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ARTICLE

Unexplained mismatch repair deficiency: Case closed

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Summary

To identify Lynch syndrome (LS) carriers, DNA mismatch repair (MMR) immunohistochemistry (IHC) is performed on colorectal cancers (CRCs). Upon subsequent LS diagnostics, MMR deficiency (MMRd) sometimes remains unexplained (UMMRd). Recently, the importance of complete LS diagnostics to explain UMMRd, involving MMR methylation, germline, and somatic analyses, was stressed. To explore why some MMRd CRCs remain unsolved, we performed a systematic review of the literature and mapped patients with UMMRd diagnosed in our center. A systematic literature search was performed in Ovid Medline, Embase, Web of Science, Cochrane CENTRAL, and Google Scholar for articles on UMMRd CRCs after complete LS diagnostics published until December 15, 2021. Additionally, UMMRd CRCs diagnosed in our center since 1993 were mapped. Of 754 identified articles, 17 were included, covering 74 patients with UMMRd. Five CRCs were microsatellite stable. Upon complete diagnostics, 39 patients had single somatic MMR hits, and six an MMR germline variant of unknown significance (VUS). Ten had somatic pathogenic variants (PVs) in *POLD1*, *MLH3*, *MSH3*, and *APC*. The remaining 14 patients were the only identifiable cases in the literature without a plausible identified cause of the UMMRd. Of those, nine were suspected to have LS. In our center, complete LS diagnostics in approximately 5,000 CRCs left seven MMRd CRCs unexplained. All had a somatic MMR hit or MMR germline VUS, indicative of a missed second MMR hit. In vitually all patients with UMMRd, complete LS diagnostics suggest MMR gene involvement. Optimizing detection of currently undetectable PVs and VUS interpretation might explain all UMMRd CRCs, considering UMMRd a case closed.

Introduction

Lynch syndrome (LS; MIM: 609310, 120435, 614350, 514337, and 613244) is the most prevalent form of hereditary colorectal cancer (CRC). LS is caused by pathogenic germline variants in DNA mismatch repair (MMR) genes *MLH1* (MIM: 120436), *MSH2* (MIM: 609309), *MSH6* (MIM: 600678), and *PMS2* (MIM: 600259) or deletion of the 3′ end of the *EpCAM* gene (MIM: 185535).[1–5] To identify LS carriers, most Western countries nowadays perform immunohistochemistry (IHC) in CRCs and endometrial carcinomas (ECs) to assess the presence of MMR proteins. The absence of at least one MMR protein is indicative of underlying LS and requires performance of further LS diagnostics, including assessment of *MLH1* promoter hypermethylation (with or without *BRAF* analysis), MMR germline analysis, and examination of the tumor for somatic MMR pathogenic variants (PVs). Upon complete LS diagnostics, the majority of MMR-deficient (MMRd) CRCs are explained. However, for the remaining MMRd tumors, their cause remains unexplained (UMMRd). Despite risk estimates for CRC development in patients with UMMRd being lower than in LS carriers, but higher than in the general population, it is currently unclear to what extent these patients are at risk to develop LS-associated tumors.[6] This makes it difficult to come to an accurate surveillance strategy for these patients and their relatives. Additionally, estimates of UMMRd percentages differ. In a recent meta-analysis performed by our group, it was shown that the number of UMMRd found largely depended on age cutoff and diagnostics performed.[7] In general, 4.2% of all CRCs remained unexplained. However, in studies where somatic PV analysis was not performed, these percentages appeared to be higher.

Notably, some controversy exists regarding patients with UMMRd: first, various terms are used to refer to patients with UMMRd, which hampers the conduct of valid research. For example, the term Lynch-like syndrome is used to define patients with UMMRd as well as patients with double-somatic MMR PVs. Second, patients with UMMRd are thought to represent a heterogeneous group of missed MMR germline PVs, somatic MMR PVs, or PVs in other, cancer-predisposing and MMR-related genes in the germline or tumor.[8] Here, we define UMMRd as follows: all patients with a CRC without an explanation of MMRd in whom germline analysis, somatic analysis, and, in the case of MLH1 protein absence, also *MLH1* hypermethylation analysis were performed.

