







# Evaluation of yield and experiences of age-related molecular investigation for heritable and nonheritable causes of mismatch repair deficient colorectal cancer to identify Lynch syndrome

Janet R. Vos<sup>1</sup>  | Ingrid E. Fakkert<sup>1</sup>  | Liesbeth Spruijt<sup>1</sup> | Riki W. Willems<sup>2</sup> | Sera Langenveld<sup>3</sup> | Arjen R. Mensenkamp<sup>1</sup>  | Edward M. Leter<sup>3</sup> | Iris D. Nagtegaal<sup>2</sup>  | Marjolijn J. L. Ligtenberg<sup>1,2</sup>  | Nicoline Hoogerbrugge<sup>1</sup>  | FINAL Group<sup>†</sup>

<sup>1</sup>Department of Human Genetics, Radboud university medical center, Nijmegen, The Netherlands

<sup>2</sup>Department of Pathology, Radboud university medical center, Nijmegen, The Netherlands

<sup>3</sup>Department of Clinical Genetics, Maastricht University Medical Center, Maastricht, The Netherlands

## Correspondence

Nicoline Hoogerbrugge, Department of Human Genetics, Radboud university medical center, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.  
Email: nicoline.hoogerbrugge@radboudumc.nl

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## Abstract

Universal mismatch repair deficiency (dMMR) testing of colorectal cancer (CRC) is promoted as routine diagnostics to prescreen for Lynch syndrome. We evaluated the yield and experience of age-related molecular investigation for heritable and nonheritable causes of dMMR in CRC below age 70 to identify Lynch Syndrome. In a prospective cohort of 3602 newly diagnosed CRCs below age 70 from 19 hospitals, dMMR, *MLH1* promoter hypermethylation, germline MMR gene and somatic MMR gene testing was assessed in daily practice. Yield was evaluated using data from the Dutch Pathology Registry (PALGA) and two regional genetic centers. Experiences of clinicians were evaluated through questionnaires. Participating clinicians were overwhelmingly positive about the clinical workflow. Pathologists routinely applied dMMR-testing in 84% CRCs and determined 10% was dMMR, largely due to somatic *MLH1* hypermethylation (66%). Of those, 69% with dMMR CRC below age 70 without hypermethylation were referred for genetic testing, of which 55% was due to Lynch syndrome (hereditary) and 43% to somatic biallelic pathogenic MMR (nonhereditary). The prevalence of Lynch syndrome was 18% in CRC < 40, 1.7% in CRC age 40-64 and 0.7% in CRC age 65-69. Age 65-69 represents most cases with dMMR, in which dMMR due to somatic causes (13%) is 20 times more prevalent than Lynch syndrome. In conclusion, up to age 65 routine diagnostics of (non-)heritable causes of dMMR CRCs effectively identifies Lynch syndrome and reduces Lynch-like diagnoses. Above age 64, the effort to detect one Lynch syndrome patient in dMMR CRC is high and germline testing rarely needed.

**Abbreviations:** d, deficiency; CRC, colorectal cancer; Hypermethylation, *MLH1* promoter hypermethylation; IHC, immunohistochemistry of the mismatch repair proteins; IQR, interquartile range, that is, 25th percentile to 75th percentile; LS, Lynch syndrome; MMR, mismatch repair; MSI, Microsatellite instability; NGS, next-generation sequencing; PALGA, Nationwide network and registry of histopathology and cytopathology in the Netherlands; U-MMR, universal mismatch repair deficiency testing workflow.

<sup>†</sup>The Finding Them All (FINAL) Group members listed in Appendix.

Marjolijn J. L. Ligtenberg and Nicoline Hoogerbrugge contributed equally to this study.

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**KEYWORDS**

colorectal cancer, mismatch repair deficiency, Lynch syndrome, germline mutation, somatic mutation

**1 | INTRODUCTION**

Recognition of Lynch Syndrome is of great importance, as patients and relatives may benefit from surveillance or risk-reducing surgery to reduce cancer risks and improve survival.<sup>1-3</sup> Lynch syndrome is the most common cause of hereditary colorectal cancer (CRC) and is also associated with increased risk of endometrial, ovarian, gastric and other cancers.<sup>4,5</sup> To enhance the detection of Lynch syndrome a prescreen by mismatch repair deficiency (dMMR) testing of patients with CRC has more and more become routine diagnostics. In addition, dMMR testing has a purpose for oncological treatment as dMMR in CRC is associated with improved prognosis, diminished Fluorouracil chemotherapy response and high sensitivity to immunotherapy with immune checkpoint inhibitors.<sup>6-8</sup> Such dMMR testing can either be done by microsatellite instability testing (MSI) or immunohistochemistry of the mismatch repair proteins (IHC).<sup>9</sup>

