Deep intronic *MSH2* variant confirms Muir-Torre subtype of Lynch syndrome

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JID Innovations (2026);**6:**100438 doi:10.1016/j.xjidi.2025.100438

Whole-genome sequencing can uncover clinically significant noncoding variants missed by standard germline testing, as demonstrated in this report in a patient with Muir–Torre syndrome, a subtype of Lynch syndrome. In this case, despite a convincing clinical phenotype and immunohistochemical loss of MSH2/MSH6 in 1 of the patient's tumors, conventional gene panel testing failed to detect a germline pathogenic variant. Whole-genome sequencing identified a deep intronic *MSH2* variant, and tumor sequencing revealed somatic *MSH2* mutations (second hits) across multiple tumors, confirming mismatch repair deficiency and establishing a Muir-Torre syndrome diagnosis. This report underscores the limitations of routine genetic testing and highlights the clinical utility of whole-genome sequencing in identifying pathogenic variants in noncoding regions. It also emphasizes the role of dermatologists in recognizing cutaneous markers of hereditary cancer syndromes and the importance of interdisciplinary evaluation in guiding both patient care and familial risk assessment.

Keywords: Basal cell cancers, Lynch syndrome, Muir–Torre syndrome, Sebaceous adenomas

INTRODUCTION

Muir–Torre syndrome (MTS) is a rare autosomal dominant disorder considered a phenotypic variant of Lynch syndrome (LS) (John and Schwartz, 2016). In addition to a predisposition to internal malignancies associated with LS, such as colorectal and endometrial cancers, individuals with MTS develop characteristic sebaceous skin tumors, including sebaceous adenomas, epitheliomas, and carcinomas (Trehan et al, 2024).

MTS is caused by germline pathogenic variants (GPVs) in the mismatch repair (MMR) genes—*MLH1*, *MSH2*, *MSH6*, and *PMS2*—as well as *EPCAM*, with *MSH2* GPVs being the most prevalent (Le et al, 2017). It accounts for an estimated 10% of all LS cases (South et al, 2008). Germline disruption of an MMR gene, followed by second somatic hits in tumor tissues, results in MMR deficiency, which leads to a high mutation rate and tumorigenesis (John and Schwartz, 2016).

Clinical genetic testing is prompted in suspected cases on the basis of factors such as age at cancer onset, number of sebaceous neoplasms, and personal or family history of LS-associated cancers (Roberts et al, 2014). Testing starts with immunohistochemistry (IHC) for MMR proteins in tumor tissue. If IHC shows loss of protein expression, germline testing is performed using targeted sequencing panels of the MMR genes and *EPCAM* (Gay et al, 2023; Roberts et al, 2014).

However, in some patients, clinical and pathological features are strongly suggestive of MTS, yet routine germline testing fails to identify a GPV. In such cases, the causal variant may lie outside the regions typically interrogated by standard sequencing approaches.

In this report, we present a case of MTS in which clinical presentation and tumor IHC strongly indicated MMR deficiency, but standard germline testing was uninformative. Whole-genome sequencing (WGS) ultimately identified a deep intronic pathogenic variant in *MSH2*, underscoring the diagnostic value of comprehensive genetic analysis in dermatology.

CASE PRESENTATION

The patient, a female aged 66 years, was first diagnosed with colon adenocarcinoma at age 43 years. Between the ages of 58 and 64 years, she developed multiple cutaneous sebaceous neoplasms, including sebaceous adenomas, sebaceous epithelioma, and sebaceous carcinoma, as well as basal cell carcinomas (BCCs) and squamous cell carcinoma (SCC). Most recently, at age 66 years, she presented with small bowel adenocarcinoma.

Family history includes 9 siblings with benign colonic neoplasia, including 1 sibling who reported 39 colorectal polyps. According to the proband, the affected family members had colorectal polyps and not colorectal cancer.

