Identification of Muir–Torre Syndrome among Patients with Sebaceous Tumors and Keratoacanthomas

Role of Clinical Features, Microsatellite Instability, and Immunohistochemistry

Giovanni Ponti, M.D.1
Lorena Losi, M.D.2
Carmela Di Gregorio, M.D.3
Luca Roncucci, M.D.1
Monica Pedroni, Ph.D.1
Alessandra Scarselli, B.Sc.1
Piero Benatti, M.D.1
Stefania Seidenari, M.D.4
Giovanni Pellacani, M.D.4
Luigi Lembo, M.D.4
Giuseppina Rossi, M.D.1
Massimiliano Marino, Ph.D.1
Emanuela Lucci-Cordisco, Ph.D.5
Maurizio Ponz de Leon, M.D.1

1 Division of Internal Medicine, University of Modena and Reggio Emilia, Modena, Italy.
2 Department of Pathology, University of Modena and Reggio Emilia, Modena, Italy.
3 Division of Pathology, Carpi General Hospital, Carpi, Modena, Italy.
4 Division of Dermatology, Department of Internal Medicine, University of Modena and Reggio Emilia, Modena, Italy.
5 Department of Medical Genetics, Catholic University, Rome, Italy.

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Address for reprints: Maurizio Ponz de Leon, M.D., Dipartimento di Medicine e Specialità Mediche, Via del Pozzo 71, Polyclinico, 41100 Modena, Italy; Fax: (011) 39 059 422958; E-mail: deleon@unimo.it

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BACKGROUND. The Muir–Torre syndrome (MTS) is an autosomal-dominant genodermatosis characterized by the presence of sebaceous gland tumors, with or without keratoacanthomas, associated with visceral malignancies. A subset of patients with MTS is considered a variant of the hereditary nonpolyposis colorectal carcinoma, which is caused by mutations in mismatch-repair genes. The objective of the current study was to evaluate whether a combined clinical, immunohistochemical, and biomolecular approach could be useful for the identification of Muir–Torre syndrome among patients with a diagnosis of sebaceous tumors and keratoacanthomas.

METHODS. The authors collected sebaceous skin lesions and keratoacanthomas recorded in the files of the Pathology Department of the University of Modena during the period 1986–2000. Through interviews and examination of clinical charts, family trees were drawn for 120 patients who were affected by these skin lesions.

RESULTS. Seven patients also were affected by gastrointestinal tumors, thus meeting the clinical criteria for the diagnosis of MTS. In the MTS families, a wide phenotypic variability was evident, both in the spectrum of visceral tumors and in the type of skin lesions. Microsatellite instability was found in five MTS patients: These patients showed concordance with immunohistochemical analysis; moreover, a constitutional mutation in the MSH2 gene was found in 1 patient. Lack of expression of MSH2/MSH6 or MLH1 proteins was evident in the skin lesions and in the associated internal malignancies of 3 patients and 2 patients with MTS, respectively.

CONCLUSIONS. The clinical, biomolecular, and immunohistochemical characterization of sebaceous skin lesions and keratoacanthomas may be used as screening for the identification of families at risk of MTS, a disease that is difficult to recognize and diagnose. Cancer 2005;103:1018–25. © 2005 American Cancer Society.

KEYWORDS: cancer, gene, hereditary nonpolyposis colorectal carcinoma, skin lesions.

Muir–Torre syndrome (MTS) is a genodermatosis with an autosomal-dominant mode of inheritance that is characterized by the association of sebaceous skin tumors, with or without keratoacanthomas (KAs), and internal malignancies.1 Sebaceous tumors included in this syndrome, which is relatively uncommon in the general population, are sebaceous adenoma and epithelioma, basal cell epithelioma with sebaceous differentiation, and sebaceous carcinoma; in addition, sebaceous hyperplasia and other benign skin lesions often are associated with MTS. Skin lesions may be the initial sign of the
The diagnosis of MTS is relevant, because affected patients are at increased risk of multiple primary malignancies. The neoplasms described above account for 90% of all malignancies found in the syndrome. Furthermore, it was found that nearly 50% of patients with MTS had ≥ 2 visceral malignancies, which frequently show a less aggressive clinical behavior, as also reported for sebaceous tumors with respect to their sporadic counterparts. Finally, because MTS is inherited as an autosomal-dominant trait, the identification of one patient with the disease warrants the accurate evaluation of all family members at risk.