In patients with Lynch syndrome, the mean age at CRC diagnosis is 45 to 60 years.<sup>5</sup> While in the general western population the median age at CRC diagnosis is 70 years.<sup>10</sup> The presence of heritable and nonheritable causes of dMMR changes with the age of CRC diagnosis, with more hereditary CRCs in the younger age groups.<sup>1,11</sup> As CRC is a very frequently occurring diagnosis and the majority of patients with CRC is above age 60 to 70 years, it is relevant to know up to what age routine diagnostics for heritable causes of dMMR is indicated. Current policies and guidelines show a broad variation up to what age diagnostics for heritable causes of dMMR is performed.<sup>9,12,13</sup> For example, in the United Kingdom, there are no age limits, and in the Netherlands, the age limit is set at below age 70 at CRC diagnosis. Therefore, more clarity is needed on age limits.

Diagnostics for heritable causes of dMMR is complex and expensive. Generally, dMMR can be diagnosed either by loss of MMR protein expression or microsatellite instability and is present in 10% to 15% of all CRCs.<sup>14</sup> In case of dMMR in CRC this can be explained by one of three causes that needs further molecular investigation: (a) a heritable pathogenic germline variant in a MMR gene (*MLH1*, *MSH2*, *MSH6* and *PMS2* or epigenetic silencing of *MSH2* through *EPCAM* deletions) rendering the diagnosis Lynch syndrome, (b) nonheritable somatic *MLH1* promoter hypermethylation (subsequently called hypermethylation) or (c) nonheritable biallelic somatic pathogenic MMR gene variants.<sup>4,14-16</sup>

In a prospective cohort of newly diagnosed CRC patients across 19 hospitals, we evaluated up to what age diagnostics for heritable causes of dMMR is efficient to identify Lynch syndrome and how this diagnostic workflow is experienced by clinicians.

**What's new?**

DNA mismatch repair deficiency (dMMR) is often found in colorectal cancer (CRC), and can be caused by hereditary Lynch syndrome (LS). In this study, the authors found that, in CRC patients younger than age 65, LS is common enough that both dMMR and germline testing are warranted. In patients older than age 64, however, non-heritable causes of dMMR are 20-fold more common than LS. It is thus reasonable to restrict germline testing to dMMR-CRC patients who are below age 65.

**2 | METHODS****2.1 | Study population and procedures**

In this prospective multicenter cohort study, the diagnostic process to assess somatic and germline causes of dMMR was evaluated in the clinical practice of 19 hospitals (two universities and 17 regional hospitals) and 13 associated pathology laboratories. This diagnostic process—the so-called universal MMR workflow (U-MMR)—consists of either IHC or MSI testing of CRC followed by *MLH1*-promoter hypermethylation testing in case of a negative *MLH1* staining or MSI in all CRC patients below age 70 (CRC < 70). Although not preferred, *BRAF* mutation analysis may serve as a surrogate marker for hypermethylation. This is followed by referral for genetic counseling of those with dMMR without hypermethylation, any CRC below age 40 (CRC < 40) or a strongly positive family history of cancer or polyposis. After germline MMR gene testing and in the absence of a germline pathogenic variant, somatic sequencing of MMR genes in tumor DNA is performed.<sup>16</sup> More details on the implementation of the U-MMR workflow are provided in the supplemental methods.

All patients with a newly diagnosed, primary CRC < 70 diagnosed by any of the participating pathology laboratories between January 2016 and July 2017 were included in the study (Figure S1). The patients were identified through a search of the nationwide network and registry of histopathology and cytopathology in the Netherlands (PALGA).<sup>17</sup> Patients with neuroendocrine tumors, squamous cell rectal cancer, or cancers of origin outside the colon were excluded. The pathology reports, including results of MMR testing, were retrieved for each of the identified patients. Patients referred for genetic counseling were identified in the two genetic centers in the region (Radboud university medical center, Nijmegen and Maastricht University Medical Center, Maastricht). For patients referred by July 2017, results of genetic testing were retrieved up to August 2018.

