Clinical and Molecular Features of Skin Malignancies in Muir-Torre Syndrome

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Abstract: Background: We investigated the mutational landscape of skin tumors in patients with Muir-Torre Syndrome (MTS) a hereditary autosomal dominant mismatch repair disorder of increased cancer susceptibility, and examined mutations other than in the DNA mismatch repair (MMR) genes. Methods: This retrospective single-center case series included seven patients with the diagnosis of Muir-Torre Syndrome with precise medical history and family history. Mutational analysis of tumor samples Formalin-fixed paraffin-embedded tissue blocks of skin lesions associated with Muir-Torre Syndrome were used for further analysis. All skin tumors were analyzed with the Oncomine Comprehensive Assay v3 (Life Technologies), which includes 161 of the most relevant cancer driver genes. Results: Eleven skin neoplasms (nine sebaceous tumors, one melanoma, one cutaneous squamous cell carcinoma) were diagnosed in seven patients. In two patients, visceral malignancies preceded the diagnosis of the skin tumors and one patient was diagnosed with a visceral malignancy after a sebaceous tumor. History of familial cancer of Lynch Syndrome (LS) was reported in three patients. The most frequently detected mutation was in the MSH2 gene, followed by mutations in the NOTCH1/2 and TP53 gene. Conclusion, this study provides a molecular analysis of Muir-Torre Syndrome associated and non-associated skin tumors in patients with Muir-Torre Syndrome. Patients with sebaceous lesions should undergo microsatellite instability analysis and accurate evaluation of personal and family history to detect a possible Muir-Torre syndrome. As secondary malignancies may appear years after the first occurrence of sebaceous tumors, lifelong screening is mandatory.

Keywords: Muir-Torre Syndrome; family history; next-generation sequencing; molecular analysis

1. Introduction

Muir-Torre Syndrome (MTS) represents a distinct variant of Lynch Syndrome (LS), previously referred to as hereditary nonpolyposis colorectal cancer (HNPCC) [1]. It is characterized as an inherited autosomal dominant disorder with early cancer susceptibility, which manifests with sebaceous adenomas or carcinomas, keratoacanthomas and tumors of visceral organs. Among visceral tumors, colorectal cancer is the most common with the prevalence of 0.5–5% [2]. Further associated malignancies are endometrial carcinoma, gastric cancer, tumors of the small bowel, transitional cell carcinoma (ureter and renal pelvis), ovarian and pancreatic tumors [3].

From a genetic perspective, all subtypes of LS underlie a microsatellite instability. This leads to an increased likelihood of the occurrence of mutations in genes, which results in oncogenic propensity [4,5]. Distinct genetic mutations are described for the MTS, most of which derive from single case studies with particular attention to mismatch repair gene
mutations. So far, genetic alterations associated with MTS have been described for the DNA mismatch repair (MMR) genes \textit{MLH1}, \textit{MSH2}, \textit{MSH6}, \textit{PMS2}, \textit{MLH3} and the non-MMR gene \textit{EBCAM}, which epigenetically silences the closely linked \textit{MSH2} gene [6]. Patients with \textit{MLH1} and \textit{MSH2} gene mutations tend to have a higher risk for malignant tumors compared to patients with a \textit{MSH6} gene mutation [7–9]. In 90% of LS patients, \textit{MLH1} and \textit{MSH2} mutation are responsible for malignant tumors with a ratio of 1:1. Only 7–10% of the LS patients develop malignancy due to \textit{MSH6} mutation, and 5% due to \textit{PMS2} mutation. In contrast, 90% of the mutations in MTS occur in the \textit{MSH2} gene, which has also shown to be responsible for more extracolonic and multiple primary cancers as compared to the \textit{MLH1} gene [10].

We aimed to detect and compare other responsible genetic alterations in MTS associated and non-associated skin tumors in our MTS patients cohort, as sebaceous tumors with locoregional and distant metastasis of up to 14% to 25% have an aggressive potential and targeted therapy in sebaceous carcinoma has not been given much thought [11]. To address this, we collected clinical information, family history and performed next-generation sequencing (NGS)-based multiple-biomarker assay on sebaceous and non-sebaceous tumors of seven MTS patients to identify responsible mutations and to compare them to the reported data.

2. Materials and Methods

2.1. Introduction

The database (KISIM) from the University Hospital of Zurich was searched for patients with the Diagnosis of Muir-Torre Syndrome. Patients who fulfilled the diagnostic criteria from the revised Bethesda Guidelines were included into analysis [12]. Retrospective data of seven patients, including their medical and family history, were analyzed and next-generation sequencing was performed on the sebaceous and non-sebaceous skin tumors of these patients.