Although several studies have demonstrated that the proportion of patients with explained MMRd increases

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by performing complete LS diagnostics,[9–12] it has not been proven yet that these analyses are sufficient to explain all patients with UMMRd. Also, little is known about the phenotype of patients with UMMRd, such as tumor development in personal and family history and fulfillment of clinical criteria. Therefore, we performed a systematic literature search to assess the phenotype of patients with UMMRd and evaluate tumor development in personal and family history. Additionally, we performed complete LS diagnostics in patients with UMMRd diagnosed in our center to gain more insight into the magnitude and characteristics of the UMMRd patient group.

Subjects and methods

Systematic review of the literature

This manuscript was drafted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[13] We systematically searched Medline, Embase, Web of Science, Cochrane CENTRAL, and Google Scholar for articles reporting on patients with UMMRd in CRC after complete LS diagnostics, namely germline and somatic analyses corresponding to IHC and, in the case of MLH1 protein absence, also *MLH1* hypermethylation analysis. Articles published until August 14, 2020, were included. On December 15, 2021, we repeated the search. A complete description of our search is shown in Table S1.

Studies were eligible for inclusion if complete LS diagnostics were performed and if UMMRd characteristics were discussed, such as age of diagnosis, family history, fulfillment of revised Bethesda criteria or revised Amsterdam criteria, or outcomes of germline and somatic analysis for PVs in MMR genes. Studies not published in English were excluded. Reference lists of retrieved papers were manually searched for additional studies to include. Upon our systematic search, duplicates were removed in Endnote X9 (Thompson Reuters, New York, NY, USA), according to the method described by Bramer et al.[14] Studies were screened by two authors (E.L.E. and S.M.) independently by first evaluating the title and abstract, followed by full-text evaluation. In case of disagreement, a senior author made the final decision (A.W.). In case of missing data, we contacted the corresponding authors for desired results and/or individual data by e-mail.

Studies meeting our inclusion criteria underwent data extraction. Data extracted include age at diagnosis, sex, clinical features (type of tumor, additional information regarding the tumor, relatives with CRC or EC, synchronous or metachronous CRC or EC, fulfillment of revised Bethesda criteria or Amsterdam criteria), outcomes of microsatellite instability (MSI) and IHC testing, and outcomes of germline and somatic PV analysis. Patients with unclear outcomes, pending analyses, or failed testing were excluded.

UMMRd patients in our center

The department of Clinical Genetics of the Erasmus Medical Center Cancer Institute Rotterdam (the Netherlands) serves as a regional referral center for the south-west of the Netherlands. Patients with a suspicion of an LS-associated tumors, for example due to age of diagnosis, family history, or IHC outcomes, were referred to the department of clinical genetics. A genetic counselor informed patients about LS, and after informed consent, further LS diagnostics were performed. LS diagnostics were performed as previously described. [15] Currently, only IHC analysis, and not MSI, is performed routinely in CRC patients. In our center, complete LS diagnostics included *MLH1* promoter hypermethylation analysis (in case of MLH1 deficiency at IHC), germline analysis (corresponding to IHC outcome), and somatic MMR PV and loss of heterozygosity (LOH) analysis (Figure 1). Sometimes, germline next-generation sequencing (NGS) analysis was also performed using a gene panel including *MLH1*, *MSH2*/*EpCAM*, *MSH6*, *PMS2*, *APC*, *MUTYH*, *POLE*, *POLD1*, *NTHL1* (founder c.268C>T [p.Gln90*]), *STK11*, and *PTEN*. Somatic NGS analysis includes the genes *MLH1*, *MSH2*, *MSH6*, *PMS2*, *BRAF* (exon 11 en 15), *POLD1* (exon 12), and *POLE* (exon 3 en 13). American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) criteria were used for the interpretation of pathogenicity of DNA MMR germline variants. Consequently, we defined UMMRd as those patients who were found to have an MMRd CRC without an identified cause after performance of at least *MLH1* promoter hypermethylation analysis (with or without *BRAF* analysis; in case of MLH1 deficiency at IHC), germline analysis (corresponding to IHC), and somatic MMR PV and LOH analysis. Patients with an identified variant of unknown significance (VUS) were functionally tested and subsequently discussed in the national meeting for "Investigation of variants of uncertain clinical significance for use in clinical practice" (InVUSE, a Dutch Cancer Foundation project, number 10645). Upon discussion, many variants were reclassified; if resulting in a class 4 or 5 variant, patients were informed about this finding and considered explained. Patients in whom no explanation for MMRd was identified were approached to perform RNA sequencing. RNA sequencing was performed on blood or skin fibroblasts, and expression was analyzed by Integrated Genome Viewer (IGV). Outlier expression was detected by the OUTRIDER algorithm comparing individual patient samples with our control cohort. [16]