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Abbreviations: BCC, basal cell carcinoma; GPV, germline pathogenic variant; IHC, immunohistochemistry; LS, Lynch syndrome; MMR, mismatch repair; MTS, Muir–Torre syndrome; SCC, squamous cell carcinoma; WGS, whole-genome sequencing

Received 26 August 2025; revised 14 November 2025; accepted 17 November 2025

Cite this article as: JID Innovations 2026;6:100438

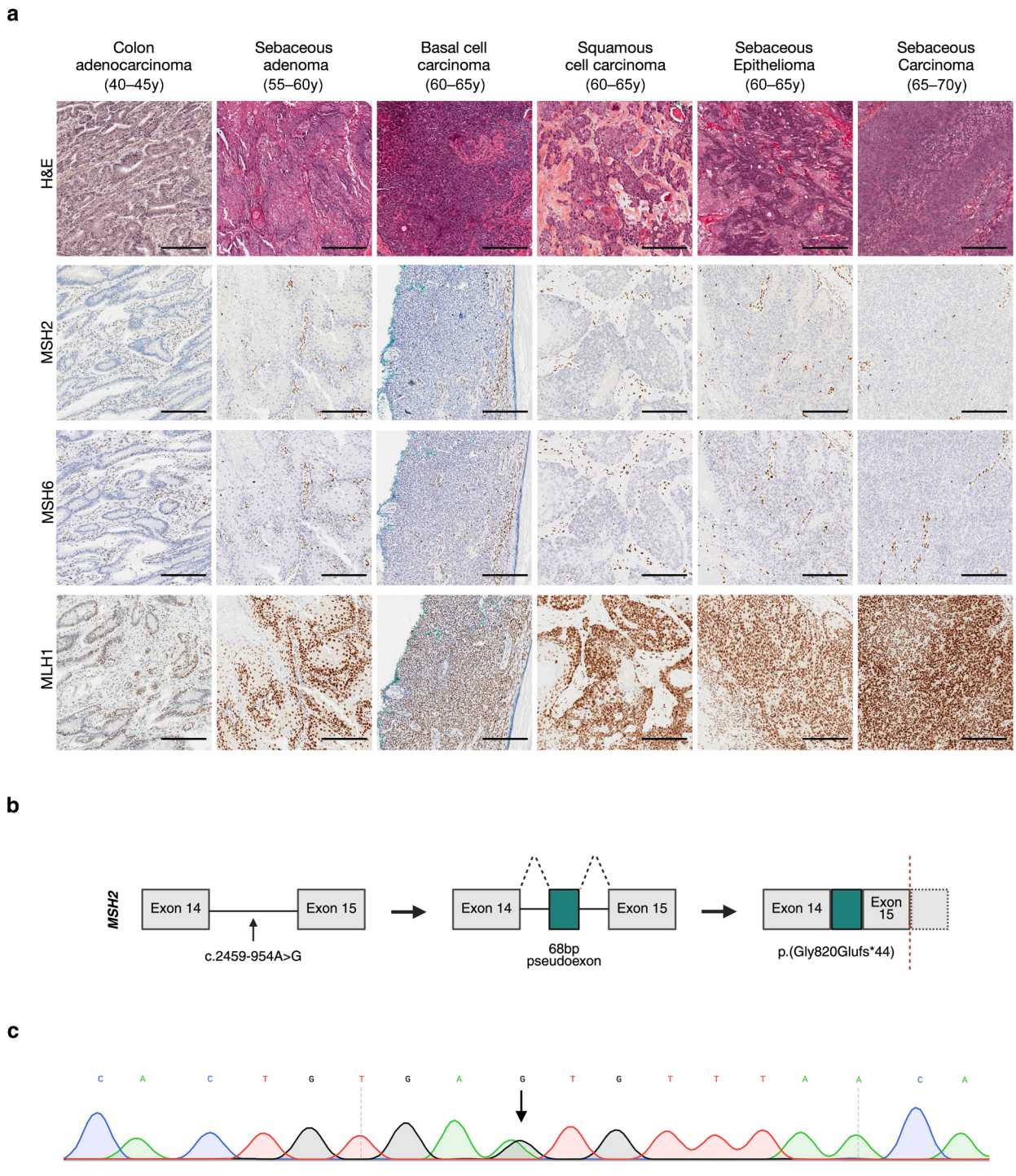

Figure 1. Aberrant splicing of *MSH2* due to c.2459-954A>G causes pseudoexon inclusion and loss of protein expression in tumor tissue. (**a**) Histology and immunohistochemistry for MSH2, MSH6, and MLH1 proteins of the proband's tumors. The neoplastic cells in all tumors show loss of nuclear staining for MSH2 and MSH6, whereas the non-neoplastic stromal and inflammatory cells demonstrate retained nuclear staining (serving as internal controls). In contrast, the neoplastic cells in all tumors retain nuclear staining for MLH1. Brown nuclear staining was interpreted as positive protein expression, whereas absence of brown staining (blue only) indicated loss of expression. Normal adjacent tissue was used as a control. Of note, the BCC showed no evidence of sebaceous differentiation (Tsalis et al, 2006). The bars shown in the figure represents 200 μm. Ages at diagnosis are shown in parentheses in 5-year ranges. (**b**) Schematic representation of the pseudoexon formation and subsequent premature truncation (red dotted line) caused by the deep intronic *MSH2* variant (on the basis of Te Paske et al [2022]). (**c**) Sanger sequencing chromatogram confirming the presence of the *MSH2* intronic variant in the tumors. Shown is a representative chromatogram from the sebaceous epithelioma. BCC, basal cell carcinoma.

We acknowledge that this reported cancer history is not consistent with LS, but without pathology reports or genetic testing, it is difficult to assess its significance.

The combination of sebaceous tumors and LS-associated internal malignancies raised strong clinical suspicion for MTS, prompting germline genetic testing. Germline sequencing was

Table 1. Summary of Sequencing Results (WGS and Panel)

Tumor samples	IHC	Germline (WGS)	Somatic (Panel Data)
Colon adenocarcinoma	Loss of MSH2/6	*MSH2*: NM_000251.3: c.2459-954A>G, p.(Gly820Glufs*44) (71.4% in blood)	*MSH2*: NM_000251.3:c.2377C>T, p.(Gln793*) (20.1%) *APC*: NM_000038.6:c.694C>T, p.(Arg232*) (19.2%) *APC*: NM_000038.6:c.4666dupA, p.(Thr1556Asnfs*3) (22.6%)