2.2. Mutational Analysis of Tumor Samples

Formalin-fixed paraffin-embedded (FFPE) tissue blocks of skin lesions associated and non-associated with MTS were used for further analysis. Representative tumor areas were marked by a dermatopathologist. Punch biopsies of 0.4 mm in diameter were taken from the corresponding areas of the FFPE blocks. DNA/RNA isolation was performed using the automated Maxwell system (Promega, Madison, WI, USA) according to the manufacturer’s manual. Library preparation was performed using 20 ng of DNA/RNA and the Oncomine Comprehensive Assay v3 (Life Technologies, Waltham, MA, USA). The Oncomine Comprehensive v3 assay was chosen as to that date it was the largest gene panel offered at our institution. The targeted genes are displayed in Table S1. It includes the most important oncogenic drivers and tumor suppressors across various types of cancers. The libraries were subsequently sequenced on the S5 sequencer (Life Technologies, Waltham, MA, USA). Data analysis was conducted using the Ion Reporter Software version 5.10 (Life Technologies, Waltham, MA, USA).

The study was conducted at the University Hospital of Zurich in Switzerland. The study protocol was approved by the institutional and regional ethical committee (KEK 2018-01565).

3. Results

3.1. Demographic Data

Seven patients were included in the analysis. The median age at diagnosis of the first MTS associated malignancy was 61 years (range 45–75 years). Five of the patients were male, two were female (Table 1).
Table 1. Demographic data.

| Total Number of Patients | 7 |
|--------------------------|---|
| Male                     | 5 |
| Female                   | 2 |
| Age (range)              | 61 (45–75) |
| Localization             |   |
| Non-head and neck        | 7 |
| Head and neck            | 4 |
| Tumor type (total number)| 11|
| Sebaceous adenoma        | 2 |
| Sebaceoma                | 1 |
| Solid-cystic sebaceous neoplasm | 1 |
| Sebaceous carcinoma      | 4 |
| Tumor with sebaceous differentiation | 1 |
| Squamous cell carcinoma  | 1 |
| Melanoma                 | 1 |

3.2. Medical History

In two patients, visceral malignancies preceded the diagnosis of the skin tumors. In our cohort the most frequent visceral malignancies were urogenital tumors (two patients; 22%). One patient was diagnosed with a small lymphocytic lymphoma due to the following staging after diagnosis of a sebaceous adenoma.

3.3. Sebaceous Lesions and Other Cutaneous Tumors

Eleven skin neoplasms were diagnosed in seven patients. Seven (64%) were located outside the head and neck region and four (36%) in the head and neck region. A photograph and histological presentation of a sebaceoma of patient 2 is illustrated in Figures 1 and 2.

Figure 1. Abdominal sebaceoma patient 2. Faintly pinkish to skin-colored papule with dermoscopically yellowish ovoid areas and arborizing vessels.

Figure 2. Histology of a sebaceoma abdomen patient 2 (hematoxylin and eosin stain). Circumscribed basaloid tumor with sebaceous differentiation and mitosis.
One patient was diagnosed with both—MTS associated and non-associated skin tumors. Additionally to a sebaceous tumors, he was diagnosed with a squamous cell carcinoma (SCC) and melanoma.

Further data are displayed in Tables 1 and 2.

### Table 2. Patients data.

| Patient | Sebaceous Tumor (Age at Diagnosis) | Visceral Tumor (Age at Diagnosis) | Family History | Localization |
|---------|-----------------------------------|-----------------------------------|----------------|--------------|
| 1       | 1. Sebaceous carcinoma (63)  
2. Tumor with sebaceous differentiation (63)  
3. Sebaceous adenoma (67) | Caecal carcinoma (44)  
Bladder carcinoma (63)  
Adenocarcinoma sigma (67) | Sister: Osteosarcoma  
Uncle (mother’s side): Lung- and brain tumor  
Sisters’ son: Brain tumor | 1. Thorax  
2. Forehead  
3. Back |
| 2       | Sebaceoma (55) | - | Brother: Cutaneous lymphoma  
Mother: Dermatofibrosarcoma  
Father and grandfather: Colorectal carcinoma | Abdomen |
| 3       | Sebaceous carcinoma (63) | Renal cell carcinoma (58) | - | Hip |
| 4       | Solid-cystic sebaceous tumor (62) | - | - | Back |
| 5       | Sebaceous carcinoma (65) | - | - | Back |
| 6       | Sebaceous carcinoma (75) | - | - | Back |
| 7       | Sebaceous adenoma (72) | Small lymphocytic lymphoma | Brother: Lung tumor | Cheek |

### 3.4. Family History

History of familial cancer of LS was reported in three patients. Seven of the tumors were associated with MTS and one tumor had no association with MTS.

The pedigree of the affected families is illustrated in Figure 3.

### 3.5. Tumor Mutations

The most frequently detected mutation in all analyzed skin tumors was in the MSH2 gene and was found in six patients (86%). The only other MMR gene mutation was MLH1 and was only present in one patient (14%). The other most frequent mutations were in the NOTCH1, NOTCH2 and in the TP53 genes and were found in three (43%), two (29%) and two patients (29%), respectively.