**TABLE 1** Comparison of characteristics of all patients with a CRC diagnosis before age 70 by MMR status

Patient and tumor characteristics	Comparison by MMR tumor status <sup>a</sup>			Comparison of dMMR subclasses <sup>a</sup>				P	
	All CRCs n = 3602	No dMMR n = 2745	dMMR n = 291	P	Germline mutation n = 26	Biallelic somatic mutation n = 20	Hyper-methylation n = 175	P germline vs biallelic hypermethylation	P germline vs somatic vs hypermethylation
Age at diagnosis									
Median (IQR; min-max)	62 (57-66; 16-69)	61 (57-66; 16-69)	65 (59-67; 17-69)	<.001	55 (40-62; 17-69)	60 (49-67; 38-69)	66 (63-68; 42-69)	.289	<.001
CRC < 40, n (%)	55 (2%)	40 (1%)	11 (4%)	.028	6 (23%)	1 (5%)	—	.805	<.001
CRC40-49, n (%)	235 (7%)	195 (7%)	22 (8%)	1.000	6 (23%)	6 (30%)	3 (2%)	1.000	<.001
CRC50-59, n (%)	960 (27%)	784 (29%)	41 (14%)	<.001	2 (8%)	3 (15%)	20 (11%)	1.000	1.000
CRC60-69, n (%)	2352 (65%)	1726 (63%)	217 (75%)	<.001	12 (58%)	10 (50%)	152 (87%)	1.000	<.001
Gender									
Male, n (%)	2151 (60%)	1650 (60%)	126 (43%)	<.001	13 (50%)	12 (65%)	52 (31%)	1.000	.350
CRC location <sup>b</sup> , n (%)									
Right	921 (26%)	612 (22%)	212 (73%)	<.001	20 (77%)	12 (60%)	144 (82%)	1.000	.189
Left	1405 (39%)	1150 (42%)	47 (16%)	—	3 (12%)	4 (20%)	22 (13%)	—	—
Rectum	1188 (33%)	924 (34%)	25 (9%)	—	3 (12%)	4 (20%)	3 (2%)	—	—
Multiple locations	34 (1%)	26 (1%)	5 (2%)	—	—	—	4 (2%)	—	—
Unknown	54 (2%)	33 (1%)	2 (1%)	—	—	—	1 (1%)	—	—
CRC histological subtype, n (%)									
Adenocarcinoma NOS	3337 (93%)	2570 (94%)	230 (79%)	<.001	19 (73%)	15 (75%)	135 (77%)	1.000	1.000
Mucinous adenocarcinoma	209 (6%)	146 (5%)	42 (14%)	—	4 (15%)	4 (20%)	29 (17%)	—	—
Signet ring cell carcinoma	30 (1%)	16 (1%)	7 (2%)	—	2 (8%)	—	3 (2%)	—	—
Medullary carcinoma	11 (0%)	1 (0%)	10 (3%)	—	1 (4%)	1 (5%)	7 (4%)	—	—
Undifferentiated carcinoma	7 (0%)	5 (0%)	1 (0%)	—	—	—	1 (1%)	—	—
Mixed adeno-neuroendocrine carcinoma	6 (0%)	5 (0%)	—	—	—	—	—	—	—
Adenosquamous carcinoma	2 (0%)	2 (0%)	—	—	—	—	—	—	—
CRC histological grade, n (%)									
Grade 1	3064 (85%)	2384 (87%)	184 (63%)	<.001	15 (58%)	17 (85%)	98 (56%)	.590	1.000
Grade 2-3	250 (7%)	152 (6%)	69 (24%)	—	6 (23%)	1 (5%)	52 (30%)	—	—
Unknown	288 (8%)	209 (8%)	38 (13%)	—	5 (19%)	2 (10%)	25 (14%)	—	—

Abbreviations: CRC, colorectal cancer; IQR, interquartile range; n, number; NOS, not otherwise specified.

<sup>a</sup>P-values were corrected for multiple group comparisons testing using a Bonferroni correction for four comparisons.<sup>b</sup>Locations: Right-sided includes cecum, ascending colon, hepatic flexure, transverse colon. Left-sided includes splenic flexure, descending colon, sigmoid, rectosigmoid. Rectum includes rectum and <15 cm from the anus.

The experience of pathologists (n = 23/81), surgeons and gastroenterologists (n = 71/226) and clinical geneticists (n = 36) were evaluated by questionnaires (Figure S1).

## 2.2 | Statistical analysis

Analyses were performed with R software.<sup>18</sup> Data were summarized using descriptive statistics. Outcomes were assessed by age and patients were stratified in three main age groups: patients diagnosed with CRC below age 40 (CRC < 40), CRC between age 40 and 65 (CRC40-64) and CRC between age 65 and 70 years (CRC65-69). Comparisons between patient groups were made using Mann-Whitney U test for nonparametric variables, and Fisher Exact test for proportions. Bonferroni correction was applied when assessing differences between patient subgroups. Values of  $P < .05$  (two-tailed) were considered significant.

## 3 | RESULTS

### 3.1 | Universal MMR deficiency testing

During the study period, 3602 eligible CRC patients were diagnosed at median age 62 (IQR 57-66; Table 1). dMMR testing was performed in 84% (3036/3602; Figure S2). The uptake of dMMR testing increased from 69% (568/827) in the first 3 months to a consistent uptake of 89% (2468/2775) in the remaining period. In the vast majority, dMMR was assessed with IHC 94% (2841/3036). dMMR was detected in 22% (11/51) CRC < 40, 7% (134/1940) in

CRC40-64 and 14% (146/1045) in CRC65-69 ( $P < .001$ ). The percentage of dMMR was lowest between age 50 to 54 and 55 to 59 with 5% (13/275 and 28/550, respectively) and increased to 14% (146/1045) between age 65 to 69 (Figures 1 and 3). Of all CRC < 70, 34% (1045/3036) were diagnosed at age 65 to 69 and 50% (146/291) of dMMR CRC was detected in this age group. Therefore, this was taken as a separate group.