Sebaceous adenoma, upper back	Loss of MSH2/6		*MSH2*: NM_000251.3: c.1861C>T, p.(Arg621*) (20.5%)
Basal cell carcinoma, left alar rim (nose)	Loss of MSH2/6		*MSH2*: NM_000251.3: c.1277-1_1277delGGinsAA (22.8%)
Squamous cell carcinoma, right alar rim (nose)	Loss of MSH2/6		No *MSH2* variant in coding region *MUTYH*: NM_001048174.2:c.214G>A, p.(Val72Met) (32.2%)
Sebaceous epithelioma, left arm	Loss of MSH2/6		*MSH2* deletion encompassing exons 9 and 10
Sebaceous carcinoma, back	Loss of MSH2/6		*MSH2*: NM_000251.3: c.2524G>T, p.(Glu842*) (34.1%) *MSH6*: NM_000179.3:c.3261dupC, p.(Phe1088Leufs*5) (28.0%)
Small bowel adenocarcinoma	N/A		*MSH2*: NM_000251.3: c.1705_1705delGA, p.(Glu569Ilefs*2) (25.1%)

Abbreviations: IHC, immunohistochemistry; N/A, not available; WGS, whole-genome sequencing.

performed using a 84-gene panel, which identified a variant of uncertain significance in *MSH3* (NM_002439.5:c.2075A>G, p.[Gln692Arg]) but no GPV in the main MMR genes. However, IHC analysis of 1 sebaceous adenoma demonstrated complete loss of MSH2 and MSH6 expression, indicating MMR deficiency and supporting the likelihood of biallelic inactivation of *MSH2*. Because MSH2 and MSH6 form a heterodimer involved in mismatch recognition, loss of MSH2 leads to the destabilization of MSH6 and consequent concurrent loss of both proteins, a pattern that is consistent with *MSH2* dysfunction (Chen and Frankel, 2019). The discordance between the patient's phenotype, IHC results, and genetic findings warranted further investigation.

RESULTS

IHC analysis was performed on all available tumor specimens—colon adenocarcinoma, sebaceous adenoma, sebaceous epithelioma, sebaceous carcinoma, BCC, and SCC—which consistently showed loss of MSH2 and MSH6 expression (Figure 1a). This uniform MMR deficiency across multiple tumors reinforced the suspicion of an underlying germline MMR defect.

With informed patient consent, WGS was then performed on peripheral blood–derived DNA. This comprehensive genomic assessment identified a deep intronic germline variant in *MSH2* (NM_000251.3:c.2459-954A>G, p.[Gly820Glufs*44]). This variant is classified as pathogenic or likely pathogenic (ClinVar ID:1475009) and has been previously reported by Te Paske et al (2022) in multiple affected family members. The variant creates a pseudoexon between exons 14 and 15, leading to the introduction of a premature termination codon (Te Paske et al, 2022) (Figure 1b).