The patient diagnosed with multiple sebaceous skin lesions (sebaceous carcinoma, sebaceous adenoma, tumor with sebaceous differentiation) additionally had a SCC and a melanoma in his history. All sebaceous lesions displayed NOTCH1 and NOTCH2 mutations. The tumor with sebaceous differentiation in this patient showed an MMR mutation in the MLH1 gene, but this was missing in the other sebaceous tumors. The melanoma and sebaceous carcinoma showed none of the above-mentioned mutations.

The detected mutations are summarized in Tables 3 and 4.
Figure 3. (a) Pedigree of patient 1 shows the autosomal dominant transmission of skin and visceral tumors; (b) patient 7 with MTS associated tumors in family members (c); patient 7 has a brother with a lung tumor; (d) Legend.

Table 3. Most frequent mutations.

| Mutated Gene | MSH2  | MLH1  | NOTCH1 | NOTCH2 | TP53  |
|--------------|-------|-------|--------|--------|-------|
| Total number (%) | 6 (86%) | 1 (14%) | 3 (43%) | 2 (29%) | 2 (29%) |
| Histological type |       |       |        |        |       |
| SC            | 4     | -     | 2      | 2      | 1     |
| SCS           | 1     | -     | 1      | -      | 1     |
| SE            | 1     | -     | -      | -      | -     |
| SA            | -     | 1     | -      | -      | -     |
| Localization  |       |       |        |        |       |
| Non-head and neck | 5     | 1     | 2      | 1      | 2     |
| Head and neck | 1     | -     | 1      | 1      | -     |

Abbreviations: SC, sebaceous carcinoma; SCS, solid-cystic sebaceous neoplasm; SE, sebaceoma; SA, sebaceous adenoma.
### Table 4. Patient’s collective and mutational burden.

| P | Sex (m/f) | Tumor Type (Tumor Cell Content) | Locus | Genes | % Frequency | Amino Acid Change | Coverage | Exon | Transcript | Coding | ACMG Class |
|---|----------|---------------------------------|-------|-------|-------------|------------------|----------|------|------------|--------|-------------|
| 1 | m        | SC (80%)                        | chr1:120462204 | NOTCH2 | 42 | p.(Arg1838*) | 2000 | 31 | NM_024408.3 | c.5512C>T | Pathogenic (class 5) |
|    |          | chr5:67591079 | PIK3R1 | 46 | p.(Glu558fs) | 1992 | 13 | NM_181523.2 | c.1674_1675del | Uncertain significance (class 3) |
|    |          | chr9:139404265 | NOTCH1 | 45 | p.(Cys963*) | 1985 | 18 | NM_017617.4 | c.2889C>A | Likely pathogenic (class 4) |
|    |          | chr9:139405616 | NOTCH1 | 44 | p.(Thr859fs) | 1923 | 16 | NM_017617.4 | c.2574_2575ins | Likely pathogenic (class 4) |
|    |          | TDS (70%) | chr1:120491661 | NOTCH2 | 41 | p.(Ser856fs) | 1623 | 16 | NM_024408.3 | c.2566_2567del | Likely pathogenic (class 4) |
|    |          | chr3:37090403 | MLH1 | 43 | p.(Trp666*) | 2000 | 18 | NM_000249.3 | c.1998G>A | Pathogenic (class 5) |
|    |          | chr3:47103763 | SETD2 | 40 | p.(Asp2064fs) | 1043 | 14 | NM_014159.6 | c.6181_6182del | Uncertain significance (class 3) |
|    |          | chr9:139397730 | NOTCH1 | 39 | p.(Gln1691*) | 810 | 27 | NM_017617.4 | c.5071C>T | Pathogenic (class 5) |
|    |          | chr10:123279674 | FGFR2 | 35 | p.(Pro253His) | 1677 | 7 | NM_000141.4 | c.758C>A | Likely pathogenic (class 4) |
|    |          | chr16:3646385 | SLX4 | 5 | p.(Gln565*) | 519 | 8 | NM_032444.3 | c.1693C>T | Pathogenic (class 5) |
|    |          | M (50%) | chr16:2135234 | TSC2 | 5 | p.(Gln1525*) | 366 | 36 | NM_000548.4 | c.4573C>T | Pathogenic (class 5) |
|    |          | SA (80%) | chr1:120464352 | NOTCH2 | 9 | p.(Gln1765fs) | 1939 | 29 | NM_024408.3 | c.5293delC | Likely pathogenic (class 4) |
|    |          | SCC (70%) | chr9:139417303 | NOTCH1 | 13 | p.(Pro247fs) | 1007 | 4 | NM_017617.4 | c.740_741ins | Likely pathogenic (class 4) |
|    |          | chr4:153249385 | FBXW7 | 22 | p.(Arg465Cys) | 2000 | 9 | NM_033632.3 | c.1393C>T | Likely pathogenic (class 4) |
|    |          | chr17:37687555 | CDK12 | 27 | p.(Gly1487*) | 2000 | 14 | NM_016507.3 | c.4459G>T | Uncertain significance (class 3) |
|    |          | chr17:37879658 | ERBB2 | 30 | p.(Arg678Gln) | 1999 | 17 | NM_004448.3 | c.2033G>A | Uncertain significance (class 3) |
| 2 | f        | SE (70%) | chr2:47698134 | MSH2 | 42 | p.(Asn566fs) | 1907 | 11 | NM_000251.2 | c.1697del | Pathogenic (class 5) |
|    |          | chr7:55241677 | EGFR | 16 | p.(Glu709Lys) | 1997 | 18 | NM_005228.4 | c.2125G>A | Uncertain significance (class 3) |
| 3 | m        | SC (80%) | chr2:47630386 | MSH2 | 59 | p.(Phe226fs) | 1835 | 1 | NM_000251.2 | c.62_63ins | Pathogenic (class 5) |
| 4 | m        | SCS (80%) | chr2:47637361 | MSH2 | 42 | p.(Tyr165*) | 1999 | 3 | NM_000251.2 | c.495T>A | Pathogenic (class 5) |
|    |          | chr3:178952085 | PIK3CA | 36 | p.(His1047Arg) | 1658 | 21 | NM_006218.3 | c.3140A>G | Pathogenic (class 5) |
|    |          | chr9:139418362 | NOTCH1 | 25 | p.(Asn708fs) | 4684 | 3 | NM_017617.4 | c.209_210ins | Likely pathogenic (class 4) |
### Table 4. Cont.