All patients with UMMRd selected from our patient registration between 1993 and 2020 were asked to provide informed consent to perform the missing diagnostic analyses. Patients who could not be approached were classified as loss of follow up and were therefore excluded for this study. In case tumors had developed prior to counseling or if treatment had been provided in another hospital, information was retrieved from the corresponding hospital. Permission of the Erasmus Medical Center Committee on Research Involving Human Subjects was not deemed necessary as included patients had previously granted informed consent to be contacted in case new diagnostic assays would be available. These assays were performed as standard of care. Patients and the public were not involved in the design, conduct, reporting, or dissemination of this research project. Due to the paucity of data and the descriptive nature of this study, no statistical analyses were performed.

Results

Literature search

Our search yielded 754 articles. Of these, 49 articles were reviewed full text. One article was retrieved manually, which led to inclusion of 11 articles. The remaining 38 articles were excluded for various reasons. Of note, to prevent bias, we excluded the publication by Geurts-Giele et al. as this publication analyzed patients diagnosed in our center. After repeating the search, another six articles were included (Figure 2).

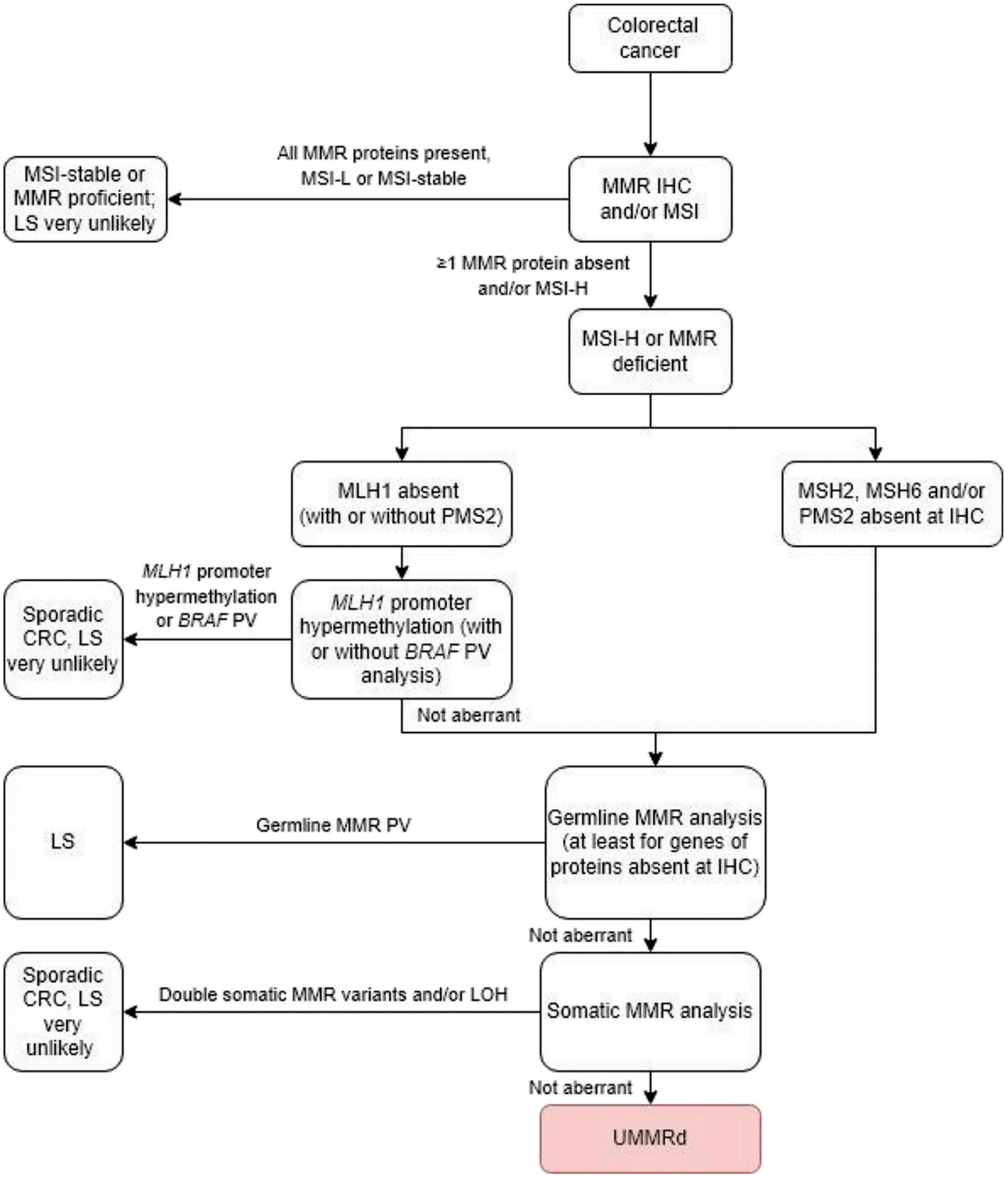

Figure 1. Flowchart of Lynch syndrome diagnostics in colorectal cancer

Characteristics of patients with UMMRd in literature

The 17 included articles identified 74 patients with UMMRd. A full overview of patient characteristics can be found in Table S2. Of the patients with known sex, nearly two-thirds (n = 23) were male, and slightly more than half of the patients (55.2%) were diagnosed with CRC under the age of 50 years (Table 1). For patients whose IHC results were available, MSH2 and MSH6 were mostly absent (n = 24, 36.9%), followed by a lack of MLH1 and PMS2 expression (n = 21, 32.3%). Of the 23 patients from whom the CRC stage was available, most (n = 11, 43.8%) patients presented with a stage III CRC. CRCs were more