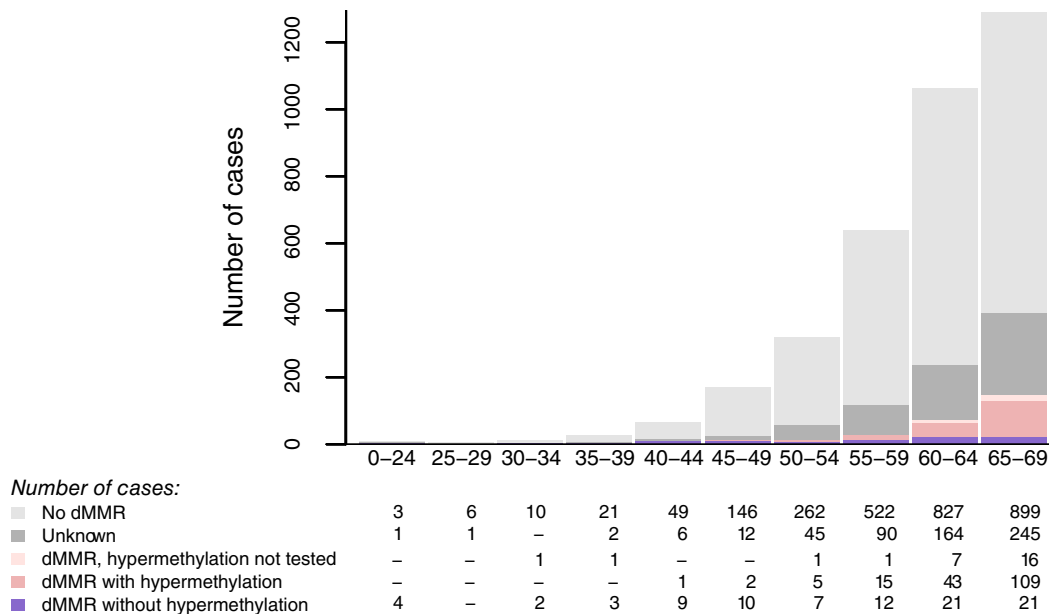
### 3.2 | Hypermethylation testing

Hypermethylation results were available for 88% (221/251) of the MLH1-negative cases and occurred in 66% (175/264) of dMMR cases. The percentage of dMMR CRC explained by hypermethylation increased with age from 0% (0/9) in dMMR CRC < 40 to 84% (109/130) in dMMR CRC65-69 ( $P < .0001$ ; Figure 3).

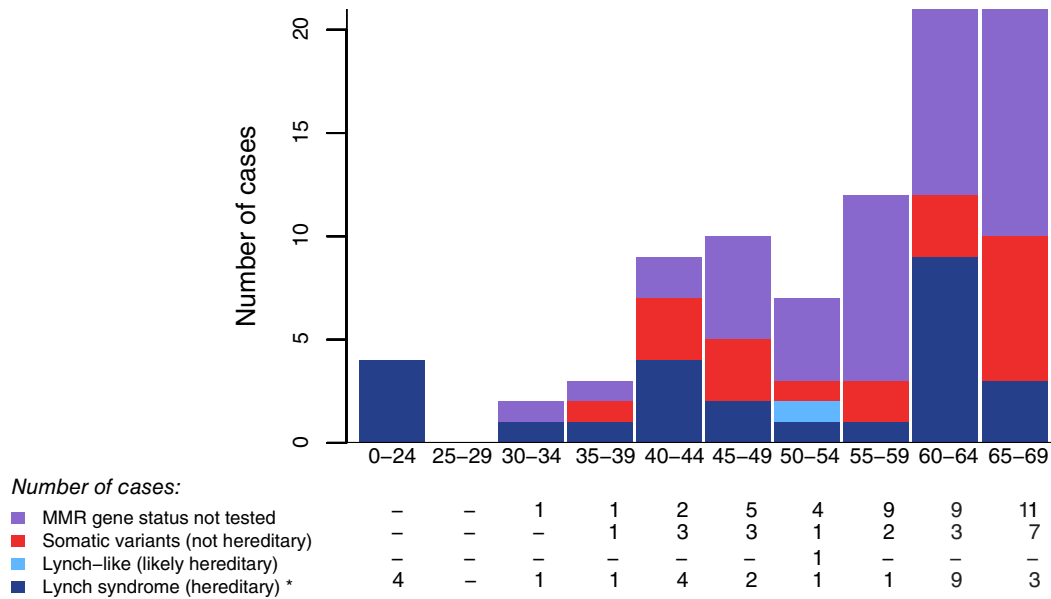
The percentage of CRCs with dMMR without hypermethylation, which represent putative Lynch syndrome patients, decreased with age from 22% (95% CI: 12%-35%) in CRC < 40 to 2% (95% CI: 2%-3%) in CRC65-69 ( $P < .0001$ ) and the percentage of CRCs with hypermethylation increased from 0% (95% CI: 0%-8%) to 12% (95% CI: 10%-14%;  $P < .0001$ ; Figure 4).