| P   | Sex (m/f) | Tumor Type (Tumor Cell Content) | Locus   | Genes     | % Frequency | Amino Acid Change | Coverage | Exon | Transcript | Coding       | ACMG Class             |
|-----|-----------|---------------------------------|---------|-----------|-------------|-------------------|----------|------|-------------|---------------|------------------------|
| 5   | f         | SC (60%)                         | chr2:47693918 | MSH2     | 42          | p.(Gln545*)      | 144      | 10   | NM_000251.2 | c.1632_1633del    | Pathogenic (class 5)   |
| 6   | m         | SC (70%)                         | chr2:47693799 | MSH2     | 36          | p.(Pro507fs)     | 2283     | 10   | NM_000251.2 | c.1518_1519ins   | Pathogenic (class 5)   |
|     |           |                                  | chr2:47693804 | MSH2     | 72          | p.(Pro507fs)     | 1200     | 10   | NM_000251.2 | c.1518_1519ins   | Pathogenic (class 5)   |
| 7   | m         | SA (30%)                         | chr1:120466424 | NOTCH2   | 40          | p.(Arg1567fs)    | 1944     | 26   | NM_024408.3 | c.4694_4695ins   | Likely pathogenic (class 4) |
|     |           |                                  | chr2:47698188 | MSH2     | 82          | p.(Asn583fs)     | 1984     | 11   | NM_000251.2 | c.1747_1748del   | Pathogenic (class 5)   |
|     |           |                                  | chr9:139402690 | NOTCH1   | 41          | p.(Arg1107*)     | 901      | 20   | NM_017617.4 | c.3319C>T        | Pathogenic (class 5)   |
|     |           |                                  | chr11:534289 | HRAS     | 43          | p.(Gly12Ser)     | 1188     | 2    | NM_00130442.2 | c.34G>A          | Uncertain significance (class 3) |

Abbreviations: P, patient number; SC, sebaceous carcinoma; TSD, tumor with sebaceous differentiation; M, melanoma; SA, sebaceous adenoma; SCC, squamous cell carcinoma; SE, sebaceoma; SCS, solid-cystic sebaceous neoplasm.
4. Discussion

4.1. Introduction

In this presented work, we investigated mutations other than in the MMR genes in sebaceous tumors and non-MTS associated skin lesions in patients diagnosed with Muir-Torre Syndrome. We showed which concomitant mutations may arise in these tumors, providing options for targeted therapy in advanced sebaceous tumors. With only few case reports in the literature, we furthermore examined the family histories of the affected patients, to highlight the importance of a precise medical history for a presumptive diagnosis of MTS in patients with sebaceous tumors [13–15].

4.2. Demographic Data

Sebaceous hyperplasia with frequent occurrence in the general population stands in contrast to sebaceous tumors, which are rare in general population, but often present in patients with MTS [16]. Most commonly, patients are diagnosed with sebaceous adenoma followed by sebaceous epithelioma and sebaceous carcinoma [17]. Concordant to previous data, we found sebaceous skin neoplasm in MTS patients to be more frequent outside the head and neck region.

