Mismatch repair deficiency in early-onset duodenal, ampullary, and pancreatic carcinomas is a strong indicator for a hereditary defect

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Abstract

Mismatch repair deficiency (dMMR) is a hallmark of Lynch syndrome (LS), but its prevalence in early-onset (diagnosed under the age of 50 years) duodenal, ampullary, and pancreatic carcinomas (DC, AC, and PC, respectively) is largely unknown. We explored the prevalence of dMMR and the underlying molecular mechanisms in a retrospectively collected cohort of 90 early-onset carcinomas of duodenal, ampullary, and pancreatic origin. dMMR was most prevalent in early-onset DCs (47.8%); more than half of those were associated with hereditary cancer syndromes (LS or constitutional mismatch repair deficiency). All dMMR AC and PC were due to LS. Concordance of dMMR with underlying hereditary condition warrants ubiquitous dMMR testing in all early-onset DC, AC, and PC.

Keywords: mismatch repair deficiency; early-onset duodenal carcinoma; early-onset ampullary carcinoma; early-onset pancreatic carcinoma; Lynch syndrome; germline variants; microsatellite instability; constitutional mismatch repair deficiency syndrome

Introduction

Duodenal, ampullary, and pancreatic carcinomas (DC, AC, and PC, respectively) are rare gastrointestinal (GI) malignancies, typically associated with late age of onset [1]. A minority develops in young patients [1], partly within Lynch syndrome (LS) [2]. LS, previously referred to as hereditary non-polyposis colorectal cancer (HNPPC), is a major hereditary cancer syndrome with an autosomal dominant pattern of inheritance, caused by heterozygous pathogenic germline variants in DNA mismatch repair (MMR) genes (MLH1, MSH2/EPCAM, MSH6, and PMS2) [2]. LS-associated malignancies arise after the subsequent somatic inactivation of the remaining wild-type allele of the affected MMR gene leading to impaired DNA MMR and accumulation of replication errors. Deficient DNA MMR (dMMR) is characterised by absence of MMR proteins and leads to microsatellite instability (MSI), a molecular phenotype characterised by accumulation of multiple alterations within microsatellite repeat regions throughout the genome [2]. The presence of MSI/dMMR in tumours can be based on germline, as in LS or constitutional mismatch repair deficiency (CMMRD) syndrome, or somatic MMR gene defects, such as silencing of MLH1 by promoter hypermethylation or due to biallelic pathogenic somatic variants in MMR genes, resulting in loss of corresponding MMR protein expression [2]. LS patients have an increased lifetime risk to develop colorectal cancer (CRC) and a variety of extracolonic malignancies [2]. Since early age of cancer onset can be a hallmark of an underlying hereditary condition [3], in this study, we investigated the incidence of dMMR in patients diagnosed with DC, AC, and PC before the age of 50 years (<50).

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Materials and methods

Patient cohorts

We performed a nationwide retrospective search (LZV977) in the Nationwide Network and Registry of Histopathology and Cytopathology in the Netherlands (PALGA) [4], with approval of their Privacy Commission and Scientific Council, to identify all patients diagnosed with primary DC and PC under the age of 50 in the Netherlands between January 2002 and December 2012. To have sufficient tissue for analyses, only resection specimens were requested. A substantial number of patients initially diagnosed with PC had, in fact, AC. Considering their high prevalence in our cohort (n = 23), and because ACs have generally better prognosis compared to conventional pancreatic adenocarcinomas [5], they were categorised separately. Normal and tumour tissue materials (formalin-fixed paraffin-embedded [FFPE] tissue blocks) were requested from eligible cases, resulting in 23 cooperating laboratories throughout the Netherlands. Additional information including the nationwide personal pathology history was requested for all included patients (contains information up to February 2020).

Findings in early-onset DC patients were compared to the internal Radboud University Medical Center cohort of late-onset (diagnosed above the age of 50 years) DC patients (DC ≥ 50) (n = 18).

Tissue materials were reviewed by pathologist MAJM-vZ, and clinicopathological characteristics were extracted from individual pathology reports.

