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ABSTRACT

Purpose: The global incidence and mortality of early-age onset colorectal cancer (EOCRC, or CRC diagnosed under 50 years) has increased in recent decades. High-risk surveillance and personalised oncological treatment may improve patients' outcomes. This study aims to characterise real-world somatic and germline molecular profiles in European EOCRC patients.

Patients and Methods: Consecutive patients across the UK, Spain, Germany and Italy from the GEOCODE and SECOC consortia were identified using electronic patient records. Clinicopathological, somatic and germline testing data were collected

Complete details about authors and their affiliations for GEOCODE and SECOC collaborators are provided in the Supporting Information S1.

on EOCRC patients. Tests included mismatch repair (MMR), somatic next generation sequencing (NGS) and germline multi-gene panels.

Results: Eight hundred ninety-three EOCRC patients were identified from 23 European centres (45.7% female, median age 42, range 14–49), predominantly in the distal colorectum: 205/893 (22.9%) patients with right-sided tumours, 302/893 (33.8%) left-sided tumours, 288/893 (32.2%) rectal tumours and 97/893 (10.8%) unknown. On somatic analysis, 735/893 (82.3%) of patients had pMMR tumours and 148/893 (16.5%) dMMR. Although 534/893 (59.7%) did not receive NGS somatic testing, somatic variants were detected in 233/359 (64.9%) of those tested. Germline variants were detected in 133/210 (63.3%) patients tested. Lynch syndrome was diagnosed in 93/210 (44.2%), of whom 17/93 (18.2%) presented with pMMR tumours. Systematic recording of family history in these real-world data was variable. In all patients with family history recorded, 153/484 (31.4%) patients reported a relative with CRC.

Conclusions: Our results support universal and paired somatic and germline multi-gene panels for all EOCRC patients, regardless of MMR status or family history. Systematic molecular testing approaches are necessary to address disparities in people with EOCRC. Larger unselected cohort studies would support validation of testing prediction models and estimates of clinically relevant variant actionability.

1 | Background

The rising incidence of early-onset colorectal cancer (EOCRC) in adults under 50 has become a significant global health concern, with rates expected to increase by 27.7% for colon cancer and 46% for rectal cancer by 2030 [1, 2]. While the majority of colorectal cancer (CRC) diagnoses are in older adults (late-onset colorectal cancer [LOCRC] diagnosed ≥ age 50), the incidence in this population is declining, possibly due to national average-risk population screening programs. Of additional concern is the trend for increased mortality in younger patients [3–5].

Diagnoses of EOCRC will more often be related to Mendelian syndromes such as Lynch or polyposis syndromes (~16% compared to < 3% in LOCRC) [6], however testing for these conditions and evaluation of family history may be variable [7]. Family history of CRC in one first-degree relative (FDR) increases the risk of developing the disease by 2–4 fold [8]; however, most sporadic cases remain unexplained, although other risk factors may also contribute a small but significant proportion of EOCRC risk, including inflammatory bowel disease and polygenic risk [9, 10]. Therefore, there exist opportunities to improve the current standard of care for patients diagnosed with EOCRC through accurate estimation of risk [10, 11]. Whilst investing in research to understand the causes of increasing incidence of CRC in the younger age group is necessary, it is important that existing gaps in clinical care, and the options for immediate change are also recognised.

Somatic molecular data may also provide therapeutic options to improve outcomes from CRC diagnosis. Recent data from the United States analysed a cohort of 10,000 cases [12], including 3185 EOCRC cases, to understand the distinct clinical and molecular profiles in comparison with LOCRC. In keeping with previous data, EOCRC more commonly presents with tumours in the left colon and rectum [5, 13], whereas LOCRC presents with proximal tumours [13]. EOCRC presents more frequently at advanced stages possibly because of delay in diagnosis and more aggressive disease biology [4, 5]. Unique mutational patterns in this study were observed in EOCRC with higher *TP53* mutations and lower *APC*, *KRAS* and *BRAF* mutations in microsatellite stable tumours, suggesting different pathogenic pathways. This diverges from previous studies which had higher rates of *KRAS* and *APC* mutations [12]. This may be due to previous data being based on smaller sample sizes and reflect the inconsistences and complex and heterogenous nature of EOCRC.