4.3. Medical History

In over half of the cases, sebaceous skin lesions develop after diagnosis of a visceral malignancy, while in 22% of cases they precede visceral malignancies by up to 25 years [18,19]. This is reflected in our patient cohort with one patient being diagnosed with small lymphocytic lymphoma as he was screened for visceral malignancies after the diagnosis of a sebaceous lesion. In two patients, visceral malignancies preceded the diagnosis of a sebaceous lesion. Apart from sebaceous tumors, squamous cell carcinoma (SCC) was reported to be associated with MMR mutations [20,21]. Additionally to sebaceous tumors, patient 1 was diagnosed with SCC and melanoma, both of which lacked MMR mutation and were most likely due to sporadic mutation. G. Ponti et al. [22] identified nine melanomas in a collective of 1057 LS patients. In only one case, MSH2 mutation was confirmed, which indicates that the MMR genes are not a driving molecular mechanism in the genesis of melanoma, although deletions of the MMR genes can be present in sporadic melanoma [22]. One patient in our study group, was treated with cyclosporine due to a severe psoriasis vulgaris 16 years prior to the diagnosis of a solid cystic sebaceous tumor. As a switch of cyclosporine to sirolimus can decrease the frequency of sebaceous lesions, this should be considered in patients with immunosuppressive therapy [23,24].

4.4. Family History

We found a positive family history in three patients for visceral malignancies associated with MTS. Families with history of MTS-associated tumors, such as colorectal cancer, should undergo further genetic testing, as immunohistochemistry without family history of colorectal cancers is inferior in sensitivity and specificity as well as in the positive and negative predictive values [25]. Dermatofibrosarcoma and cutaneous T cell lymphoma (CTCL), as found in the relative of patient 2, who had a sebaceoma with a MSH2 mutation, have been described in association with Lynch families with germline MSH2 mutations [26]. There are also case reports of patients with CTCL, such as mycosis fungoides (MF) with MTS [27]. Scarisbrick et al. found microsatellite instability in 27% of patients with MF. Reduced expression of the hMLH1 protein was found in 56% and normal expression of the hMLH1 protein in patients without MSI, suggesting that hMLH1 promoter methylation may provide a potential future therapeutic target [28].

4.5. Mutational Analysis

In our collective, most commonly mutations in the mismatch repair protein (MMR) in the MSH2 gene were detected (six patients; 86%), which is consistent with the current
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literature [10,29]. Besides these MMR mutations, we found diverse further mutations: The second most frequently mutated gene was NOTCH1, present in three patients. Reflecting the tumor suppressing function of NOTCH signaling in keratinocytes, loss of function mutations are found in SCC of the head and neck, basal cell carcinoma but also in visceral carcinomas, such as breast cancer, small cell lung carcinoma and urothelial carcinoma [30]. In contrast, gain of function mutations in the NOTCH genes have been described for different lymphomas [30–32]. Interestingly, patient 7 was diagnosed with small lymphocytic lymphoma after diagnosis of a sebaceous adenoma, which comprised mutations in NOTCH1 and 2 additionally to the MSH2 gene. However, mutational analysis of the bone marrow using the “TruSight Myeloid Sequencing Panel” showed no mutations. Mutations in TP53, coding for the tumor suppressor protein p53, for instance, was found in two patients. Yongyang Bao et al. found TP53 mutations in 83% of the patients with sebaceous carcinoma of the eyelid [33]. This result is similar to previous studies of sebaceous carcinomas, which display a high rate for TP53 mutations [34,35]. Patient 4 had a solid-cystic sebaceous tumor, with a NF1 mutation. Somatic mutations in the NF1 gene are found in a wide variety of malignant neoplasms that are not associated with Neurofibromatosis type 1 such as desmoplastic, cutaneous and mucosal melanoma and various visceral malignancies [36]. Especially in desmoplastic melanoma, NF1 gene mutations are commonly observed with frequencies of up to 93% [37]. Tetzlaff and colleagues found two patients with NF1 gene mutations in a collective of 27 sebaceous carcinomas [35]. An EGFR mutation was identified in one patient with a sebacea of the abdomen. EGFR mutations in sebaceous tumors are described in previous studies. Harvey et al. found, in a collective of 24 sebaceous lesions, nine to have an EGFR mutation [34]. Regarding the mutations we found in our patients collective, targeted therapy has been used with success in adenocarcinoma of the lung with EGFR mutations, PIK3 inhibitors in stage four melanoma or MEK inhibitors in inoperable plexiform neurofibromas with NF1 mutations [38–40].

4.6. Diagnostic Criteria

Our findings highlight the necessity of physical examination of the skin and the lymph nodes, as well as the common screening recommendations for patients with MTS and their first-degree relatives. This includes annually physical examination (including breast in women and testicular and prostate in men), laboratory tests, colonoscopy every one to two years from the age of 25 years or five years before the youngest age of diagnosis of colorectal cancer in the family, gastroscopy and ultrasonography every one to two years [41]. As an association between sebaceous tumors and iatrogenic immunosuppression is described in the literature, particularly under the treatment of cyclosporine, patients under immunomodulatory therapy should receive regularly physical examinations and sebaceous lesions should be analyzed for microsatellite instability, as it could unmask a latent Muir-Torre phenotype [23]. In accordance with the Bethesda guidelines, patients diagnosed with colorectal cancer at an early age (<50 years), with a suggestive personal (presence of synchronous, metachronous colorectal, or other Lynch-associated tumors) or family history, should undergo further genetic testing [12].