This study (CMO-2017-3780) was approved by the local ethical committee of the Radboud University Medical Center. Personal data concerning individual patients were anonymised prior to obtaining patient data and tissue materials, thereby preventing identification of the individuals included in the study. In consequence, patient consent was not required, and the results of germline analyses could not be shared with patients, their families, and physicians.

Research strategy

Immunohistochemical staining for MLH1, MSH2, MSH6, and PMS2 was used to determine dMMR status. In cases with aberrant staining, MSI analysis was performed, followed by methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) to detect somatic MLH1 promoter hypermethylation in cases with MLH1 and/or PMS2 loss. Single-molecule molecular inversion probes (smMIP) sequencing for the MMR genes was performed on normal and tumour DNA. MLPA analysis to detect exon deletions and duplications was performed on cases without pathogenic variants or with only one somatic event [6].

IHC, MSI analysis, and somatic hypermutation

Immunohistochemistry (IHC) for MMR (MLH1, MSH2, MSH6, and PMS2) protein expression was performed using standard procedures. Tissue microarray slides were stained with antibodies against MLH1 (clone G168-15; BD Biosciences, San Jose, CA, USA), MSH2 (clone GB12; Calbiochem/Merck, Darmstadt, Germany), MSH6 (clone EPR3945; Abcam, Cambridge, UK), and PMS2 (clone A16-4; BD Biosciences, San Jose, CA, USA). Based on the IHC pattern, all tumours were classified as MLH1-deficient (aberrant MLH1 and PMS2 staining), PMS2-deficient (loss of PMS2 staining), MSH2-deficient (aberrant MSH2 and MSH6 staining), and MSH6-deficient (loss of MSH6 staining). Scoring was performed by two blinded observers (MAJM-vZ and IDN) as described by Overbeek et al [7].

Genomic DNA was extracted from deparaffinised FFPE normal and tumour tissue for all cases with aberrant expression of at least one MMR protein, according to standard procedures. Regions with at least 30% neoplastic cells (when possible) were dissected for tumour DNA, as required for sensitive MSI detection. MSI was assessed using five mononucleotide markers BAT25, BAT26, NR21, NR24, and NR27 as described previously [8]. Tumours without any unstable markers were categorised as microsatellite stable (MSS), and cases with more than one unstable marker as having a high degree of MSI (MSI-H). In cases with doubtful findings or low tumour cell percentage, MSI assessment was performed using targeted smMIP-based next-generation sequencing (NGS) PATHv2D panel including 55 sensitive markers for MSI detection. Library preparation and sequencing using smMIP-based libraries were performed on a NextSeq 500 (Illumina, San Diego, CA, USA) according to the manufacturer’s instructions as described previously [9]. The presence of ≥30% of unstable markers out of the total assessable was scored as MSI.

To detect somatic MLH1 promoter hypermethylation, MS-MLPA was performed on tumour DNA from MLH1- and PMS2-deficient cases using standard procedures during routine diagnostic procedures (MRC-Holland, Amsterdam, The Netherlands).
Germline and somatic mutation analyses

Targeted sequencing

Targeted smMIP-based NGS was performed on normal and tumour DNA. Library preparation and sequencing using NextSeq 500 approach were performed as described previously [9]. The entire coding regions of four MMR genes, MLH1 (NM_000249.3), MSH2 (NM_000251.2), MSH6 (NM_000179.2), and PMS2 (NM_000535.5) were sequenced. MLH1- and PMS2-
decient cases were initially sequenced for MLH1, followed by PMS2 if no MLH1 variants were detected. All MSH2- and MSH6-deficient cases were sequenced for MSH2 and MSH6. Sequencing reads were aligned to the reference genome (human genome 19). Variants were called and sequencing results were analysed using Sequence Pilot (JSI Medical Systems, Ettenheim, Germany) software for genetic analysis as described previously [9]. Identified variants were evaluated with Alamut Visual

Table 1. Clinicopathological characteristics and MSI status of the patients.