### 3.3 | Germline and somatic MMR genetic testing

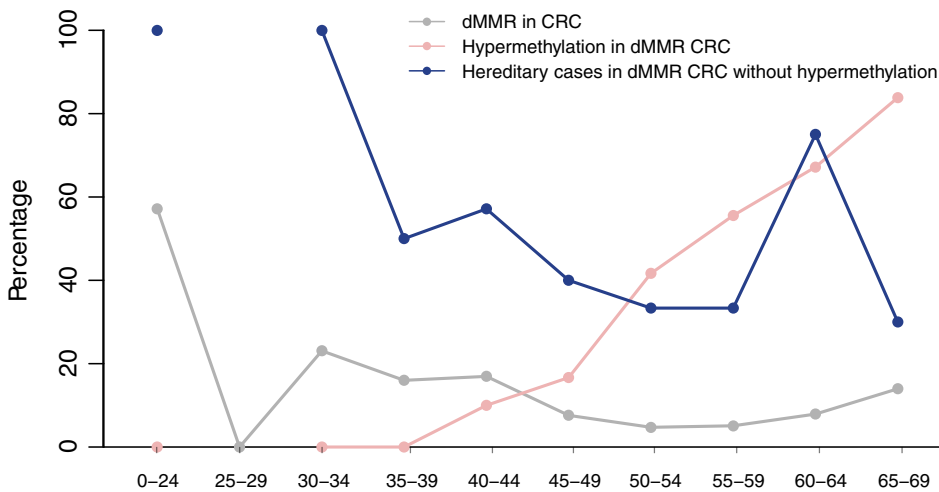
Of the CRC patients with dMMR without hypermethylation, 78% (7/9) with CRC < 40, 51% (30/59) with CRC40-64 and 48% (10/21) with CRC65-69 was tested for pathogenic MMR gene variants after genetic counseling (Figure S2). The referral rate was higher for



**FIGURE 1** Mismatch repair deficiency and hypermethylation in CRC by age. The group “dMMR CRC unknown” refers to patients without any dMMR tumor test results and “dMMR, unknown hypermethylation” to dMLH1 cases without any results on MLH1 promoter hypermethylation [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**FIGURE 2** Cause of mismatch repair deficiency without hypermethylation in CRC by age. \*The CMMRD cases are included in count of Lynch syndrome [Color figure can be viewed at wileyonlinelibrary.com]



**FIGURE 3** Frequency of mismatch repair deficiency, hypermethylation and Lynch syndrome by age [Color figure can be viewed at wileyonlinelibrary.com]

patients diagnosed more than 1 year before the end of the study 71% (29/41) compared to those within 1 year of the end of the study 44% (21/48). The median time between CRC diagnosis and referral for genetic counseling was 56 days (IQR 26-78 days, range 5-442 days) for patients diagnosed in the first period.

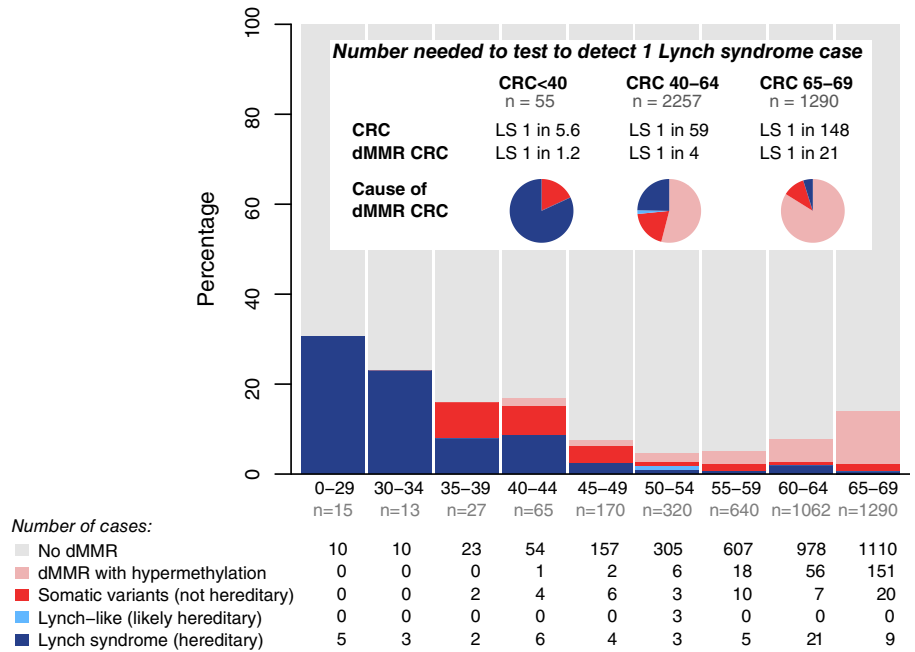
In 55% (26/47) patients tested for MMR gene variants, a pathogenic MMR germline variant was detected, of which 31% (8/26) in *MLH1*, 27% (7/26) in *PMS2*, 19% (5/26) in *MSH2*, 8% (2/26) in *EPCAM* and 15% (4/26) in *MSH6* (Table S1). Only 77% (20/26) fulfilled revised Bethesda guidelines for referral for clinical genetic testing. Two of these 26 patients were diagnosed with Constitutional Mismatch Repair Deficiency (CMMRD; CRC at age 22 and 24) as they had inherited a pathogenic variant from both parents (Table S1). Of patients with dMMR CRC without hypermethylation, 86% (6/7) with CRC < 40, 57% (17/29) with CRC 40-64 and 30% (3/10) with CRC 65-69 had a pathogenic MMR germline variant (Figures 2 and 3).