4.7. Limitations

The limitation of the study is the restriction of patients number and tumor samples’ origin. Furthermore, the evaluation of the precise mutations being responsible for the visceral tumors in our patients collective would be important to draw a connection to the skin tumors. As only skin samples were analyzed for MMR mutations, and we did not look for germline mutations, we cannot rule out that the here described mutations are somatic. Germline mutations in the MMR genes could provide information about the cause of the tumors. Further molecular analysis of the tumors in the patients’ relatives or genetic testing of normal tissue or blood of the patients could give an indication of possible inherited mutations causing the Muir-Torre syndrome in this family.
5. Conclusions

This study provides an extensive molecular analysis of MTS associated and non-associated skin tumors in patients with MTS. We showed how different MTS-associated tumors can appear throughout generations in patients with mutations in the MSH genes. Therefore, along with MSI analysis, accurate evaluation of personal and family history is mandatory for patients with sebaceous lesions to detect a possible Muir-Torre syndrome. As secondary malignancies may appear years after the first occurrence of sebaceous tumors, lifelong screening is required. Furthermore, the importance of physical examination of the skin is undeniable.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/genes12050781/s1, Table S1: List of gene targets in the Oncomine Comprehensive Assay v3. (Thermo Fisher Scientific, Waltham, MA, USA)—161 gene panel.

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Conflicts of Interest: Reinhard Dummer has intermittent, project focused consulting and/or advisory relationships with Novartis, Merck Sharp and Dhome (MSD), Bristol-Myers Squibb (BMS), Roche, Amgen, Takeda, Pierre Fabre, Sun Pharma, Sanofi, Catalym, Second Genome, Regeneron, Alligator outside the submitted work. Egle Ramelyte has intermittent, project focused consulting relationships with Amgen, BMS and Sanofi outside the submitted work. Other authors have no interests to declare.

References
1. Joly, M.-O.; Attignon, V.; Saurin, J.-C.; Desseigne, F.; Leroux, D.; Martin-Denavit, T.; Giraud, S.; Bonnet-Dupeyrón, M.-N.; Faivre, L.; Auclair, J.; et al. Somatic MMR gene mutations as a cause for MSI-H sebaceous neoplasms in Muir–Torre syndrome-like patients. *Hum. Mutat.* 2015, 36, 292–295. [CrossRef]
2. Ligtenberg, M.J.; Kuiper, R.P.; Geurts van Kessel, A.; Hoogerbrugge, N. EPCAM deletion carriers constitute a unique subgroup of Lynch syndrome patients. *Fam. Cancer* 2013, 12, 169–174. [CrossRef]
3. Muir, E.G.; Bell, A.J.; Barlow, K.A. Multiple primary carcinomata of the colon, duodenum, and larynx associated with kerato-acanthomata of the face. *Br. J. Surg.* 1967, 54, 191–195. [CrossRef]
4. Thibodeau, S.N.; French, A.J.; Roche, P.C.; Cunningham, J.M.; Tester, D.J.; Lindor, N.M.; Moslein, G.; Baker, S.M.; Liskay, R.M.; Burgart, L.J.; et al. Altered expression of hMSH2 and hMLH1 in tumors with microsatellite instability and genetic alterations in mismatch repair genes. *Cancer Res.* 1996, 56, 4836–4840. [CrossRef]
5. Entius, M.M.; Keller, J.J.; Driltenburg, P.; Kuypers, K.C.; Giardiello, F.M.; Offerhaus, G.J. Microsatellite instability and expression of hMLH-1 and hMSH-2 in sebaceous gland carcinomas as markers for Muir-Torre syndrome. *Clin. Cancer Res.* 2000, 6, 1784–1789. [CrossRef]
6. Steinke, V.; Engel, C.; Büttner, R.; Schackert, H.K.; Schmiegel, W.H.; Propping, P. Hereditary nonpolyposis colorectal cancer (HNPCC)/Lynch syndrome. *Disch. Arztbl. Int.* 2013, 110, 32–38. [CrossRef] [PubMed]
7. Senter, L.; Clendenning, M.; Sotamaa, K.; Hampel, H.; Green, J.; Potter, J.D.; Lindblom, A.; Lagerstedt, K.; Thibodeau, S.N.; Lindor, N.M.; et al. The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. *Gastroenterology* 2008, 135, 419–428. [CrossRef]
8. Baglietto, L.; Lindor, N.M.; Dowty, J.G.; White, D.M.; Wagner, A.; Gomez Garcia, E.B.; Vriends, A.H.; Cartwright, N.R.; Barnetson, R.A.; Farrington, S.M.; et al. Risks of Lynch syndrome cancers for MSH6 mutation carriers. *J. Natl. Cancer Inst.* 2010, 102, 193–201. [CrossRef] [PubMed]

9. Bonadonna, V.; Bonaiti, B.; Olschwang, S.; Grandjouan, S.; Huwart, L.; Longy, M.; Guimbaud, R.; Buecher, B.; Bignon, Y.J.; Caron, O.; et al. Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. *JAMA* 2011, 305, 2304–2310. [CrossRef]

10. Le, S.; Ansari, U.; Muntaz, A.; Malik, K.; Patel, P.; Doyle, A.; Khachemoune, A. Lynch Syndrome and Muir-Torre Syndrome: An update and review on the genetics, epidemiology, and management of two related disorders. *Dermatol. Online J.* 2017, 23.