|                      | Early-onset cases (<50 years) | P values | Late-onset cases (≥50 years) | P value |
|----------------------|------------------------------|----------|-------------------------------|---------|
|                      | All, n = 90                  | Duodenal, n = 23 | Ampullary, n = 23 | Pancreatic, n = 44 | DC versus AC | AC versus PC | DC versus PC | Duodenal, n = 18 |     |
| Gender, n (%)        |                              |           |                              |          |           |           |           |           |           |  |
| Male                 | 53 (58.9)                    | 15 (65.2) | 10 (43.5)                    | 28 (63.6) | 0.139     | 0.114     | 0.898     | 13 (72.2)    | 0.632 |
| Female               | 37 (41.1)                    | 8 (34.8)  | 13 (56.5)                    | 16 (36.4) |           |           |           | 5 (27.8)     |       |
| Age, median (range)  | 43 (17–49)                   | 46 (17–49)| 44 (33–49)                   | 43 (30–49)| 0.691     | 0.005     | 0.174     | 70 (57–77)   | <0.001|
| Histological type, n (%) |                          |           |                              |          |           |           |           |           |         |
| Adenocarcinoma NOS   | 84 (93.3)                    | 20 (87)   | 21 (91.3)                    | 43 (97.7) | >0.999    | 0.114     | 0.113     | 14 (77.8)    | 0.654 |
| Mucinous             | 3 (3.3)                      | 1 (3.4)   | 2 (8.7)†                     | 0        |           |           |           | 2 (11.1)     |       |
| Signet ring cell     | 1 (1.1)                      | 1 (3.4)   | 0                            | 0        |           |           |           | 2 (11.1)     |       |
| Adenosquamous        | 1 (1.1)                      | 0         | 0                            | 1 (2.3)  |           |           |           | 0           |       |
| Medullary            | 1 (1.1)                      | 1 (3.4)   | 0                            | 0        |           |           |           | 0           |       |
| Diameter, median (range) (cm)† | 3 (0.5–7) | 4.4 (0.9–6) | 2 (0.5–7) | 3 (1.5–6.5) | 0.004 | 0.01 | 0.028 | 4 (1.4–13) | 0.946 |
| Differentiation grade, n (%) |                    |           |                              |          |           |           |           |           |         |
| Well/moderate        | 57 (63.3)                    | 14 (60.9) | 13 (56.5)                    | 30 (68.2) | 0.765     | 0.345     | 0.549     | 12 (66.7)    | 0.702 |
| Poor                 | 33 (36.7)                    | 9 (39.1)  | 10 (43.5)                    | 14 (31.8) |           |           |           | 6 (33.3)     |       |
| T-stage, n (%)†      |                              |           |                              |          |           |           |           |           |         |
| T1                   | 5 (5.6)                      | 2 (8.7)   | 3 (13)                       | 0        | 0.018     | <0.001    | <0.001    | 1 (5.6)      | 0.304 |
| T2                   | 10 (11.1)                    | 0         | 6 (26.1)                     | 4 (9.1)  |           |           |           | 3 (16.7)     |       |
| T3                   | 58 (64.4)                    | 10 (43.5) | 9 (39.1)                     | 39 (88.6)|           |           |           | 7 (38.9)     |       |
| T4                   | 15 (16.7)                    | 11 (47.8) | 4 (17.4)                     | 0        |           |           |           | 7 (38.9)     |       |
| Unknown              | 2 (2.2)                      | 0         | 1 (4.3)                      | 1 (2.3)  |           |           |           | 0           |       |
| N-stage, n (%)†      |                              |           |                              |          |           |           |           |           |         |
| N0                   | 28 (31.1)                    | 11 (47.8) | 7 (30.4)                     | 10 (22.7) | 0.014     | 0.328     | <0.001    | 9 (50)       | 0.065 |
| N1                   | 52 (57.8)                    | 6 (26.1)  | 13 (56.5)                    | 33 (75)  |           |           |           | 9 (50)       |       |
| N2                   | 5 (5.6)                      | 5 (21.7)  | 0                            | 0        |           |           |           | 0           |       |
| Unknown              | 5 (5.6)                      | 1 (4.3)   | 3 (13)                       | 1 (2.3)  |           |           |           | 0           |       |
| Overall MSI/dMMR, n (%) |                          |           |                              |          |           |           |           |           |         |
| MSS/pMMR             | 76 (84.4)                    | 12 (52.2) | 22 (95.7)                    | 42 (95.5) | 0.002     | >0.999    | <0.001    | 17 (94.4)    | 0.005 |
| MSI/dMMR             | 14 (15.6)                    | 11 (47.8) | 1 (4.3)                      | 2 (4.5)  |           |           |           | 1 (5.6)      |       |
| Immunohistochemical analysis of MSI/dMMR cases, n (%) |            |           |                              |          |           |           |           |           |         |
| MLH1/PMS2            | 4 (28.6)                     | 3 (27.3)  | 1 (100)                      | 0        | 0.5       | 0.333     | 0.026     | 1 (100)      | 0.5   |
| PMS2                 | 6 (42.9)                     | 6 (54.5)  | 0                            | 0        |           |           |           | 0           |       |
| MSH2/MSH6            | 2 (14.3)                     | 2 (18.2)  | 0                            | 0        |           |           |           | 0           |       |
| MSH6                 | 2 (14.3)                     | 0         | 0                            | 2 (100)  |           |           |           | 0           |       |
| Molecular background of MSI/dMMR cases, n (%) |            |           |                              |          |           |           |           |           |         |
| CMMRD                | 2 (14.3)                     | 2 (18.2)  | 0                            | 0        | >0.999    | NA        | >0.999    | 0           | 0.583 |
| LS                   | 8 (57.1)                     | 5 (45.5)  | 1 (100)                      | 2 (100)  |           |           |           | 0           |       |
| Unclassified         | 2 (14.3)                     | 2 (18.2)  | 0                            | 0        |           |           |           | 0           |       |
| Non-hereditary       | 2 (14.3)                     | 2 (18.2)  | 0                            | 0        |           |           |           | 1 (100)      |       |