The observation of rare cutaneous lesions in patients affected by hereditary nonpolyposis colorectal carcinoma (HNPCC) led Lynch et al. to hypothesize in 1981 that MTS may be a variant of HNPCC; this intuition subsequently was confirmed by the identification of DNA mismatch-repair (MMR) gene mutations—the same mutations involved in the pathogenesis of HNPCC—also in many patients with MTS.

MMR gene mutations cause microsatellite instability (MSI), which is evident in > 95% of HNPCC-associated neoplasms. This biomolecular alteration also has been observed in a subset of sebaceous lesions and KAs from patients affected by MTS, thus demonstrating a possible common pathogenesis of colorectal and cutaneous lesions. Recently, several studies showed the efficacy of immunohistochemical analysis for the identification of MMR gene alterations in cutaneous sebaceous lesions in patients with MTS and the usefulness of this technique for screening purposes.

For the current work, we collected sebaceous skin lesions and KAs recorded during the period 1986–2000 from the files of the Pathology Department of the University of Modena. The specific objective of our study was to evaluate whether a combined clinical, immunohistochemical, and biomolecular screening could be useful for the identification of MTS among patients with a diagnosis of sebaceous tumors and KAs. The criterion for the diagnosis of MTS was the presence of at least one sebaceous tumor or KA in association with at least one visceral malignancy.

**MATERIALS AND METHODS**

**Patients and Tumor Samples**

From 1986 through 2000, a total of 152 skin lesions (embedded in paraffin) from 120 patients were selected by consulting the archives of the Pathology Department of the University of Modena. Thirty-three lesions were sebaceous adenomas, 11 were sebaceous epitheliomas, 15 were sebaceous carcinomas, and 93 were KAs (we have collected multiple KAs and/or early-onset KAs). A tumor was classified as sebaceous when well defined, enlarged, sebaceous lobules were present that comprised fully mature sebocytes, frequently demonstrating an attachment to the epidermis with epidermal thinning. Sebaceous adenoma is composed of variably sized and incompletely differentiated sebaceous lobules that contain “basalike” cells at the periphery and mature sebaceous cells (with characteristic cytoplasmic vacuoles) toward the center. A diagnosis of sebaceous epithelioma (which is synonymous of sebaceoma) is established when more than half of the cells are undifferentiated basaloid elements with rare nuclear atypia but with significant aggregates of sebaceous and transitional cells. Sebaceous epitheliomas are not organized in well demarcated sebaceous lobules and are diagnosed only after the exclusion of basal cell carcinoma. In sebaceous carcinomas, variably atypical, polyhedral cells are present that are separated from one another by fibrovascular stroma, sometimes with spread of pagetoid epithelial cells into the epidermis. KA is a distinctive, benign skin tumor that is characterized by an initial, rapid growth during several months followed by gradual involution; the most important microscopic feature is the architecture of the lesions on a cross section, which shows overhanging edges, keratin-filled craters, and a hemispheric shape. In some patients, KAs are difficult to distinguish from squamous cell carcinomas only on the basis of histology.

**Family History**

Detailed family histories were collected for each patient by interviewing the patients and/or their relatives. Verification of cancer occurrence among family members could be obtained in the majority of patients through clinical charts, pathologic records, or death certificates. Through the reconstruction of the genealogic tree, we identified seven patients with MTS.

**Microsatellite Analysis**

For microsatellite analysis, DNA was extracted from microdissected neoplastic and paired normal mucosa specimens of dermatologic lesions and visceral malignancies according to standard procedure. MSI was evaluated with 5 microsatellite markers (BAT25, BAT26, BAT40, D2S123, and D5S346) using a fluorescence-based polymerase chain reaction (PCR) method. DNA samples from normal tissues and tumor tissues were amplified in a 10-μL volume containing...
30–50 ng of DNA; 5 ng of dye-labeled forward and unlabeled reverse primers; 200 μM each of deoxyguanine triphosphate, deoxythymidine triphosphate, deoxyadenosine triphosphate, and deoxycytidine triphosphate; 1.5 mM MgCl2; 50 mM KCl; 10 mM Tris, pH 8.3; and 0.3 units of Taq polymerase. All samples were run on a CEQ 8000 sequencer and were analyzed using a fragment analysis system (Beckman Coulter). MSI-positive tumors were defined as tumors in which instability was detected in at least two microsatellite loci.15

**Immunohistochemistry**

Immunohistochemical analysis of MSH6, *MLH1*, and *MSH2* proteins were carried out on paraffin-embedded tumor samples. Immunoperoxidase staining using diaminobenzidine as a chromogen was run with the NEX-ES Automatic Staining System (Ventana, Strasbourg, France). The mouse monoclonal antibodies used were anti-MSH6 (clone 44; Transduction Laboratories) at 1:40 dilution, anti-*MLH1* (G168-15; Pharmingen) at 1:40 dilution, and anti-*MSH2* (G129-1129; Pharmingen) at 1:40 dilution. Nuclei were counterstained with hematoxylin. Adjacent normal tissues from each sample served as positive controls.