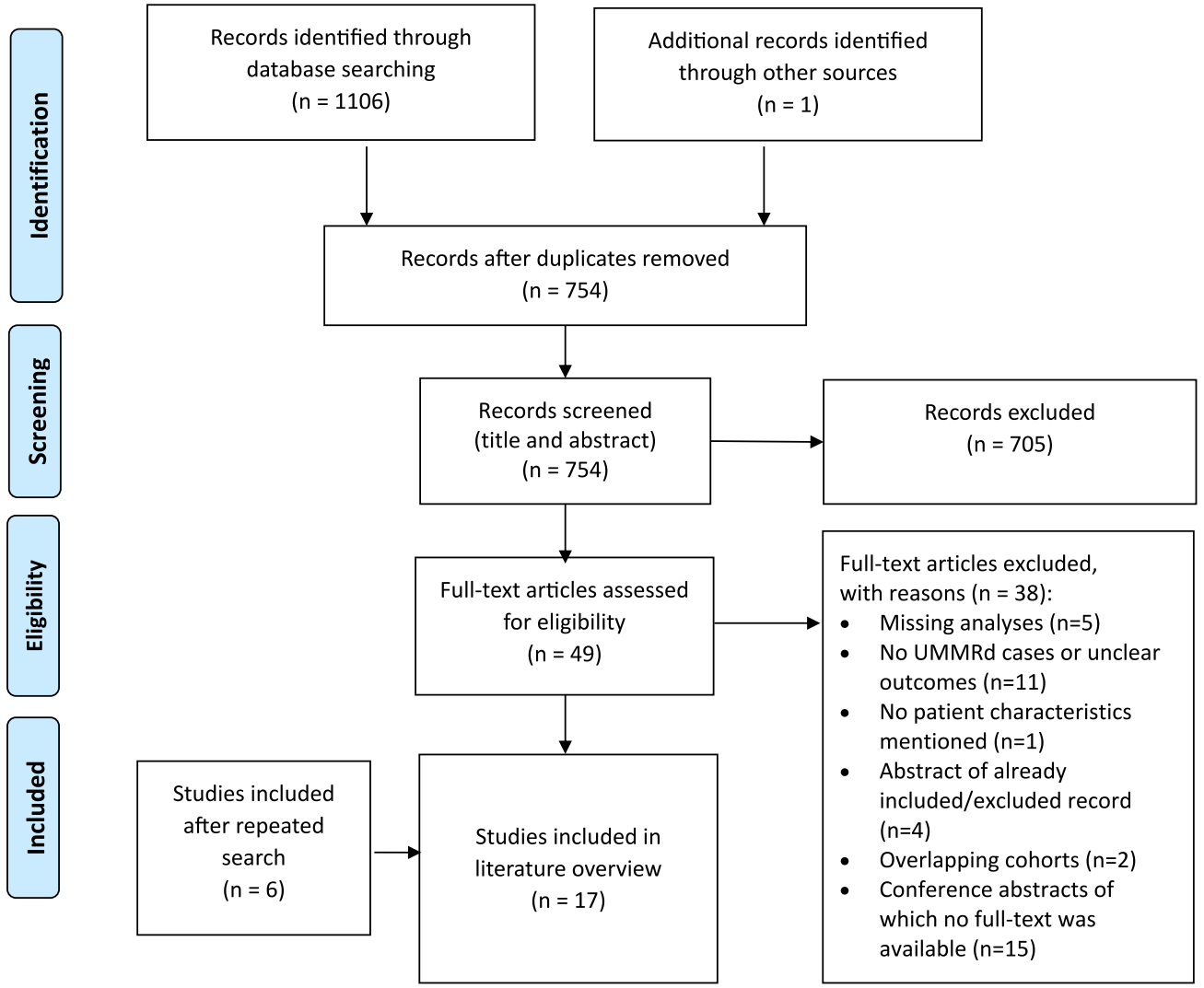

Figure 2. PRISMA chart of literature search

often (n = 16, 57.1%) located in the left colon, including the rectum. In most of the 74 patients (n = 39), a single somatic MMR hit was identified upon further investigation (Table 2), of which nine had a somatic VUS. In six patients, a germline VUS was identified, of whom four had a somatic PV or VUS in the corresponding gene. In 10 patients, somatic PVs were found in *APC*, *MLH3*, *MSH3*, and *POLD1*, genes that are known to be associated with MMR or are causative in cancer development.

In the remaining 19 patients, no MMR hits were identified. Of note, five of these 19 patients had a microsatellite stable (MSS) tumor, indicating probably false IHC results. Of the 14 remaining patients, 10 (partially) lacked MLH1 at IHC (Table S2). In nine of the patients with UMMRd, CRC was diagnosed under age 50; 10 fulfilled revised Bethesda criteria. Of 10 patients with known sex, four were female. Upon assessment of available personal and family history, nine of these 14 patients were indicative for a missed LS germline PV and were therefore deemed suspect LS. In three patients, missed somatic hits were most likely; consequently, these patients are expected to have had a sporadic tumor. For two patients, more clinical information was required to assess the likelihood of missed germline or sporadic PVs.

Erasmus MC cohort