Fisher’s exact test was used when at least one expected or observed value was below 5; in other cases, chi-square (χ²) test was used. Values in bold indicate statistically significant results (significance considered at p < 0.05).

NA, not applicable; NOS, not otherwise specified.

†Calculated only using patients with sufficient data available for certain characteristics.

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| Patient ID | Histological type and differentiation | Sex | Age | Gene | Germline | Somatic | Diagnosis | Other tumours (age) |
|------------|-------------------------------------|-----|-----|------|----------|---------|-----------|-------------------|
| DC11       | Adenocarcinoma NOS, intestinal       | M   | 17  | PMS2 | c.137G>T p.(Ser46Ile) (class 4, likely pathogenic) and c.2174+1G>A p.? (splice site) (class 5, pathogenic) | None | CMMRD     | Serrated adenoma colon (15), 3× tubulovillous adenoma duodenum (16, 17, 17), jejunal adenocarcinoma (19), 8× colon adenomas (20), tubulovillous adenoma jejunum (20), T-cell acute lymphoblastic leukaemia (21), multiple adenomas colon (22) |
| DC12       | Adenocarcinoma NOS, intestinal       | M   | 32  | PMS2 | c.[63+1_164–1]_ [603+1_804–1] (exons 3–7 deletion) (class 5, pathogenic) and c.87_2174+2175–1 (exons 1–12 deletion) (class 5, pathogenic) | None | CMMRD     | Colorectal adenomas (10, 10, 12, 15, 16, 17, 19, 21), CRC (21), adenomatous polyposis colon (21), duodenal adenoma (32), jejunal adenocarcinoma (32), ileal adenoma (32), diffuse astrocytoma with progression towards secondary glioblastoma (34) |
| DC16       | Adenocarcinoma NOS, intestinal       | M   | 46  | PMS2 | c.247_250dup p.(Thr84Ile) (class 5, pathogenic) and c.1639dup p.(Glu547Lysfs*15) (class 5, pathogenic) | LOH and c.1639dup p.(Ser547Phefs*15) (class 5, pathogenic) | LS         | Tubular adenoma rectum (60) |
| DC13       | Adenocarcinoma NOS, intestinal       | F   | 46  | MSH2 | c.1139delT p.(Leu380Tyrfs*32) (class 5, pathogenic) | c.233dup p.(Cys778Leufs*9) (class 5, pathogenic) | LS         | CRC (24), CRC (26), endometrioid ovarian carcinoma (39), tubulovillous adenoma rectum (56), 3× tubular adenomas colon (58) |
| DC10       | Adenocarcinoma NOS, intestinal       | M   | 48  | PMS2 | c.736_741delTGTGTGAAG p.(Pro246Cysfs*3) (class 5, pathogenic) | c.859dup p.(Glu287Lysfs*12) (class 5, pathogenic) | LS         | Hyperplastic polyp sigmoid (52), hyperplastic polyp sigmoid and rectum (54), 2× tubular adenomas colon ascendens (57), prostate adenocarcinoma (58) |