Germline variant validation and second-hit analysis were subsequently performed. Sanger sequencing confirmed the presence of the intronic *MSH2* variant in the patient's blood DNA and in all 7 tumors (Figure 1c). Targeted panel sequencing of the tumors revealed somatic *MSH2* pathogenic mutations in 6 of the 7 tumors analyzed (Table 1), confirming the MTS diagnosis. The absence of a second hit in the seventh tumor may reflect an intronic variant not detected by panel sequencing.

DISCUSSION

This case highlights the limitations inherent in routine clinical genetic testing methods, particularly in detecting deep intronic variants. Although standard germline panel testing focuses on coding regions of known cancer susceptibility genes, it may fail to identify pathogenic variants outside these regions. In contrast, WGS offers a comprehensive genomic assessment capable of detecting deep intronic, regulatory, and structural variants missed by conventional methods (Horti-Oravecz et al, 2025). For example, previous WGS studies have identified GPVs in 18.8% and 21.4% of cases, respectively, that were previously undetected by targeted panel and Sanger sequencing (Pope et al, 2021; Te Paske et al, 2022).

While no GPV was identified through panel testing in the patient described in this report, WGS revealed a known likely pathogenic deep intronic variant in *MSH2*. Across multiple tumors, skin and visceral lesions, IHC consistently demonstrated loss of *MSH2* and *MSH6*, and somatic second-hit mutations in *MSH2* were identified, confirming biallelic *MSH2* inactivation, MMR deficiency, and establishing the diagnosis of MTS.

Although sebaceous tumors are the hallmark cutaneous manifestation of MTS, other skin malignancies, including SCC and BCC, have also been reported in association with LS/MTS (Grob et al, 2016). In a limited number of cases, SCCs and BCCs in patients with confirmed germline MMR GPVs have shown loss of MMR protein expression (Adan et al, 2019; Amjad et al, 2014; Grob et al, 2016; Kientz et al, 2017; Sorscher, 2015). In 1 reported SCC, the patient carried a germline *MLH1* deletion, and tumor sequencing identified a somatic second hit in *MLH1* (Khaddour et al, 2020). However, second-hit mutations in *MSH2* in MTS-related SCCs/BCCs have not been reported previously but would be expected on the basis of IHC results (Adan et al, 2019; Amjad et al, 2014; Kientz et al, 2017). The consistent MMR deficiency and somatic *MSH2* alteration observed

in the BCC in our patient provide additional molecular evidence that this tumor type falls within the clinical spectrum of MTS.

The identification of a deep intronic *MSH2* GPV provided definitive confirmation of a MTS diagnosis, directly informing patient care, clinical management, and surveillance strategies. Beyond the individual diagnosis, recognizing MTS has significant implications for family members because the autosomal dominant inheritance pattern means that first-degree relatives may also carry the variant. However, given the absence of LS-associated cancers among her siblings and the lack of genetic testing in family members, the proband's pathogenic variant remains a possible de novo variant. This underscores the importance of genetic counseling and cascade genetic testing for family members, which are essential not only to clarify whether the variant arose de novo but also to enable early identification and proactive management of at-risk relatives (Syngal et al, 2015).

This case demonstrates how WGS can uncover a deep intronic *MSH2* GPV in a patient with MTS when standard panel testing is negative. It highlights the essential role of dermatologists in recognizing sebaceous neoplasms as sentinel lesions for LS/MTS and emphasizes the importance of comprehensive genomic analysis when clinical suspicion is strong, but standard testing is inconclusive. Implementing such advanced diagnostic approaches can significantly improve outcomes for patients and inform risk assessment and management for their families.

MATERIALS AND METHODS

Patient samples

A blood sample and formalin-fixed, paraffin-embedded tumor blocks for the patient's colon adenocarcinoma, sebaceous adenoma, BCC, SCC, sebaceous epithelioma, sebaceous carcinoma, and small bowel adenocarcinoma were collected. All tumors except the small bowel adenocarcinoma were available for both IHC and DNA extraction for sequencing. The small bowel tumor block was returned to the referring hospital before IHC could be performed; however, DNA was successfully extracted and used for sequencing analysis.