**Mutational Analysis**

Germline mutations in *hMSH2*, *hMLH1*, and *hMSH6* were studied by single-strand conformation polymorphism (SSCP) on DNA derived from blood leukocytes. Samples that showed an altered SSCP mobility pattern were sequenced by means of the Sequenase® PCR product sequencing kit (Amersham Life Science, Buckinghamshire, United Kingdom) using a Beckman sequencer (model CEQ 8000).

**RESULTS**

Clinical characterization and family history of all patients (*n* = 120 patients) was obtained. Seven patients had a history of intestinal or other visceral carcinomas; these patients fulfilled the criteria for a clinical diagnosis of MTS (four males and three females). MTS was diagnosed in 5 of 36 patients who had sebaceous neoplasms (13.9%) and in 2 of 84 patients with KAs (2.4%). All of the remaining 113 patients were categorized with sporadic cutaneous lesions. In the MTS group, there were 34 skin lesions, including 15 sebaceous adenomas, 6 sebaceous epitheliomas, 7 sebaceous carcinomas, and 6 KAs. The average age at onset of the first skin malignancy was 54 years (range, 34–86 years) compared with 68.2 years (range, 37–91 years) in the sporadic group (Table 1). In patients with MTS, skin lesions often were multiple, synchronous, or metachronous, and they were located preferentially in the head and neck. Histologically, most of the lesions were sebaceous tumors rather than sporadic neoplasms, which more often were KAs.

In four patients with MTS, sebaceous lesions occurred as the first neoplasm; in the remaining three patients, these lesions developed after an internal malignancy. Among the seven MTS probands, five colorectal carcinomas and two gastric carcinomas were found. The average age at onset of the first visceral malignancy was 56.3 years. In addition to skin and visceral malignancies, the tumor spectrum included melanoma, one thyroid tumor, two carcinomas of the renal pelvis, and one breast tumor. Finally, in two MTS probands, psoriatic arthropathy was diagnosed. Among first-degree relatives, adenomatous polyps; colonic, gastric, bladder, and renal pelvis carcinomas; non-Hodgkin lymphoma; and laryngeal tumors were detected (Table 2).

For all patients, we examined sebaceous skin tumors and KAs for microsatellite status and immunohistochemical expression of the *MLH1*, *MSH2*, and *MSH6* proteins. The skin lesions showed MSI and lack of expression of at least one protein was evident in the head and neck. In patients with apparently sporadic lesions, these lesions developed after an internal malignancy. Among the seven MTS probands, five colorectal carcinomas and two gastric carcinomas were found. The average age at onset of the first visceral malignancy was 56.3 years. In addition to skin and visceral malignancies, the tumor spectrum included melanoma, one thyroid tumor, two carcinomas of the renal pelvis, and one breast tumor. Finally, in two MTS probands, psoriatic arthropathy was diagnosed. Among first-degree relatives, adenomatous polyps; colonic, gastric, bladder, and renal pelvis carcinomas; non-Hodgkin lymphoma; and laryngeal tumors were detected (Table 2).

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MTS group</th>
<th>Sporadic group</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>7</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>4/3</td>
<td>71/42</td>
<td>0.76&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age in yrs at diagnosis (mean ± SD)</td>
<td>54.0 ± 18.8</td>
<td>68.2 ± 11.7</td>
<td>0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. of lesions</td>
<td>34</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>Lesions per patient (mean ± SD)</td>
<td>4.9 ± 4.6</td>
<td>1.0 ± 0.2</td>
<td>0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Site of lesions (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head</td>
<td>86</td>
<td>39</td>
<td>&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chest</td>
<td>13</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Arms/legs</td>
<td>1</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Histology (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sebaceous carcinoma</td>
<td>21</td>
<td>7</td>
<td>&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sebaceous adenoma</td>
<td>43</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Sebaceous epithelioma</td>
<td>18</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Keratoacanthoma</td>
<td>18</td>
<td>74</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Statistical analyses were performed using chi-square tests.
<sup>b</sup> Statistical analyses were performed using t tests.