| DC17       | Adenocarcinoma NOS, intestinal       | F   | 48  | PMS2 | c.[23+1_24–1]_ [163+1_164–1] (exon 2 deletion) (class 5, pathogenic) | c.1A>G p.Met1? (class 4, likely pathogenic) | LS         | Adenocarcinoma NOS breast (52) |
| DC19       | Adenocarcinoma NOS, intestinal       | F   | 49  | MLH1 | c.1896G>T p.(Ser632Ile) (class 5, pathogenic) | NA*       | LS         | Endometrial adenocarcinoma (55) |
| DC14       | Adenocarcinoma NOS, intestinal       | M   | 29  | MSH2 | NA*      | c.679_687delTCTCTCTAAAAA p.(Arg227Phefs*5) (class 5, pathogenic) | Unclassified | None |
| DC23       | Adenocarcinoma NOS, intestinal       | M   | 49  | MLH1 | NA*      | c.531_532delinsCT p.(Leu177_ Glu178delinsPhe*) (class 5, pathogenic) | Unclassified | CRC (49), colon adenomas (49, 50, 53, 57, 57), hyperplastic polyp sigmoid (57) |
| DC25       | Adenocarcinoma, medullary           | M   | 34  | PMS2 | None     | c.338C>A p.(Ser113*), biallelic (class 5, pathogenic) | Sporadic DC | Tubular adenoma colon (34) |
| Patient ID | Histological type and differentiation | Sex | Age | Gene | Germline | Somatic | Diagnosis | Other tumours (age) |
|------------|--------------------------------------|-----|-----|------|----------|---------|-----------|-------------------|
| DC1        | Adenocarcinoma NOS, intestinal       | M   | 46  | MLH1 | None     | c.198_193del (entire gene deletion), biallelic (class 5, pathogenic) | Sporadic DC | Seminoma testis (24), colon adenomas (51, 52, 52, 55, 55, 57, 59, 59), 9 × colorectal adenomas (62) |
| DC ≥ 50   | Adenocarcinoma NOS, intestinal       | M   | 66  | MLH1 | None     | c.2074_2078del p. (Ser692Glyfs*10) (class 5, pathogenic) and LOH | Sporadic DC | Nil |
| AC < 50   | Adenocarcinoma NOS, intestinal       | M   | 49  | MLH1 | c.677+1_678-1_ (884+1_885-1) (exons 9–10 deletion) (class 5, pathogenic) | LS       | 2 × CRC: transversum and caecum (43), colon adenomas (43, 44), 2 × CRC: sigmoid and rectosigmoid (56), adenocarcinoma lung (57) |
| PC < 50   | Adenocarcinoma NOS, pancreatobiliary | M   | 41  | MSH6 | c.3438+1G>A p.? (splice site) (class 4, likely pathogenic) | NA*     | LS       | None |
| PC13      | Adenocarcinoma NOS, pancreatobiliary | M   | 42  | MSH6 | c.2982C>G p.(Tyr994*) (class 5, pathogenic) | NA*     | LS       | None |

Transcripts (hg19): MLH1 (NM_000249.3), MSH2 (NM_000251.2), MSH6 (NM_00179.2) and PMS2 (NM_000535.5). Malignant tumours are highlighted in bold.