Since 1993, patients with familial or early-onset CRC are seen by a genetic counselor in our center. On average, this comprises about 200 patients each year. Upon complete LS diagnostics, MMRd of most patients could be explained, for example by identification of previously missed germline or somatic PVs due to diagnostics that were not available at the time of counseling. In a total of seven patients from a cohort of approximately 5,000 patients with CRC, MMRd remained unexplained (Table 3).

Erasmus MC cohort: Unexplained patients

In six of the seven patients with UMMRd (patients 1424, 1844, 1846, 1860, 1947, and 1949), the tumor harbored

Table 1. Clinical characteristics of patients with UMMRd summarized

Total	74 (100)
Age, n (% of known total)	
≤50 years	32 (55.2)
>50 years	26 (44.8)
Unknown	16
Sex, n (% of known total)	
Male	23 (62.2)
Female	14 (37.8)
Unknown	37
IHC, n (% of known total)	
MLH1/PMS2−	21 (32.3)
MSH2/MSH6−	24 (36.9)
MLH1−	4 (6.2)
MSH2−	3 (4.6)
MSH6−	2 (3.1)
PMS2−	4 (6.2)
Other	2 (3.1)
None (all MMR proteins present at IHC but MSI-H)	3 (4.6)
No IHC available but MSI-L	1 (1.5)
No information available or unknown	9
CRC	67
Tumor presumed CRC	7
Stage	
I	4 (15.6)
II	6 (21.9)
III	11 (43.8)
IV	2 (18.8)
Unknown	51
Right colon	10 (35.7)
Transverse colon	2 (7.1)
Left colon (including rectum)	16 (57.1)
Unknown	46
Family history, n (% of total)	
Revised Bethesda criteria+ (≥1/5)	42
No family history	6
Unknown family history	26

