MSH-6: extending the reliability of immunohistochemistry as a screening tool in Muir–Torre syndrome

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The subtype of Muir–Torre syndrome, allelic to hereditary nonpolyposis colorectal cancer is typically associated with germline mutations in the mismatch repair proteins *MSH-2* and/or *MLH-1*. More recently, mutation in an additional mismatch repair protein *MSH-6* has been documented in a patient with Muir–Torre syndrome. Given this, the aim of the present study was to ascertain the frequency of the same in unselected sebaceous gland neoplasms. Overall, we found that 59% of sebaceous neoplasms exhibited a mutation in at least one mismatch repair protein gene—a prevalence rate similar to that reported previously by others. Of interest, we found *MSH-6* to be the mismatch repair protein most commonly lost 17/41 (41%), followed by *MSH-2* 14/41 (34%) and *MLH-1* 18/41 (20%) and the positive predictive value of each were as follows: *MLH-1* 88%, *MSH-6* 67% and *MSH-2* 55%. The frequency of a *MSH-6* germline mutation in our cohort indicates that it is not a rare finding. Evidence indicating microsatellite stability in three of 17 patients with a clinical history indicative of Muir–Torre syndrome and a mutation in only *MSH-6* suggests that the phenotype of a germline *MSH-6* mutation differs from that of *MLH-1* and *MSH-2* mutations and further supports the use of immunohistochemistry as a screening tool in patients with Muir–Torre syndrome with an extended panel that includes *MSH-6*.

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Muir–Torre syndrome, an autosomal dominant inherited disorder, is characterized by the synchronous or metachronous occurrence of at least one sebaceous gland neoplasia such as an adenoma, epithelioma (sebaceoma) or carcinoma and an internal malignancy.^{1,2} The molecular alteration characteristic of neoplasms that develop in patients with Muir–Torre syndrome is microsatellite instability that is believed to be secondary to the coexistence of germline mutations in some mismatch repair protein genes.^{3,4} Several early reports have shown that the spectrum of internal malignancies seen in patients with Muir–Torre syndrome is identical to those observed in patients with hereditary nonpolyposis colorectal cancer or the Lynch syndrome and exhibits microsatellite instability and germline mutations similar to those encountered in hereditary nonpolyposis colorectal cancer.^{5–8}

These mutations typically involve the mismatch repair proteins *MLH-1* and *MSH-2* with the majority located in the latter.^{8,9}

More recently, a mutation in *MSH-6* has been documented in a patient with Muir–Torre syndrome.^{10,11} The rarity of a germline mutation in *MSH-6* in patients with Muir–Torre syndrome allelic to hereditary nonpolyposis colorectal cancer is somewhat unusual as mice carrying a null mutation in the same have been shown to develop a spectrum of tumors similar to that occurring in patients with Lynch syndrome.¹² Also, a germline mutation in one mismatch repair protein gene has been shown to be typically complemented by a second somatic mutation, often a deletion, in the contralateral allele, with consequent lack of expression of the gene.¹² Given that studies in eukaryotes indicate that *MSH-2* and *MSH-6* normally form a stable heterodimer, mutations in *MSH-2* should technically result in instability of the *MSH-2/MSH-6* heterodimer and consequentially absence of *MSH-6*.¹² However, despite the high prevalence of a germline mutation in *MSH-2*, the contribution of *MSH-6* in Muir–Torre syndrome is sporadic and hitherto confined to rare case reports.^{10,11,13} In the current study, we sought to

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ascertain the frequency of a germline mutation in *MSH-6* in unselected sebaceous gland neoplasias, using immunohistochemistry, a fairly inexpensive tool that allows both for rapid sample screening and precise identification of the mismatch repair protein involved in the disease process.

Materials and methods

The study was approved by the UMass Medical Center institutional review board (IRB # H-11842). Archival materials between 1996–2007 with a diagnosis of sebaceous adenomas and epitheliomas (sebaceomas) were retrieved from the pathology files of UMass Medical Center (Worcester, MA, USA). The frequency of occurrence of sebaceous hyperplasias in the general population precluded targeting these as diagnostic indicators of MTS for which reason they were excluded from the study. A total of 41 paraffin-embedded skin lesions from 36 patients were obtained: 31 sebaceous adenomas and 10 sebaceous epitheliomas (sebaceomas). The histologic sections of all cases were re-reviewed and the diagnoses confirmed. All patient data were de-identified.