F, female; M, male; NA, not assessable; NI, no information; NOS, not otherwise specified.

*Sequencing on tumour tissue has not worked out; therefore, the presence of second somatic hit could not be assessed.

†Unclassified cases with pathogenic somatic variants; molecular analysis on normal DNA was not possible due to the limitations of material.

‡MSI status as well as the presence of LOH could not be assessed due to low tumour cell percentage.
version 2.13 (SOPHiA GENETICS, Lausanne, Switzerland) software and publicly available databases such as ClinVar [10] and InSiGHT [11], and categorised based on the current guidelines for variant classification defined by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [12].

**MLPA assays**

All MSI/dMMR cases without detectable pathogenic variant or with one identified somatic event underwent MLPA analysis to detect exon deletions and duplications depending on the affected MMR gene based on NGS. MLPA was performed on normal and tumour DNA using the MRC-Holland SALSA probe mix assays (MRC-Holland) according to the manufacturer’s instructions.

**Diagnostic criteria**

Based on germline and somatic mutation analyses, all cases were classified as having: (1) **CMMRD syndrome** when there were biallelic pathogenic germline variants in one of the MMR genes. Aberrant IHC expression of the affected protein in normal tissue confirmed the presence of biallelic hits. The personal history of patients was studied to identify other malignancies concordant with CMMRD to support the diagnosis; (2) **LS** when a pathogenic germline variant was detected in normal DNA, confirmed (when possible) by a somatic inactivating event (second somatic pathogenic variant or loss of heterozygosity [LOH]) in tumour DNA; (3) **Unclassified** when there was a pathogenic somatic event in tumour DNA but germline DNA could not be assessed due to the limitations of material; (4) **Non-hereditary or sporadic** when the causative pathogenic variant was present in tumour, but not in normal DNA, together with the second inactivating event, by means of second somatic hit or LOH.

**Statistical analysis**

Demographics, clinical data, and pathological characteristics were analysed. Chi-square ($\chi^2$) test or Fisher’s exact test (if observed or expected sample size in the contingency table was less than 5) was used for categorical data; the Kruskal–Wallis test was used for continuous variables. Two-sided $P$ values of <0.05 were considered as statistically significant. Statistical analyses were calculated only using patients with sufficient available data. All analyses were performed using the SPSS software (IBM SPSS Statistics, version 25 [SPSS Inc., Chicago, IL, USA]).

**Results**

Of the 162 identified patients, 90 were included (Figure 1A), and their characteristics are summarised in Table 1. Tumours diagnosed before the age of 30 years were only present in the DC group (supplementary material, Table S1).

Almost half of DC $\geq 50$ (11/23) exhibited dMMR (Figure 1B, Table 2). Two patients (2/11) had CMMRD syndrome caused by biallelic pathogenic germline variants in MMR genes, five patients (5/11) had LS, two (2/11) were categorised as unclassified due to insufficient quality of normal tissue for germline testing, and two (2/11) had biallelic somatic MMR aberrations (Table 2). Both CMMRD patients had a personal history of associated malignancies (Table 2). Only 1 of 18 DC $\geq 50$ was dMMR due to biallelic somatic inactivation of MLH1 (Figure 1E, Table 2).

In contrast, dMMR was rare in AC $< 50$ (1/23) and PC $< 50$ (2/44), but indicative of LS in all three cases (Figure 1C,D, Table 2).

All but one dMMR tumours had adenocarcinoma, not otherwise specified histology. A single dMMR early-onset DC (patient DC25) had medullary histology (Table 2); this case was sporadic (i.e. non-hereditary) based on the presence of biallelic somatic PMS2 variant. Among all early-onset carcinomas, dMMR tumours were significantly larger in diameter ($p = 0.031$) compared to MMR proficient (pMMR) cases; however, the difference was not significant across specific cancer types, likely due to the small numbers of dMMR cases in separate groups (supplementary material, Table S2). No significant differences in other clinicopathological features were detected between dMMR versus pMMR tumours across all cancer types (supplementary material, Table S2).