A full overview can be found in Table S1.

at least one somatic hit, namely a somatic PV in at least one of the corresponding MMR genes or LOH (Table 3). In two patients with a somatic MMR VUS (patients 1860 and 1947), other somatic hits were identified in the tumor (LOH and PVs, respectively). In two patients with UMMRd (1837 and 1844), a germline MMR gene VUS was identified; one of them also had LOH in the tumor in the corresponding gene (Table 3). Age at the time of diagnosis of these seven patients with UMMRd varied between 32 and 62 years; five of them were female, and six patient families fulfilled the revised Bethesda criteria (Table 3). No mutations were detected in other CRC-related genes in patients in whom a CRC germline analysis panel was performed. Four patients (patients 1424, 1860, 1947, and 1949) consented to RNA sequencing of MMR genes. In one (patient 1860), 50% reduced expression was seen for *MSH2*, indicative for skewed or monoallelic expression, probably due to an intronic PV affecting mRNA expression or splicing and causing non-sense-mediated mRNA decay. Indeed, long-range sequencing later detected an intronic PV c.2458 + 976A>G in this patient (patient LLP004).[17] These innovative diagnostics also resulted in MLH1 PV c.306 + 1001_307-642delinsTA detection in patient 1947 (patient LP0032).[17]

Discussion

In the current study, we assessed UMMRd CRC cases from both the literature and from our own center. Upon an extensive, systematic literature search, we identified 17 articles describing 74 patients with known outcomes that supposedly had UMMRd. After performance of complete LS diagnostics, in the majority of these patients, a possible explanation for the UMMRd could be found, namely a missed or yet to be classified germline or somatic MMR hit or PVs in other cancer-predisposing genes. Only 14 patients could be identified in literature who remained truly unexplained. In addition to the literature search, we also assessed to what extent patients diagnosed in our center remained with UMMRd. Over more than 25 years, corresponding to a cohort of approximately 5,000 patients with CRC, only seven patients remained supposedly unexplained after performance of complete LS diagnostics. In all our patients with UMMRd, we found evidence for at least one MMR hit, indicative of a missed or hitherto undetectable second PV in the tumor and/or germline. Afterward, innovative diagnostics indeed detected intronic MMR PVs in two of these patients with UMMRd.[17]

Consequently, we imply that UMMRd cases could virtually all be solved, considering UMMRd a case closed. To our knowledge, this study is the first to show that remaining patients with UMMRd can be explained by defects in one of the MMR genes after all when complete LS diagnostics are applied.

Previous literature

Several research papers have been published concerning the outcomes of universal IHC to detect LS. Approximately 3%–5% of these CRCs turn out to be caused by LS, and the percentages of remaining UMMRd cases differ among

Table 2. Specification of the 74 patients with UMMRd with known outcomes identified in literature

Variants identified	n
Single somatic hit	39
Single somatic MMR variant	29
Partial LOH without variant identified	1
Somatic VUS	9
Germline VUS[a]	6
Germline or somatic PV in other cancer-predisposing genes	10
No germline, somatic variants, or LOH identified (UMMRd)	19
MSS	5
Suspect LS	9
Not suspect LS/probably sporadic	3
Unclear phenotype	2

[a]Four patients were identified with a germline VUS and a somatic variant.