11. Nelson, B.R.; Hamlet, K.R.; Gillard, M.; Rainall, D.; Johnson, T.M. Sebaceous carcinoma. *J. Am. Acad. Dermatol.* 1995, 33, 1–15. [CrossRef]

12. Umar, A.; Boland, C.R.; Terdiman, J.P.; Syngal, S.; de la Chapelle, A.; Ruschoff, J.; Fishel, R.; Lindor, N.M.; Burgart, L.J.; Hamelin, R.; et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J. Natl. Cancer Inst.* 2004, 96, 261–268. [CrossRef]

13. Burris, C.K.H.; Rodriguez, M.E.; Raven, M.L.; Reddy, D.N.; Xu, Y.G.; Wiggs, J.L.; Potter, H.D.; Albert, D.M. Muir-Torre Syndrome: The importance of a detailed family history. *Case Rep. Ophthalmol.* 2019, 10, 180–185. [CrossRef]

14. Hatta, N.; Takata, A.; Ishizawa, S.; Niida, Y. Family with MSH2 mutation presenting with keratoacanthoma and precancerous skin lesions. *J. Dermatol.* 2015, 42, 1087–1090. [CrossRef]

15. Kacerovska, D.; Cerna, K.; Martinek, P.; Grossmann, P.; Michal, M.; Ricar, J.; Kazakov, D.V. MSH6 mutation in a family affected by Muir-Torre syndrome. *Am. J. Dermatopathol.* 2012, 34, 648–652. [CrossRef]

16. Abbas, O.; Mahalingam, M. Cutaneous sebaceous neoplasms as markers of Muir-Torre syndrome: A diagnostic algorithm. *J. Cutan. Pathol.* 2009, 36, 613–619. [CrossRef]

17. Gay, J.T.; Troxell, T.; Gross, G.P. Muir-Torre Syndrome; StatPearls: Treasure Island, FL, USA, 2020.

18. Akhtar, S.; Oza, K.K.; Khan, S.A.; Wright, J. Muir-Torre syndrome: Case report of a patient with concurrent jejunal and ureteral cancer and a review of the literature. *J. Am. Acad. Dermatol.* 1999, 41, 681–686. [CrossRef]

19. Cohen, P.R.; Kohn, S.R.; Kurzrock, R. Association of sebaceous gland tumors and internal malignancy: The Muir-Torre syndrome. *Am. J. Med.* 1991, 90, 606–613. [CrossRef]

20. Gray, S.E.; Kay, E.W.; Leader, M.; Mabruk, M.J. Enhanced detection of microsatellite instability and mismatch repair gene expression in cutaneous squamous cell carcinomas. *Mol. Diagn. Ther.* 2006, 10, 327–334. [CrossRef] [PubMed]

21. Kiento, C.; Joly, M.-O.; Faivre, L.; Clemenson, A.; Dalac, S.; Lepage, C.; Chapusot, C.; Jacquot, C.; Schiappa, R.; Lebrun, M. A case report of Muir-Torre syndrome in a woman with breast cancer and MSI-Low skin squamous cell carcinoma. *Hered. Cancer Clin. Pract.* 2017, 15, 6. [CrossRef]

22. Ponti, G.; Losi, L.; Pellacani, G.; Wannesson, L.; Cesinaro, A.M.; Venesio, T.; Petti, C.; Seidenari, S. Malignant melanoma in patients with hereditary nonpolyposis colorectal cancer. *Br. J. Dermatol.* 2008, 159, 162–168. [CrossRef] [PubMed]

23. Harwood, C.A.; Swale, V.J.; Bataille, V.A.; Quinn, A.G.; Ghali, L.; Patel, S.V.; Dove-Edwin, I.; Cerio, R.; McGregor, J.M. An association between sebaceous carcinoma and microsatellite instability in immunosuppressed organ transplant recipients. *J. Investig. Dermatol.* 2001, 116, 246–253. [CrossRef]

24. Griffard, E.A.; McCoppin, H.H.; Wieberg, J.; Feldman, M. The cutaneous effects of post-transplant immunosuppression with cyclosporine in Muir-Torre Syndrome. *J. Am. Acad. Dermatol.* 2011, 64, e86–e87. [CrossRef] [PubMed]

25. Roberts, M.E.; Riegert-Johnson, D.L.; Thomas, B.C.; Thomas, C.S.; Heckman, M.G.; Krishna, M.; DiCaudo, D.J.; Bridges, A.G.; Hunt, K.S.; Rumilla, K.M.; et al. Screening for Muir-Torre syndrome using mismatch repair protein immunohistochemistry of sebaceous neoplasms. *J. Genet. Couns.* 2013, 22, 393–405. [CrossRef] [PubMed]