**Discussion**

In young patients with DC, AC, and PC, dMMR is a good indicator for a germline MMR defect, with a remarkably high incidence of CMMRD and LS in DC $< 50$. None of the dMMR tumours exhibited hypermethylation of the MLH1 promoter. A minority of cases was due to biallelic somatic aberrations. Our study focuses on patients with early-onset tumours, as they carry an increased chance of an associated hereditary condition. Indeed, no cases of CMMRD or LS were detected in DC $\geq 50$. 

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In our cohort of 90 young patients, MSI/dMMR was present in half of DCs, while ACs and PCs rarely showed dMMR. Because of rarity, large studies on DC are either missing or include DC in cohorts of perianampullary or other small bowel carcinomas (SBCs). The observed dMMR frequency in early-onset DCs is more than two-fold higher compared to 13–23% dMMR in unselected DCs [13,14]. The high dMMR yield in our cohort could be explained by preselection on the basis of young age, resulting in an increased number of patients with hereditary predisposition. Our findings are consistent with significantly lower age at diagnosis of LS-related SBCs (mean age: 54.6 years) compared to sporadic dMMR (68.8 years) and pMMR (66.6 years) SBCs [14]. Indeed, we identified LS solely in the early-onset group, where it was the most common molecular background in dMMR tumours (5/11). No LS was detected in any DC ≥ 50, and the single dMMR case was sporadic. Our findings suggest that age-based preselection of young (<50 years) DC patients can significantly improve the yield of dMMR and LS screening.

Next to LS, two dMMR DC < 50 patients had CMMRD, a very rare autosomal recessive syndrome, so far reported in only ~200 patients worldwide [15]. CMMRD is caused by biallelic pathogenic germline variants in MMR genes [16]. Our patients had PMS2 aberrations, which is the most frequently affected gene in CMMRD [15,16]. The spectrum of CMMRD-associated neoplasms differs from LS and most typically includes haematological malignancies, brain and central nervous system tumours, and GI cancers [15]. GI polyps and CMMRD-related cancers usually manifest in childhood and early adolescence [17]. Accordingly, CMMRD patients in this study developed multiple polyps since childhood (starting at age 10 and 15 years, respectively) and presented with multiple malignancies, consistent with the CMMRD phenotype, at a young age. Because of its rarity and, as is typical for autosomal recessive syndromes, lack of family history for the index patient, CMMRD is often unrecognised [18]. As the incidence of CMMRD is extremely low, hindering the possibility of large-scale studies and proper estimation, the presence of two such patients should be considered as an extra incentive to analyse DC for MSI/dMMR. It is crucial to recognise CMMRD in view of the severity of this condition, as these patients are prone to develop multiple tumours during their lifetime and their parents are obligatory LS patients [16]. Particular hallmarks that are strongly suggestive of CMMRD, and were also present in our patients, include very early age at cancer onset, typical CMMRD spectrum malignancies in the personal history, germline MSI, and loss of IHC expression of the affected MMR protein also in normal tissue [18].

dMMR was rare in AC < 50 and PC < 50 but, when present, was fully concordant with LS. Although rare, a detected frequency of 4.5% dMMR in PC < 50 was still higher compared to unselected PCs (1–2.5%) [13,19–21]. About 10% of unselected ACs are dMMR, but frequencies vary among studies [13]. Only a minority of unselected ACs and PCs occurs within LS [13], further emphasising that unselected dMMR testing in ACs and PCs would have limited utility for identification of LS patients. Recognising LS is crucial for surveillance of affected individuals and their relatives. MSI/dMMR is a sensitive biomarker for LS detection in all LS spectrum malignancies, including SBC, AC, and PC, detected in ~96, 100, and ~76% of these cancers, respectively, in LS patients [13].