Immunohistochemical staining

The patient's tumors were stained by IHC using the following ready-to-use antibodies from Dako Agilent (Agilent Technologies, Santa Clara, CA) on the Dako Omnis platform: MLH1 (clone ES05, mouse monoclonal, incubation time of 25 minutes), MSH2 (clone FE11, mouse monoclonal, incubation time of 20 minutes), MSH6 (clone EP49, mouse monoclonal, incubation time of 25 minutes), and PMS2 (clone EP51, mouse monoclonal, incubation time of 30 minutes). Brown nuclear staining was interpreted as positive protein expression, whereas absence of brown staining (blue only) indicated loss of expression. Normal adjacent tissue was used as a control.

DNA extraction

Blood DNA was extracted using the Gentra PureGene Blood Kit (Qiagen, Hilden, Germany), and the formalin-fixed, paraffin-embedded tumors were extracted using the QIAmp DNA FFPE Tissue Kit (Qiagen), according to the manufacturer's instructions.

WGS

WGS was performed on the blood sample using the NovaSeq platform (Illumina, San Diego, CA), generating 150 bp paired-end reads. The sequencing yielded an average alignment coverage of 32.4× across the human genome. Primary data processing, including base calling, demultiplexing, and quality control, was conducted using Illumina's DRAGEN pipeline (Illumina). Reads were aligned to the human reference genome (GRCh38/hg38), and duplicate reads were marked. Variant calling for single nucleotide variants and small insertions/deletions was performed following GATK best practices.

Secondary analysis and variant interpretation were carried out using Illumina Connected Insights – Germline (Illumina). Variants were annotated, filtered, and prioritized on the basis of pathogenicity, population frequency, inheritance patterns, and clinical relevance.

Panel sequencing

DNA from the proband's blood and tumors were enriched for targeted regions using a hybrid capture-based protocol and sequenced on an Illumina MiSeq (Illumina). The following transcripts were used in the analysis: *APC* (NM_00038.4), *MUTYH* (NM_001128425.1), *MLH1* (NM_000249.3), *MSH2* (NM_000251.2), *MSH6* (NM_000179.2), *PMS2* (NM_000535.5), and *EPCAM* (NM_002354.2). Variants were analyzed using NextGENe v2.4.2.3 (Soft Genetics, LLC, State College, PA) with alignment performed against the hg19 reference genome. Variants were annotated and subsequently filtered on the basis of the following criteria: (i) ≥3 reads supporting the variant, (ii) ≥6× coverage at the genomic locus, (iii) variant allele frequency ≥5%, (iv) population allele frequency ≤1% across multiple databases (1000 Genomes, ExAC, ESP6500), (v) variants located within coding or splice-site regions (missense, nonsense, no-stop, and insertions/deletions), and (vi) passing strand bias filters (strand balance ratio >0.10 and frequency ≤80%). Synonymous and variants outside target regions were excluded.

Sanger sequencing

The *MSH2* GPV was validated in the patient's tumors and blood DNA by Sanger sequencing using the following primer set: forward 5′ GGAACTTTTAGTAGATCTGGCAGC 3′ and reverse 5′ ACAGCTG TTTTTGCCACAAT 3′.

ETHICS STATEMENT

Written informed consent was obtained from the patient, and the study protocol was approved by the McGill University Health Centre Research Ethics Board (project number MP-37-2019-4865).

DATA AVAILABILITY STATEMENT

All relevant data supporting the findings of this study are included in the manuscript. Raw sequencing data are available upon reasonable request from the corresponding author.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This study was funded by the Canadian Institutes of Health Research grant (FDN-148390) to WDF. BR is a Miguel Servet Fellow (CP21/00038) from the Instituto de Salud Carlos III (PI20/01721). This research as well as biobanking of biological material and data were made possible in part through a collaboration with the Réseau de recherche sur le cancer financially supported by the Oncopole, the FRQ cancer division, which receives funding from Merck Canada, GSK, Pfizer, and the Ministère de l'Économie, de

l'Innovation et de l'Énergie du Québec. The Réseau de recherche sur le cancer is affiliated to the Canadian Tumor Repository Network.