MTS: Muir-Torre syndrome; SD: standard deviation.
of the MMR proteins was evident in the same patient. In 1 patient (Patient 2, Table 3), a constitutional mutation (2133 C→T) in exon 13 of the MSH2 gene could be detected. Two more patients (Patients 3 and 5) still are under investigation; however, to date, no constitutional abnormalities of the main DNA MMR genes have been detected. One patient (Patient 1) denied to provide consent for blood sampling. Finally, as shown in Table 3, 3 patients were deceased at the time of our investigation. The 118 sporadic lesions all were MSI-negative and showed normal expression of all MMR proteins.

**DISCUSSION**

The results of the current study can be summarized as follows: First, on a clinical basis, 7 of 120 patients with sebaceous lesions or KAs were identified with MTS (5.8%); thus, an accurate personal and family history

**TABLE 2**

Clinical Features of the Seven Patients with Muir-Torre Syndrome

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gender</th>
<th>Age at first skin lesion (yrs)</th>
<th>No. of skin lesions</th>
<th>Skin lesion histology</th>
<th>Disease site</th>
<th>Visceral tumor in the proband (age in yrs)</th>
<th>Other tumors in the proband (age in yrs)</th>
<th>Tumors in the family (age in yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>51</td>
<td>15</td>
<td>SEB CA</td>
<td>Head</td>
<td>Colon dx (49)</td>
<td>Colon (38), colon (59), brain (60), lung (65)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>34</td>
<td>4</td>
<td>SEB AD</td>
<td>Chest</td>
<td>Colon dx (35)</td>
<td>Bladder (65), breast (60)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Female</td>
<td>37</td>
<td>2</td>
<td>SEB AD</td>
<td>Head</td>
<td>Colon (32)</td>
<td>Thyroid (37)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>47</td>
<td>5</td>
<td>SEB AD</td>
<td>Arms</td>
<td>Multiple adenomas</td>
<td>Bladder (57), lymphoma (68)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>50</td>
<td>2</td>
<td>SEB AD</td>
<td>Head</td>
<td>Colon (51)</td>
<td>Kidney (45)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>73</td>
<td>4</td>
<td>SEB CA</td>
<td>Neck</td>
<td>Colon (75)</td>
<td>Lymphoma (22), lymphoma (46), larynx (54)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Female</td>
<td>86</td>
<td>2</td>
<td>KA</td>
<td>Head</td>
<td>Gastric (89)</td>
<td>Breast (45) Gastric (72)</td>
<td></td>
</tr>
</tbody>
</table>

Dx: diagnosis; SEB CA: sebaceous carcinoma; SEB AD: sebaceous adenoma; KA: keratoacanthoma.

**FIGURE 1.** Example of a Muir–Torre syndrome-positive analysis in skin and visceral tumors in the same patient.
Sebaceous tumors, which are relatively rare in the general population, can be found as sporadic neoplasms or in the tumor spectrum of MTS. Their clinical characterization led us to realize that these lesions in MTS usually were multiple, recurrent, showed an early onset, and occurred in 60% of patients before visceral malignancies. Moreover, sebaceous adenomas and carcinomas in particular were frequent, representing 36% and 23%, respectively, of all sebaceous lesions detected in MTS.

In the current study, biomolecular and immunohistochemical characterization showed MSI and lack of expression of MSH2/MSH6 proteins in 3 patients (Patients 1, 2, and 5) in the MTS group in both skin lesions and visceral malignancies; in 1 of those patients, a constitutional mutation in the MSH2 gene was detected. Moreover, we found MSI and lack of expression of MLH1 protein in 2 other patients (Patients 3 and 7). Along with germline mutations, lack of expression of MLH1 can be due to other causes, including hypermethylation of the MLH1-promoter region.

MSI is the “hallmark” of HNPCC-associated tumors, although it occurs in 5–15% of sporadic carcinomas, including skin malignancies. MSI was described previously in MTS malignancies; thus, Kruse et al. found MSI in all MTS visceral and cuta-
neous lesions. Moreover, in other studies,\textsuperscript{23,6} MSI was detected in 46% and 69% of MTS-associated tumors, respectively. In the current study, MSI was found in 71% of MTS lesions. In microsatellite-stable neoplasms, other molecular genetic mechanisms may lead to the MTS phenotype through an MMR-independent pathway. Therefore, on the basis of these clinical and biomolecular differences, it is possible to identify two variants of MTS: The first, which is MSI-positive, shares its pathophysiology and genetic base with HNPCC and is characterized by early onset colorectal carcinoma and a strong family history of cancer; the second, MSI-negative, which includes late-onset cancers and a less pronounced family history, may be linked to other molecular mechanisms and to their interaction with unknown environmental factors. In the latter form, other\textsuperscript{27} MLH1 variants or MMR minor genes, such as PMS2, may be involved.\textsuperscript{28} Alternatively, inactivation of the MMR system by 2 somatic mutations or by\textit{MLH1} promoter hypermethylation may be hypothesized.