In 95% (20/21) patients tested negative for a pathogenic germline variant two somatic pathogenic MMR aberrations (Table S2) were detected in the tumor. One patient remained 'Lynch-like' with only one somatic pathogenic variant detected in *PMS2*, but the analysis was incomplete due to the presence of the *PMS2CL* pseudogene (Figure S2).

Patients with a pathogenic germline variant had a median age of 55 (IQR 40-62) at diagnosis which is significantly younger than those with dMMR CRC due to hypermethylation (age 66 [IQR 63-68],  $P < .001$ ; Table 1; Figure S3). Those with biallelic somatic aberrations (age 60 [IQR 49-67]) were also younger than those with hypermethylation ( $P = .012$ ).

Extrapolations indicate a detection rate of pathogenic MMR germline variants of 17.8% (95% CI 9.2-30.9%) in CRC < 40, 1.7% (95% CI 1.2-2.3) in CRC40-64 and 0.7% (95% CI 0.3-1.3) in CRC65-69 (Figure 4). To detect one Lynch syndrome patient the number needed to test is 5.6 (95% CI 3.2-10.8) in CRC < 40, 59 (95% CI 43-82) in CRC40-64 and 148 (95% CI 74-306) in CRC65-69, and after

**FIGURE 4** Detection of Lynch syndrome (LS) by age. For each of the presented age groups, the detection rates of mismatch repair deficiency (dMMR) and LS are extrapolated by imputing the diagnostic probabilities from the cases with completed or partial diagnostics to those cases without complete diagnostics. For the total cohort (n = 3602), the extrapolation provides a detection rate of Lynch syndrome (pathogenic MMR germline variants) of 17.8% in CRC < 40 and 1.7% in CRC40-64 and 0.7% in CRC65-69. CRC, colorectal cancer; dMMR, mismatch repair deficient; LS, Lynch syndrome [Color figure can be viewed at wileyonlinelibrary.com]



prescreen for solely dMMR CRC this is 1.2 (95% CI 1.0-2.0), 4.1 (95% CI 3.1-5.5) and 21 (95% CI 11-43), respectively.

### 3.4 | Experiences of clinicians

Of the 23 pathologists, all performed universal IHC or MSI testing in CRC < 70. Only 5% (1/21) of them considered universal dMMR testing difficult to organize.

Of the 71 surgeons and gastroenterologists, 89% (54/61) considered the guideline on universal MMR testing clear and practicable, and 70% (37/53) considered the advices of the genetic services to be good and useful. The majority of the physicians (78%; 43/55) used the tool kit that was developed to facilitate the referral process, including a website with short videos for patients (cancergenetics.eu), mobile-app and a printed guideline pocket card. Clinicians most often mentioned the following five reasons for not referring CRC patients with an indication based on the U-MMR workflow: (a) refused by patients (mentioned by 98%; 52/53), (b) missed referrals due to delay in availability of hypermethylation results (mentioned by 87%; 47/55), (c) no clinical consequences as there are no relatives (mentioned by 65%; 34/52), (d) delay until after CRC treatment (mentioned by 56%; 31/55) and (e) psychological burden (mentioned by 33%; 18/55). Gastroenterologists and surgeons suggested the following facilitating factors to further increase the referral rate: (a) patient empowerment by sharing results and conclusion of the dMMR test directly with the patient, (b) a dedicated Lynch syndrome expert in all clinical teams and (c) including the results of the dMMR-test in the multidisciplinary team discussion.