AUTHOR CONTRIBUTIONS

Conceptualization: BR, WDF; Formal Analysis: FC-P-C; Investigation: FC-P-C, EW, LF; Writing — Original Draft Preparation: AYS; Writing — Review and Editing: AYS, FC-P-C, EW, LF, BR, WDF

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) OR LARGE LANGUAGE MODELS (LLMs)

The author(s) did not use AI/LLM in any part of the research process and/or manuscript preparation.

REFERENCES

Adan F, Crijns MB, Dekker E, Bastiaansen BAJ, Lapid O, Snaebjornsson P, et al. A squamous cell carcinoma in a young woman with Lynch syndrome. Fam Cancer 2019;18:193—6.

Amjad AI, Singhi AD, Balaban EP, Dudley B, Brand RE, Bahary N. First reported case of a squamous cell carcinoma arising in the duodenum in a patient with Lynch syndrome. Int J Clin Exp Pathol 2014;7:8988—95.

Chen W, Frankel WL. A practical guide to biomarkers for the evaluation of colorectal cancer. Mod Pathol 2019;32:1—15.

Gay JT, Troxell T, Gross GP. Muir-Torre syndrome. Treasure Island: StatPearls; 2023.

Grob A, Feser C, Grekin S. Muir-Torre syndrome: a case associated with an infrequent gene mutation. J Clin Aesthet Dermatol 2016;9:56—9.

Horti-Oravecz K, Bozsik A, Pócza T, Vereczkey I, Strausz T, Tóth E, et al. Whole genome sequencing completes the molecular genetic testing workflow of patients with Lynch syndrome. NPJ Genom Med 2025;10:5.

John AM, Schwartz RA. Muir-Torre syndrome (MTS): an update and approach to diagnosis and management. J Am Acad Dermatol 2016;74:558—66.

Khaddour K, Fields RC, Ansstas M, Rosman IS, Ansstas G. Metachronous cutaneous squamous cell carcinoma in a young patient as the only presenting symptom to uncover Lynch syndrome with MLH1 germline mutation. Hered Cancer Clin Pract 2020;18:23.

Kientz C, Joly MO, Faivre L, Clemenson A, Dalac S, Lepage C, et al. 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.

Le S, Ansari U, Mumtaz A, Malik K, Patel P, Doyle A, et al. Lynch syndrome and Muir-Torre Syndrome: an update and review on the genetics, epidemiology, and management of two related disorders. Dermatol Online J 2017;23:13030/qt8sg5w98j.

Pope BJ, Clendenning M, Rosty C, Mahmood K, Georgeson P, Joo JE, et al. Germline and tumor sequencing as a diagnostic tool to resolve suspected Lynch syndrome. J Mol Diagn 2021;23:358—71.

Roberts ME, Riegert-Johnson DL, Thomas BC, Rumilla KM, Thomas CS, Heckman MG, et al. A clinical scoring system to identify patients with sebaceous neoplasms at risk for the Muir-Torre variant of Lynch syndrome. Genet Med 2014;16:711—6.

Sorscher S. A case of squamous cell carcinoma of the skin due to the molecularly confirmed Lynch syndrome. Hered Cancer Clin Pract 2015;13:12.

South CD, Hampel H, Comeras I, Westman JA, Frankel WL, de la Chapelle A. The frequency of Muir-Torre syndrome among Lynch syndrome families. J Natl Cancer Inst 2008;100:277—81.

Syngal S, Brand RE, Church JM, Giardiello FM, Hampel HL, Burt RW, et al. ACG clinical guideline: genetic testing and management of hereditary gastrointestinal cancer syndromes. Am J Gastroenterol 2015;110:223—62 [quiz 263].

Te Paske IBAW, Mensenkamp AR, Neveling K, ERN-GENTURIS Lynch-Like Working Group, Hoogerbrugge N, Ligtenberg MJL, et al. Noncoding aberrations in mismatch repair genes underlie a substantial part of the missing heritability in Lynch syndrome. Gastroenterology 2022;163:1691—4.e7.

Trehan S, Singh G, Goswami K, Farooq A, Kalpana F, Antil P, et al. Muir-Torre syndrome: a case report and a literature review of genetic insights and cancer surveillance. Cureus 2024;16:e65828.

Tsalis K, Blouhos K, Vasiliadis K, Tsachalis T, Angelopoulos S, Betsis D. Sebaceous gland tumors and internal malignancy in the context of Muir-Torre syndrome. A case report and review of the literature. World J Surg Oncol 2006;4:8.