we believe that there is a sporadic mechanism most likely responsible for many of these patients' abnormal MMR IHC. We would reiterate the importance of the personal and family history. In one scenario, the patient that would be considered high-risk and in needed of more frequent colonoscopies (high-risk screening) despite negative germline MMR gene testing, might present with a personal history of one or more sebaceous neoplasms and either a personal and/or family history of a Lynch syndrome related cancers (≥ 2 relatives). On the other hand, the patient who presents with a single sebaceous neoplasm later in life (>60 years of age) or has a history of solid organ transplantation with no personal or family history of a Lynch syndrome cancer and negative germline MMR gene testing would be considered low-risk, with general population colon cancer screening recommended. .

Thus, we recommend that screening be based on personal and family history and not the abnormal MMR IHC. Due to the high rate of abnormal MMR IHC (57 % overall) and predominant abnormal staining pattern being loss of MSH2 and MSH6 (47 % overall), we feel that we cannot base colon cancer screening recommendations off of previously published data, as both of the statistics are not what have previously been observed in colon (MSI-H 15-20 %) and endometrial (MSI-H in 21.7 %) cancer research (de la Chapelle and Hampel 2010; Hampel et al. 2006).

Many pathologists indicate on their SN reports the possibility of MTS. At this time there is no standard reporting recommendation for SNs by any professional organization. Based on our results and conclusions, we recommend considering the following statement be addended to reports for SNs:

> "This neoplasm may be sporadic or associated with Muir-Torre syndrome (MTS), a variant of Lynch syndrome (Hereditary Non-Polyposis Colorectal Cancer). If this patient has a personal or family history of colon cancer, Mismatch Repair (MMR) Immunohistochemistry (IHC) could be considered to further evaluate for the presence of a defective MMR gene. However, abnormal MMR IHC alone is not sufficient for making the diagnosis of MTS. "

When abnormal MMR IHC is found on a SN we recommend considering the following statement be addended to pathology reports:

> "This neoplasm was found to have absence of XXX and retention of XXX Mismatch Repair (MMR) gene protein products. This finding can be an indicator of Muir-Torre syndrome (MTS) but is not diagnostic for this syndrome. A referral to a genetic counselor is recommended for additional workup as genetic testing is the standard for diagnosing MTS."

When normal MMR IHC is found on a SN we recommend considering the following statement be addended to pathology reports:

"This neoplasm was found to retain expression of all four Mismatch Repair (MMR) gene proteins (MLH1, MSH2, MSH6 and PMS2). This finding does not provide evidence for a diagnosis of Muir-Torre syndrome (MTS). However for patients who have a personal or family history of colorectal cancer (CRC) it is recommended that they be referred to a genetic counselor due to the possibility of another hereditary CRC cancer syndrome."

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