The 36 clinical geneticists reported that U-MMR testing made counseling more efficient as for 42% to 64% of patients with U-MMR results one appointment was sufficient compared to two appointments for 14% to 36% of patients without a U-MMR result.

## 4 | DISCUSSION

In this large prospective cohort of consecutive CRC patients diagnosed below age 70, we demonstrate that routine testing for MMR deficiency combined with *MLH1* promoter methylation testing is an efficient and well accepted manner to select patients for MMR gene mutation analysis in patients diagnosed up to age 65. The prevalence of Lynch syndrome is 18% in CRC below 40 and 1.7% in CRC between age 40 and 65 and, in both age groups, 5% of all CRCs is MMR deficiency due to somatic causes. In CRC patients between age 65 and 70, the prevalence of Lynch syndrome is only 0.7%, whereas 13% of all CRCs is MMR deficient due to a somatic cause, mainly *MLH1* promoter methylation.

With increasing age, the proportion of dMMR increases and the balance between hereditary and nonhereditary causes of dMMR continues to shift toward nonhereditary. Evaluation of dMMR CRC in patients diagnosed above age 65 to 69 to detect Lynch Syndrome has a substantial impact on the amount of MMR deficiency and especially *MLH1* promoter methylation analyses that need to be performed with limited yield. Our study showed that patients between age 65 and 70 years represent 36% of CRC < 70 and 50% of CRC < 70 with dMMR, and that they represent about half of all CRC patients and the majority of patients with dMMR that need genetic testing.<sup>1,11,19</sup> Another recent study estimated a prevalence of Lynch syndrome of 0.7% in CRC > 60 and 0.3% in CRC > 70,<sup>19</sup> which is similar to the overall population prevalence of 0.3% to 0.7%.<sup>20-22</sup> This indicates that for patients with CRC over age 65-69 years the risk of Lynch syndrome is not increased compared to that of the general population. To reduce costs and anxiety for hereditary diseases in those not at increased hereditary risk, an age above 65 can be a good reason not to evaluate the underlying cause of dMMR.<sup>23</sup> However, the analyses of dMMR itself should remain in place because the dMMR status may be informative for oncological care. Taken together, we consider dMMR in CRC found by routine resting above age 65 not a good biomarker for Lynch syndrome, and plea to consider not to

test for the underlying cause of dMMR in patients unless dMMR is tested because of a family history directing toward Lynch syndrome.<sup>24</sup>

It has been shown that pathogenic *MLH1* germline variants and *MLH1*-promoter methylation may co-occur in less than 2% to 7% of *MLH1* germline cases.<sup>25-27</sup> With the universal Mismatch repair workflow, these cases may be missed. However, given the low co-occurrence rate, the higher hypermethylation rates and the lower prevalence of germline variants with increasing age and the advice still to refer cases with a strong family history, this will hardly affect the estimated Lynch syndrome prevalence in our study.

Lynch syndrome and two pathogenic somatic MMR aberrations in tumor DNA explained each about 15% of dMMR cases. Tumor DNA MMR gene testing in the absence of any pathogenic germline MMR variant doubles the explained cases. In our study, a high proportion of somatic aberrations was detected (20 of 21) compared to previous studies with rates of 50% and 69%, which is due to the improved sequencing techniques and the additional loss of heterozygosity analyses.<sup>16,28</sup> Such somatic testing is introduced in our lab in 2011, but not yet part of recommendations for routine diagnostics.<sup>16</sup> It significantly reduces the number of patients and relatives considered Lynch-like and prevents unnecessary surveillance colonoscopies in patients and relatives who would otherwise be advised a Lynch-like surveillance protocol.<sup>29</sup> Other studies have suggested that upfront NGS assessment of tumor mutations, including MMR genes, in all CRCs would be preferable as it simplifies the workflow, provides more detailed tumor mutation information which may be used to select systemic therapy, and improves detection of LS.<sup>30</sup> Although, cost-effectiveness analyses should yet be performed for both workflows, cost-effectiveness of upfront tumor NGS is debatable as only 1% of CRC is LS-associated and the therapeutic implications of predictive somatic mutations are limited in early-stage disease.