The significance of benign skin lesions—such as

\begin{table}[h]
\centering
\caption{Skin and Visceral Tumors in Patients with Muir-Torre Syndrome, including Tumor Site, Histologic Diagnosis, and Age at Onset: Results of Immunohistochemical Staining with Antibodies Against MSH2, MSH6, and MLH1 Proteins; Microsatellite Status; and Results of Mutation Analysis of Mismatch-Repair Genes}
\begin{tabular}{|c|c|c|c|c|}
\hline
Tumor site & Histology (age in yrs) & IHC analysis (loss of) & MS status & Mutation analysis \\
\hline
Patient 1 & Head & Sebaceous carcinoma (51) & MSH2/MSH6 & MSI & Test refused \\
& Head & Sebaceous adenoma (53) & MSH2/MSH6 & MSI & \\
& Right colon & Adc (49) & MSH2/MSH6 & MSI & \\
Patient 2 & Neck & Keratoacanthoma (34) & MSH2/MSH6 & MSI & \textit{MSH2} mutation \\
& Chest & Keratoacanthoma (44) & MSH2/MSH6 & MSI & \\
& Right Colon & Adc (35) & MSH2/MSH6 & MSI & \\
Patient 3 & Head & Sebaceous adenoma (37) & MLH1 & MSI & Under investigation \\
& Right colon & Adc (32) & MLH1 & MSI & \\
& Thyroid & Adc (37) & MLH1 & MSI & \\
Patient 4 & Back & Sebaceous adenoma (47) & None & MSS & Not tested (proband deceased) \\
& Back & Sebaceous adenoma (54) & None & MSS & \\
& Gastric & Adc (55) & None & MSS & \\
Patient 5 & Head & Sebaceous adenoma (50) & MSH2/MSH6 & MSI & Under investigation \\
& Kidney & Adc (57) & MSH2/MSH6 & MSI & \\
Patient 6 & Head & Sebaceous carcinoma (73) & None & MSS & Not tested (proband deceased) \\
& Rectum & Adc (75) & None & MSS & Not done \\
Patient 7 & Head & Keratoacanthoma (86) & MLH1 & MSI & Not tested (proband deceased) \\
& Gastric & Adc (88) & MLH1 & MSI & \\
\hline
\end{tabular}
\end{table}

IHC: immunohistochemical analysis; MS status: microsatellite status; MSI: microsatellite instability; MSS: microsatellite stability; Adc: adenocarcinoma; right colon: from cecum to splenic flexure.
sebaceous hyperplasia, sebaceous and trichilemmal cysts, and psoriatic skin lesions—diagnosed in patients with MTS should be investigated further. There is evidence of MSI in these benign lesions both in patients with HNPCC and in patients with MTS. These data suggest that the detection of MSI in common benign skin lesions of patients who belong to MTS families could have clinical implications and may be used in clinical practice to screen individuals or families at risk. Skin lesions also can be useful for the possible detection of MSI in family members at risk who have not yet developed cancer.

In general terms, the sebaceous tumors characteristic of MTS can serve as premonitory physical stigmata for an underlying cancer predisposition; in our opinion, every patient with a sebaceous tumor (or multiple KAs and/or early-onset KAs) should be evaluated further and, if necessary, put under a strict cancer surveillance. The clinical-biomolecular approach to recognizing patients who may be affected by the MMR-deficient type of MTS includes the examination of tumor tissues for MSI or with immunohistochemical analysis: In fact, both techniques are extremely useful as the initial procedures for screening purposes. This strategy is illustrated in Figure 4.

In conclusion, sebaceous lesions are rare in the general population, and their diagnosis warrants the search for associated internal malignancies. The peculiarity of these skin lesions and their biomolecular and immunohistochemical characterization may be used in screening to identify families with suspected Muir–Torre syndrome. This may allow physicians to propose timely and effective clinical-endoscopic schedules of surveillance.


REFERENCES