Five-micrometer-thick sections were cut for immunohistochemical studies, which were performed using the standard techniques, heat-induced epitope retrieval buffer, and mouse antihuman monoclonal antibodies for detection of the mismatch repair proteins (*MLH-1* clone G168-15 1:50 Pharmingen, San Diego, CA, USA; *MSH-2* clone G219-1129 1:200 Pharmingen, San Diego, CA, USA and *MSH-6* clone = BC/44 1:50 Biocare, Concord, CA, USA). A second panel for immunohistochemical analysis of *PMS-2* was performed selectively on cases that demonstrated loss of only *MSH-6* (per courtesy of Genzyme). Analysis for microsatellite instability was selectively performed on cases with loss of only *MSH-6*, on matched pairs of tumor DNA and normal DNA (latter entailed using non-lesional tissue from prior unrelated biopsies on the same patient) using the Bethesda panel of microsatellite markers per courtesy of Genzyme as described previously.¹⁴

Tumors were considered as having no expression of either/all of the repair proteins when their nuclei showed no immunostaining, whereas a positive internal control was given by staining of the surrounding normal tissue.

Retrospective analyses of clinical histories for each of the cases were performed. A history suggestive of Muir-Torre syndrome was defined by the presence of either of the following: Keratoacanthoma, non-cutaneous malignancy or an adenomatous colonic polyp. An indeterminate clinical history was defined by lack or limited availability of follow-up information. Standard statistical methods were used to ascertain the sensitivity, specificity,

positive and negative predictive value of results obtained.

Results

Overall, 24/41 lesions (59% of total) (from 21 patients) exhibited a mutation in at least one mismatch repair protein gene. Three cases (34, 37, and 42) with at least one mutation in a mismatch repair protein gene were excluded from the study because of lack of availability of clinical history.

MLH-1

A total of 8/24 cases (33% of total) exhibited a mutation in *MLH-1* (Table 1, Figure 1). Of the seven of these with a clinical history suggestive of Muir-Torre syndrome, five had mutations in only *MSH-1*, one had an additional mutation in *MSH-6* and one had mutations in both *MSH-2* and *MSH-6* (see Table 2). The positive predictive value for *MLH-1* was 88%.

MSH-2

A total of 11/24 cases (46% of total) exhibited a mutation in *MSH-2* (Table 1, Figure 1). In six of these, all of which were also associated with mutations in *MSH-6*, the clinical history was suggestive of Muir-Torre syndrome (see Table 2). The positive predictive value for *MSH-2* was 55%.

MSH-6

A total of 15/24 cases (63% of total) exhibited a mutation in *MSH-6* (Table 1, Figure 1). Of the 10 of these with a clinical history suggestive of Muir-Torre syndrome, three had mutations in only *MSH-6*, five had an additional mutation in *MSH-1* and one had additional mutations in both *MSH-2* and *MSH-6* (see Table 2). The positive predictive value for *MSH-6* was 67%.

Table 1 Frequency of mismatch repair protein mutations (ascertained by immunohistochemistry) in unselected sebaceous neoplasias

Mutation	Case number	Proportion with a mutation
1	4,5,6,9,15,16,41,46	8/24 (33%)
2	1,2,6,11,12,14,17,21,25,44,45	11/24 (46%)
6	1,2,4,6,11,12,13,14,17,21,25, 26,36,44,45	15/24 (63%)
1&2	6	1/24 (4%)
1&6	4,6	2/24 (8%)
2&6	1,2,6,11,12,14,17,21,25,44,45	11/24 (46%)
1&2&6	6	1/24 (4%)

Figure 1 Representative examples of immunohistochemical staining of the DNA mismatch repair proteins *MLH-1*, *MSH-2* and *MSH-6* in sebaceous neoplasms. Case 5, Loss of *MLH-1*; Case 36, Loss of *MSH-6*; Case 4, Loss of *MLH-1* and *MSH-6*; Case 2, Loss of *MSH-2* and *MSH-6*; Case 6, Loss of *MLH-1*, *MSH-2* and *MSH-6*.

MLH-1* and *MSH-2

No case had mutations in only both of these mismatch repair proteins.

MLH-1* and *MSH-6

A total of 2/24 cases (8% of total) exhibited a mutation in *MLH-1* and *MSH-6* (see Table 1). Both of these had a clinical history suggestive of Muir-Torre syndrome (see Table 2). The positive predictive value for *MLH-1* and *MSH-6* together was 100%.

MSH-2* and *MSH-6

A total of 11/24 cases (46% of total) exhibited a mutation in *MSH-2* and *MSH-6* (see Table 1). In six of these, the clinical history was suggestive of Muir-Torre syndrome (see Table 2). The positive predictive value for *MSH-2* and *MSH-6* together was 55%.

MLH-1*, *MSH-2* and *MSH-6

Only one patient (case 6) was noted to have a mutation in all three markers by immunohistochemistry. This patient's clinical history was suggestive of Muir-Torre syndrome. The positive predictive value for a lesion with a mutation in all three markers by immunohistochemistry was 100%.