In 1999, the Bethesda guidelines were introduced to select CRC patients for dMMR testing.<sup>31</sup> In the current study, only 78% of the Lynch syndrome cases in CRC < 65 would have been detected by the revised Bethesda guidelines.<sup>32</sup> This confirms that universal dMMR testing in CRC < 65 improves the detection of Lynch syndrome families. Additionally, universal dMMR testing is feasible as routine dMMR testing was performed in the majority of CRCs (84%). In our study based on day-to-day practice, pathologists were shown to be very effective in tracing the 3% dMMR-tumors without *MLH1*-promoter methylation in newly diagnosed CRC patients. Gastroenterologists and surgeons substantially improved their referral rates for genetic counseling as compared to the workflow based on age, family history and restricted MMR testing in early-onset cancer or multiple cancer cases which was previously reported in the same study region (69% vs 33%,  $P = .004$ ).<sup>33</sup> This is mainly attributable to the increasing attention for Lynch syndrome in pathology reports, and may be partly due to increased clinical awareness of Lynch syndrome over time.<sup>34</sup>

To conclude, routine testing of mismatch repair deficiency in CRC as biomarker for Lynch syndrome and evaluating somatic and germline (nonheritable and heritable) causes of mismatch repair deficiency is feasible in daily practice and appreciated by clinicians. It increases genetic testing in those CRC patients at high risk for Lynch syndrome and yields more Lynch syndrome families than the revised Bethesda criteria. Routine analysis of the cause underlying dMMR, including *MLH1* promoter

methylation analysis and germline genetic testing, may be restricted to patients with dMMR CRC < 65 or with a strong family history.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## DATA ACCESSIBILITY

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

## ETHICS STATEMENT

This study was performed with the approval of the privacy committee and the scientific board of PALGA and the medical ethical board of the Radboudumc, Nijmegen, the Netherlands (number 2015-2171). As part of the diagnostic process patients have provided oral informed consent for cancer predisposition testing.

## ORCID

Janet R. Vos  <https://orcid.org/0000-0001-8802-7002>

Ingrid E. Fakkert  <https://orcid.org/0000-0002-2430-2711>

Arjen R. Mensenkamp  <https://orcid.org/0000-0003-3805-877X>

Iris D. Nagtegaal  <https://orcid.org/0000-0003-0887-4127>

Marjolijn J. L. Ligtenberg  <https://orcid.org/0000-0003-1290-1474>

Nicoline Hoogerbrugge  <https://orcid.org/0000-0003-2393-8141>

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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## APPENDIX

The Finding Them All (FINAL) Group consists of the following members: H. Küsters-Vandeveldel, Canisius-Wilhelmina Ziekenhuis, Nijmegen, The Netherlands; J. Meijer, Rijnstate, Arnhem, The Netherlands; M. Tebar, Laboratorium Pathologie Oost-Nederland, Hengelo, The Netherlands; H. Shirango, Deventer Ziekenhuis, Deventer, The Netherlands; J. Stavast, Elisabeth-TweeSteden Ziekenhuis, Tilburg, The Netherlands; C. Bronkhorst, Jeroen Bosch Ziekenhuis, s Hertogenbosch, The Netherlands; C. Huysentruyt, Laboratorium voor Pathologie en Medische Microbiologie, Eindhoven, The Netherlands; H. van Herk, Elkerliek Ziekenhuis, Helmond, The Netherlands; D. Rupa, St. Laurentius, Roermond, The Netherlands; S. Sastrowijoto, Zuyderland Medisch Centrum, Heerlen and Sittard-Geleen, The Netherlands; A. de Bruine, Vie Curi Medisch Centrum, Venlo, The Netherlands; H.I. Grabsch, Maastricht University Medical Center, Maastricht, The Netherlands; K. van Meerten, Radboud university medical center, Nijmegen, The Netherlands; L. Geurts, Canisius-Wilhelmina Ziekenhuis, Nijmegen, The Netherlands; F. Kuijpers, Maasziekenhuis Pantein, Boxmeer, The Netherlands; M. Smaak, Rijnstate, Arnhem, The Netherlands; M. van Schijndel, Jeroen Bosch Ziekenhuis, 's Hertogenbosch, The Netherlands; H. Kleiweg, Ziekenhuis Gelderse Vallei, Ede, The Netherlands; M. Castelijns, Catharina Ziekenhuis,



Eindhoven, The Netherlands; M. Borremans-Simons, Elisabeth-TweeSteden Ziekenhuis, Tilburg, The Netherlands; C. Kort, Medisch Spectrum Twente, Enschede, The Netherlands; D. Ekkel, Ziekenhuis Groep Twente, Almelo and Hengelo, The Netherlands; J. Nieuwenhuis, Deventer Ziekenhuis, Deventer, The Netherlands; C. Gielen, Maastricht University Medical Center, Maastricht, The Netherlands; K. Peeten, St Anna Ziekenhuis, Geldrop, The

Netherlands; A. Briels, Laurentius Ziekenhuis, Roermond, The Netherlands; J. Ophorst, Maxima Medisch Centrum, Veldhoven and Eindhoven, The Netherlands; B. Simons, Zuyderland Medisch Centrum, Heerlen and Sittard, The Netherlands; M. van Berkom, Elkerliek Ziekenhuis, Helmond, The Netherlands; E. Cuijpers, St Jan Gasthuis, Weert, The Netherlands; N. Metselaars, VieCuri Medisch Centrum, Venlo, The Netherlands.