these papers. Recently, the contribution of somatic MMR analysis to solve these remaining cases has been stressed: high percentages of UMMRd cases can be explained by performing somatic analysis of the tumor.[7,9–11] However, all studies reported a substantial proportion of cases remaining unexplained. Most of these patients with supposed UMMRd are, however, diagnosed with one somatic hit in the tumor, for example one MMR PV or LOH only, similarly as in our cohort. Additionally, several studies reported on patients with UMMRd having incomplete LS diagnostics or failed tests. We therefore conclude that complete LS diagnostics can explain virtually all UMMRd cases, assuming one somatic hit to be indicative for another missed or hitherto undetectable hit in the tumor or germline. PV analysis can be hampered by old tissue, and also IHC could be falsely indicative of MMRd. *PMS2* PV analysis is known to be difficult due to the presence of pseudogenes. Additionally, detection of cryptic MMR gene variants or mosaicism is challenging. Based on personal and family history, it is possible to classify remaining patients with UMMRd as probably sporadic MMRd or suspect LS patients. The latter patients and their direct relatives should be advised of LS surveillance. Furthermore, novel techniques such as RNA sequencing and long-range DNA sequencing should be applied to provide more clarity into missed MMR germline PVs.

Explanation of supposedly UMMRd cases

Sporadically, PVs in other cancer-predisposing genes can be causative of UMMRd. [18–21] In 10 cases identified in our literature search, somatic PVs in the *MSH3*, *APC*, *POLD1*, and *MLH3* gene were identified. The authors assumed these PVs to be causative for the CRCs. Of note, it is debatable to what extent these patients are also at risk to have an undetected MMR (germline) variant contributing to or causative for the CRC.

Nevertheless, the majority of patients with UMMRd, both in the literature and in our cohort, were identified with one single MMR hit in their tumor. This makes it likely that these patients carry a second, still undetected, germline or somatic MMR PV. Examples of a likely second hit could be inversions in MMR genes,[22,23] intronic PVs in MMR genes,[24,25] variants in the 3′ untranslated region (UTR) affecting MMR gene expression,[26] PVs still considered as VUSs,[27] or mosaicism.[28] However, if a tumor is found to be ultra-mutated, *POLD1*/*POLE* genes should be assessed, as these could also drive development of secondary MMR variants.[29,30] Additionally, patients with germline PVs in these genes are advised of intensive surveillance to prevent tumor development as well.[31]

Conclusively, our results suggest we should focus on performing complete LS diagnostics to find undetected MMR hits first. Subsequently, multigene panel testing could be performed, as a minority of unexplained cases was found to be explained by PVs in other cancer-predisposing genes. However, focusing on genes without a proven relation to MMR genes does not seem to be indicated in UMMRd cases.

Importance of solving UMMRd

It is very important to gain more insight into the extent to which UMMRd cases can be solved. This does not only appear from the demand for accurate surveillance in patients having UMMRd but also from the question of to what extent germline genetic testing—and, consequently, LS surveillance—is required in their relatives. As most patients with supposed UMMRd turn out to be most likely explained by sporadic MMR PVs, this could provide a more accurate and lower-risk estimate regarding the development of subsequent CRCs or other LS-related tumors. One could even debate to what extent surveillance is required in older relatives of patients with non-suspicious UMMRd whose tissue is not available for reliable somatic testing. Based on these individual patients' phenotype and family history, surveillance advice should be tailored.

Limitations

Our study had several limitations. First, we encountered a lot of missing data in our literature search even after having extensively contacted corresponding authors for the remaining information. This could have led to some patients with UMMRd being overlooked, for example because no characteristics or type of tumor were available whatsoever. Although this is inherent in retrospective research, we have tried to document this as properly as possible in our center. Second, several included studies found somatic PVs in cancer-predisposing genes and assumed this to be causative for the UMMRd. It is debatable to what extent these patients' CRCs are truly explained by these PVs or which of these developed secondary to undetected MMR PVs. Third, one could hypothesize that there might has been some ascertainment bias in the papers published on this topic. Included studies were