***PMS-2* and Microsatellite Analysis**

Of the three cases with loss of only *MSH-6*, two showed no evidence of a mutation in *PMS-2* (lack of lesional tissue precluded further studies on the thirds). Mutational analysis of these two cases indicated that they were microsatellite stable.

Discussion

Muir-Torre syndrome, characterized by the development of cutaneous neoplasms and internal malignancies.

Table 2 Clinical details of patients studied

Case (age in years, sex F/M)	Microscopic diagnosis	Mutation	Clinical history
1 (57, M)	Sebaceous adenoma	2,6	Negative
2 (75, F) ^a	Sebaceous adenoma	2,6	Negative
4 (53, M) ^a	Sebaceous epithelioma (sebaceoma)	1,6	Sebaceous carcinoma, adenocarcinoma of the colon, hepatocellular carcinoma, multiple tubulovillous adenomas
5 (53, M) ^a	Sebaceous epithelioma (sebaceoma)	1	Adenocarcinoma of the colon, Hepatocellular carcinoma, tubulovillous adenoma
6 (65, F) ^a	Sebaceous epithelioma (sebaceoma)	1,2,6	Tubulovillous adenoma
9 (70, M)	Sebaceous adenoma	1	Esophageal adenocarcinoma, bronchial adenocarcinoma, keratoacanthoma, multiple tubulovillous adenomas
11 (35, M)	Sebaceous adenoma	2,6	Negative
12 (35, M)	Sebaceous adenoma	2,6	Negative
13 (73, M) ^{b,c}	Sebaceous epithelioma (sebaceoma)	6	Adenocarcinoma of the colon, Tubulovillous adenoma
14 (70, F)	Sebaceous adenoma	2,6	Adenocarcinoma of the colon, multiple tubulovillous adenomas with high grade dysplasia
15 (79, F)	Sebaceous adenoma	1	Negative
16 (84, M)	Sebaceous epithelioma (sebaceoma)	1	Esophageal adenocarcinoma, squamous cell carcinoma of vocal cords
17 (90, M)	Sebaceous adenoma	2,6	Gastric adenocarcinoma
21 (62, M)	Sebaceous adenoma	2,6	Multiple tubulovillous adenomas
25 (82, M)	Sebaceous adenoma	2,6	Negative
26 (53, M) ^b	Sebaceous adenoma	6	Tubular adenoma
34 (84, M)	Sebaceous adenoma	2,6	Not available
36 (86, M) ^{a,b,c}	Sebaceous adenoma	6	Acute lymphoblastic leukemia, Keratoacanthoma
37 (58, M)	Sebaceous epithelioma (sebaceoma)	2	Not available
41 (35, F)	Sebaceous adenoma	1	Tubulovillous adenoma
42 (68, M)	Sebaceous adenoma	2,6	Not available
44 (63, M)	Sebaceous adenoma	2,6	Sebaceous carcinoma (× 2), keratoacanthoma, adenocarcinoma of the colon, multiple tubulovillous adenomas with high grade dysplasia
45 (63, M)	Sebaceous adenoma	2,6	Sebaceous carcinoma (× 2), keratoacanthoma, adenocarcinoma of the colon, multiple tubulovillous adenomas with high grade dysplasia
46 (81, F)	Sebaceous epithelioma (sebaceoma)	1	Adenocarcinoma of the breast

^aSee Figure 1.

^bCases with loss of only *MSH-6*.

^cCases with no *PMS-2* mutation and no microsatellite instability.

nancies, is typically inherited in an autosomal dominant manner with varied clinical manifestations.¹⁵ A subtype of Muir-Torre syndrome, associated with microsatellite instability and a defective DNA mismatch repair protein system, is closely linked to the development of hereditary nonpolyposis colorectal cancer or Lynch syndrome.^{16,17} A clinical diagnosis of this subtype can be made in a patient with concurrent or sequential documentation of a sebaceous neoplasm and a minimum of one internal malignancy or, multiple keratoacanthomas and a visceral malignancy and a positive family history.^{3,4,18} The significance of a negative history is perhaps mitigated by reports indicating that patients with Muir-Torre syndrome typically develop their first internal malignancy at an older age.¹⁸ The clinical relevance of identifying patients with an inherited cancer predisposition, necessitates the use of alternative laboratory-based screening methods to identify microsatellite instability or germline

mutations in the mismatch repair proteins. The former involves molecular analysis of tumoral tissue, while the latter, ascertained immunohistochemically, looks for loss vs maintenance of the more commonly implicated mismatch repair proteins.^{3,4,19,20} Several reports attest to the reproducibility of these two methods.^{13,21} Briefly, these include studies on patients with Muir-Torre syndrome showing that expression of both *MLH-1* and *MSH-2* by immunohistochemistry is highly predictive of microsatellite stability (93%) while loss of either is associated with microsatellite instability (100%).^{3,4,20} A good correlation between the two methods has been observed in patients with hereditary nonpolyposis colorectal cancer and also in patients with sporadic carcinomas of the colon and the endometrium.²² In one study of 19 cutaneous tumors from patients with Muir-Torre syndrome and a germline mutation in *MLH-1* or *MSH-2* by molecular analysis, immunohistochemistry