26. Huang, R.L.; Chao, C.F.; Ding, D.C.; Yu, C.P.; Chang, C.C.; Lai, H.C.; Yu, M.H.; Liu, H.S.; Chu, T.Y. Multiple epithelial and nonepithelial tumors in hereditary nonpolyposis colorectal cancer: Characterization of germline and somatic mutations of the MSH2 gene and heterogeneity of replication error phenotypes. *Cancer Genet. Cytoenet.* 2004, 153, 108–114. [CrossRef]

27. Lewis, D.J.; Duvic, M. A possible association between mycosis fungoides and Muir-Torre syndrome: Two disorders with microsatellite instability. *JAAD Case Rep.* 2017, 3, 358–361. [CrossRef] [PubMed]

28. Scarisbrick, J.J.; Mitchell, T.J.; Calonje, E.; Orchard, G.; Russell-Jones, R.; Whittaker, S.J. Microsatellite instability is associated with hypermethylation of the hMLH1 gene and reduced gene expression in mycosis fungoides. *J. Investig. Dermatol.* 2003, 121, 894–901. [CrossRef]

29. Kuwabara, K.; Suzuki, O.; Chika, N.; Kumamoto, K.; Minabe, T.; Fukuda, T.; Arai, E.; Tamaru, J.I.; Akagi, K.; Eguchi, H.; et al. Prevalence and molecular characteristics of DNA mismatch repair protein-deficient sebaceous neoplasms and keratoacanthomas in a Japanese hospital-based population. *Jpn. J. Clin. Oncol.* 2018, 48, 514–521. [CrossRef]

30. Aster, J.C.; Pear, W.S.; Blacklow, S.C. The varied roles of notch in cancer. *Annu. Rev. Pathol.* 2017, 12, 245–275. [CrossRef]

31. Dotto, G.P. Notch tumor suppressor function. *Oncogene* 2008, 27, 5115–5123. [CrossRef]

32. Aster, J.C.; Pear, W.S.; Blacklow, S.C. Notch signaling in leukemia. *Annu. Rev. Pathol.* 2008, 3, 587–613. [CrossRef] [PubMed]

33. Bao, Y.; Selfridge, J.E.; Wang, J.; Zhao, Y.; Cui, J.; Guda, K.; Wang, Z.; Zhu, Y. Mutations in TP53, ZNF750, and RB1 typify ocular sebaceous carcinoma. *J. Genet. Genom.* 2019, 46, 315–318. [CrossRef]

34. Harvey, N.T.; Tabone, T.; Erber, W.; Wood, B.A. Circumscribed sebaceous neoplasms: A morphological, immunohistochemical and molecular analysis. *Pathology* 2016, 48, 454–462. [CrossRef] [PubMed]
35. Tetzlaff, M.T.; Singh, R.R.; Seviour, E.G.; Curry, J.L.; Hudgens, C.W.; Bell, D.; Wimmer, D.A.; Ning, J.; Czerniak, B.A.; Zhang, L.; et al. Next-generation sequencing identifies high frequency of mutations in potentially clinically actionable genes in sebaceous carcinoma. *J. Pathol.* 2016, 240, 84–95. [CrossRef]

36. Philpott, C.; Tovell, H.; Frayling, I.M.; Cooper, D.N.; Upadhyaya, M. The NF1 somatic mutational landscape in sporadic human cancers. *Hum. Genom.* 2017, 11, 13. [CrossRef]

37. Wiesner, T.; Kiuru, M.; Scott, S.N.; Arcila, M.; Halpern, A.C.; Hollmann, T.; Berger, M.F.; Busam, K.J. NF1 Mutations are common in desmoplastic melanoma. *Am. J. Surg. Pathol.* 2015, 39, 1357–1362. [CrossRef]

38. Vaassen, P.; Durr, N.; Rohrig, A.; Willing, R.; Rosenbaum, T. Trametinib induces neurofibroma shrinkage and enables surgery. *Neuropediatrics* 2019, 50, 300–303. [CrossRef]

39. Tryggvason, G.; Bayon, R.; Pagedar, N.A. Epidemiology of sebaceous carcinoma of the head and neck: Implications for lymph node management. *Head Neck* 2012, 34, 1765–1768. [CrossRef]

40. Pao, W.; Miller, V.; Zakowski, M.; Doherty, J.; Politi, K.; Sarkaria, I.; Singh, B.; Heelan, R.; Rusch, V.; Fulton, L.; et al. EGF receptor gene mutations are common in lung cancers from “never smokers” and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc. Natl. Acad. Sci. USA* 2004, 101, 13306–13311. [CrossRef]

41. Boardman, L.A. Heritable colorectal cancer syndromes: Recognition and preventive management. *Gastroenterol. Clin. N. Am.* 2002, 31, 1107–1131. [CrossRef]