Table 3. Patients with UMMRd in Erasmus MC cohort

Patient number	Tumor, age, sex	rBET	IHC and/or MSI analysis outcome	Germline variant in associated MMR gene(s)[a]	Germline variant in CRC panel[b]	MMR gene RNA-seq anomaly	Somatic hit(s)[c]	Probable cause UMMRD
1424	CRC, 38, female	+	MSH2-/MSH6-	none	none	none	LOH *MSH2* and *MSH6*	missed germline or somatic PV *MSH2*
1837	CRC, 40, female	+	MSI-H, no aberrant IHC	*PMS2* VUS c.2528G>C (p.C843S)	Np	Np	none	VUS is PV
1844	CRC, 43, female	+	MSH6-	*MSH6* VUS c.73G>T (p.Ala25Ser)	Np	Np	LOH *MSH6*	missed PV *MSH6* (germline/ somatic)
1846	CRC, 44, female	+	MSH2-/MSH6-	none	Np	Np	PV *MSH2* c.2527delT; p.C843fs	missed germline or somatic PV *MSH2*
1860	CRC, 32, female	+	MSH2-/MSH6-	none	none	abnormal *MSH2* pattern	*MSH6* VUS c.3788G>A (p.R1263H) and LOH *MSH2* and *MSH6*	missed germline PV *MSH2*
1947	CRC, 43, male	+	MLH1-/PMS2-	none	none	none	PV *MLH1* c.1732-2A>G; p.?, PV *MSH2* c.387_388del; p.Q130Vfs*2, *MSH6* VUS c.3163G>A (p.A1055T)	missed germline or somatic PV *MLH1*
1949	CRC, 62, male	–	MLH1-/PMS2-	none in *MLH1*	Np	none	PV *MLH1* c.1838_1854del; p.E613Gfs*2	missed germline or somatic PV *MLH1*

Np, not performed; rBET, revised Bethesda criteria; CRC, colorectal cancer; IHC, immunohistochemistry; LOH, loss of heterozygosity; MSI, microsatellite instability; RNA-seq, RNA sequencing; sCRC, synchronous CRC; VUS, variant of unknown significance.
[a]In MMR genes, at least in MMR genes of which proteins were absent at IHC. All patients with IHC MLH1– did not have *MLH1* promoter hyper methylation.
[b]Germline next-generation sequencing analysis for the genes *MLH1*, *MSH2*/*EpCAM*, *MSH6*, *PMS2*, *APC*, *MUTYH*, *POLE*, *POLD1*, *MSH3*, *NTHL1* (founder c.268C>T [p.Gln90*]), *STK11*, and *PTEN*.
[c]Somatic next-generation-targeted sequencing analysis MMR panel, includes the genes *MLH1*, *MSH2*, *MSH6*, *PMS2*, *BRAF* (exon 11 en 15), *POLD1* (exon 12), and *POLE* (exon 3 en 13).

performed in predominantly Western countries (Europe, US, Canada, Australia, South Korea), which more often perform complete LS diagnostics, and additionally, some studies used an age limit as inclusion criterion. However, we believe that these factors have only slightly affected our results. Fourth, we were hindered by old tissue in one patient in our cohort, which also prevented us from performing complete LS diagnostics. We assumed this to be a problem as well in other included (retrospective) studies.

A key strength of our study, however, was the large cohort of patients derived from both the systematic literature search and our cohort of patients tested in our center for more than 25 years.

In conclusion, by performing complete LS diagnostics, we showed that an explanation for virtually all UMMRd cases can be found. Therefore, other institutions should strive to implement complete LS testing as much as possible to minimize the number of UMMRd CRCs. Based on phenotype and family history, analyses for cryptic or intronic MMR gene PVs should be performed in patients with LS-suspect UMMRd. In less suspect cases, indicative of a missed second hit in the tumor and thus a tumor from sporadic origin, less stringent surveillance could be considered in our opinion. Only occasionally are other cancer-predisposing genes involved in the explanation of UMMRd. Hence, we consider UMMRd to be a case closed.

Data and code availability

The dataset supporting the current study has not been deposited in a public repository but are available from the corresponding author upon request.

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.xhgg.2022.100167.

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Author contributions

Conceptualization, M.C.W.S. and A.W.; data curation, E.L.E., S.M., L.v.L., W.R.R.G.-G., C.M.J.T., T.J.v.H., W.N.M.D., and H.J.D.; formal analysis, E.L.E., S.M., asnd L.v.L.; investigation, E.L.E. and S.M.; methodology, E.L.E., A.W., and M.C.W.S.; project administration, A.W. and M.C.W.S.; resources, W.R.R.G.-G., C.M.J.T., T.J.v.H., W.N.M.D., and H.J.D.; supervision, M.C.W.S. and A.W.; validation, E.L.E., S.M., M.C.W.S., and A.W.; visualization, E.L.E.; writing – original draft, E.L.E.; writing – review & editing, E.L.E.,

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Declaration of interests

The authors declare no competing interests.

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