revealed loss of one or the other in 89% (17 of 19 patients).²⁰ This prevalence is similar to our findings which show 21 of 24 cases (88%) exhibiting a mutation in at least one of the three mismatch repair proteins. While the high sensitivity attests to the utility and reliability of immunohistochemistry as a screening test, we found the specificity of immunohistochemistry to be lower (70%). Perhaps the discrepancy is a function of a non-genetic or sporadic mutation.^{23,24} The pathogenesis of tumors found in Muir-Torre syndrome indicates that patients with a germline mutation ('first hit') in a mismatch repair protein, need a somatic mutation ('second hit') to inactivate the mismatch repair protein function at a cellular level. Thus, a sporadic mutation would result in a defective protein that, in the absence of the second hit, did not translate into having an impact of any clinical significance. Therefore, the high sensitivity may be due to the inclusion of false negatives.

Of significance, we found absence of *MSH-6* to be the most common anomaly (15/24 patients) in our cohort. Germline mutations in *MSH-2* and *MLH-1* account for the vast majority of the mutations in Muir-Torre syndrome kindreds and the contribution of mutations in other mismatch repair proteins such as *MSH-6* has hitherto been sporadic.^{25,26} *MSH-2* and *MLH-1* are human homologs of the eukaryotic *MutS* and *MutL* components of the *Escherichia coli* *MutHLS* mismatch repair system.^{12,27} The eukaryotic *MutS*-related protein involves heterodimeric complexes of which one is a complex of *MSH-2* and *MSH-6*.¹² Given that a germline mutation in one mismatch repair protein gene is complemented by a second, somatic mutation in the contralateral allele with consequent lack of expression of the gene, inactivating mutations in *MSH-2* should, and indeed has been shown to, in the presence of wild-type *MSH-6*, lead to simultaneous loss of *both* proteins.^{10,12} That 11 of our 15 patients (73%) demonstrating absence of *MSH-6* also demonstrated loss of *MSH-2* further supports this. However, three of the 15 (cases 13, 26 and 36) showed isolated loss of *only MSH-6* (see Table 2). Additional studies performed on two of these (lack of lesional tissue precluding studies from being performed on the third), indicated maintenance in expression of the mismatch repair protein *PMS-2* and microsatellite stability. Published literature to date indicates that *PMS-1* and *PMS-2* are rarely mutated in hereditary non-polyposis colorectal cancer kindreds, therefore the former finding is not entirely surprising.²⁵ While we found the latter to be somewhat intriguing, studies on murine models indicate that tumors in *MSH-6* mutant mice do not show evidence of microsatellite instability.¹² Of interest, the spectrum of tumors, originating in the gastrointestinal or hematopoietic systems, developing in these *MSH-6* mutant mice were similar to those seen in our two cases with isolated loss of *MSH-6*.¹² The presence of a germline mutation in *MSH-6* in a Japanese hereditary

nonpolyposis colorectal cancer family which did not meet the Amsterdam criteria has led to the suggestion that the phenotype of a germline *MSH-6* mutation differs from that of *MLH-1* and *MSH-2*.²⁸ The ability of *MSH-6* to correct base-base mismatches and single nucleotide deletion loops, but not the larger deletion loops may help explain the weaker phenotype associated with *MSH-6* mutations.^{29,30} Further in support of this, patients with early onset colorectal cancer and germline mutations in *MSH-6*, have also been shown not to be associated with classical microsatellite instability.^{29,30}

While identification of mismatch repair protein genes associated with Muir-Torre syndrome makes presymptomatic diagnosis a reality, the presence of multiple genes and the heterogeneity of mutations present challenges to the development of diagnostic tests for this disease. Our data on unselected sebaceous neoplasias reiterates the prevalence of a germline mutation in the commonly implicated mismatch repair protein genes *MLH-1* and *MSH-2*. We also show that a germline mutation in *MSH-6* is not entirely uncommon and is likely to be missed by a standard (Bethesda) panel with dinucleotide markers. While extending the panel by inclusion of mononucleotide markers will increase the sensitivity of microsatellite instability analysis and minimize the chances of missing a germline mutation in *MSH-6*, this will be associated with an increase in costs incurred on mutation analysis. Thus, our findings justify the use of an expanded panel that includes *MSH-6* and reinforce the utility of immunohistochemistry as a screening tool.

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