Two standard reference methods for MSI/dMMR detection, namely MSI analyses by polymerase chain reaction or NGS and screening for MMR protein loss by IHC, are valid initial screening modalities for MSI/dMMR detection in tumour specimens [22]. Based on the proven cost-effectiveness and high correlation between dMMR and MSI, when all four MMR proteins are tested [22], our screening strategy included IHC staining for all four MMR proteins, followed by MSI analyses for dMMR cases. IHC is a highly sensitive, low time-intensive, and routinely used technique, and is feasible with small and low percentage neoplastic cells tumour specimens [22], as were some tumours in our series. Furthermore, IHC is capable of identifying the affected protein, providing a direction for subsequent genetic testing [22]. However, false negative results may occur in the presence of antigenically intact, but catalytically inactive protein, which otherwise would demonstrate MSI [22].

In our cohort of early-onset carcinomas, dMMR was present in almost half of DC < 50, but was rarely observed in AC < 50 and PC < 50. This difference may potentially be attributed to high proliferation rates of intestinal tissues, as GI epithelium has the highest proliferation rate across all tissue types, enabling accumulation of multiple mutations in each replication cycle [23,24]. Another factor potentially contributing to high dMMR rates in DCs, similar to CRCs, is exposure to dietary mutagens that might have direct toxic effects on GI epithelium [23]. Furthermore, the presence of highly mutable sequences across genes critical for specific tissues can contribute to the observed differences in dMMR rates across various cancer types; however, the exact mechanisms underlying cell- and tissue-type specificity of dMMR cancers are yet to be fully understood [23].

Remarkably, no dMMR tumours developed as a consequence of somatic MLH1 promoter hypermethylation.
This could potentially be explained by an early age at cancer diagnosis in our cohort, resulting in 10/14 dMMR early-onset carcinomas having developed in the context of a hereditary syndrome, either CMMRD or LS. For instance, in a consecutive unselected cohort of 400 SBCs (22.3 and 4.4% dMMR in resected and biopsy SBCs, respectively), MLH1 promoter hypermethylation was the most common cause of the dMMR phenotype, explaining 40.5% of dMMR resections and 66.7% of dMMR biopsy specimens. This is potentially related to an overrepresentation of patients with coeliac disease, known to be associated with dMMR, particularly in the context of MLH1 promoter hypermethylation [14]. The mean age at diagnosis in the reported cohort was significantly higher in sporadic dMMR SBCs (68.8 years) compared to LS-associated SBCs (54.6 years) [14].

Testing for dMMR status is crucial not only for identification of patients with hereditary predisposition, but also in view of patient prognostication and application of novel targeted therapies. MSI/dMMR CRCs [25,26] and SBCs [27,28] have been shown to have improved prognosis compared to MSS/pMMR tumours. The presence of dMMR is a predictive biomarker for sensitivity to immunotherapies, such as the programmed cell death 1 (PD-1) immune checkpoint inhibitor pembrolizumab [29]. MSI/dMMR tumours demonstrated the highest response rates to PD-1 blockade with durable responses and significantly improved overall survival [29]. Currently, application of pembrolizumab is approved for all advanced unresectable solid tumours with MSI/dMMR in a tissue-agnostic manner [30].

In conclusion, the presence of dMMR in early-onset AC, PC, and, particularly, DC was strongly associated with hereditary MMR gene defects, the majority presenting with LS. These findings, together with relevance for patient prognostication and eligibility for immunotherapies, support dMMR and MSI testing in young patients with these types of cancer.

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Author contributions statement

VK acquired the data, analysed and interpreted the data, drafted the manuscript and critically revised the manuscript for important intellectual content. LAAB analysed and interpreted the data, drafted the manuscript, critically revised the manuscript for important intellectual content and supervised the study. MAJM-vZ collected samples, acquired the data, analysed and interpreted the data, and critically revised the manuscript for important intellectual content. MJLL conceived and designed the study, analysed and interpreted the data, drafted the manuscript, critically revised the manuscript for important intellectual content, and supervised the study. IDN conceived and designed the study, analysed and interpreted the data, drafted the manuscript, critically revised the manuscript for important intellectual content.

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Table S1. An overview of the patient cohorts

Table S2. Clinicopathological characteristics of MSI/dMMR versus MSS/pMMR cases