This study collected real-world data of somatic and germline molecular testing outcomes in cases of EOCRC from the European GEOCODE together with SECOC (Spanish EOCRC) consortia. By characterising gaps in testing, we may identify opportunities for improvements in care and surveillance for patients diagnosed with EOCRC [14].

2 | Methods

2.1 | Study Design and Data Sources

This is a retrospective, descriptive, multicentre European study where consecutive, unselected patients diagnosed with EOCRC were identified at 23 sites across the following European countries: England, Spain, Germany and Italy. The study aims to identify real world data and understand disparities across European centres. This built upon the previously collected datasets from the Global Early-Onset Colorectal Cancer Database (GEOCODE) and the Spanish Early-Onset Colorectal Cancer Cohort (SECOC) [6]. GEOCODE is a worldwide consortium study exploring global patterns of EOCRC; data were collected on patients from participants from the following European countries: Spain, Italy, UK and Germany. SECOC is a Spanish group that integrates hospitals from Madrid, Barcelona, the Basque Country, Castilla y Leon and Navarra. Data from GEOCODE and SECOC were curated to avoid duplicates.

2.2 | Study Population and Inclusion/Exclusion Criteria

Patients were included with a histologically confirmed diagnosis of colon or rectal adenocarcinoma with diagnosis at age < 50. This consisted of consecutive diagnoses presented to each

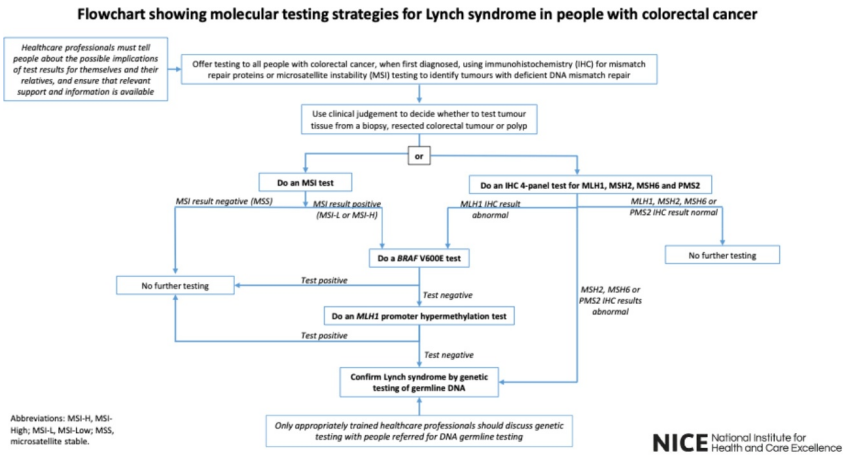
Key Summary

- Established knowledge of the subject
  - Early-onset colorectal cancer (EOCRC), diagnosed before age 50, is rising globally.
  - Previous studies have described the presence of microsatellite instability (MSI) and germline mismatch repair (MMR) mutations in a subset of EOCRC cases, but real-world data on comprehensive somatic and germline testing in unselected EOCRC populations remain limited.
- Significant and/or new findings of this study
  - We analysed real-world molecular testing data from EOCRC patients across multiple European centres, providing a picture of current germline and somatic testing practices outside of research settings.
  - While MMR immunohistochemistry (IHC) was widely implemented, germline and somatic testing rates were significantly lower, with only 23% of patients receiving germline results and 40% undergoing somatic profiling, highlighting underutilisation of comprehensive testing despite clinical guidelines.
  - Among tested patients, pathogenic germline variants were identified in 14.8% of the entire cohort, noting that only 23% of patients were tested. Of patients diagnosed with Lynch syndrome 18% of them presented with pMMR tumours. Somatic testing revealed common alterations (e.g. *KRAS*, *TP53*, *BRAF*), and a subset of patients harboured actionable mutations, 64.9% of those tested.
  - These data underline the need for broader and more consistent implementation of molecular testing in EOCRC and suggest potential missed opportunities for targeted therapy, genetic counselling, and family risk assessment.

centre, that is, every case that was diagnosed as a colon or rectal cancer, regardless of the following information availability and whether they had treatment or not. Patients were excluded if they did not have a diagnosis of colon or rectal cancer or were older than 50 years at the time of diagnosis. Benign tumours, or patients with other types of tumours found in the bowel for example, lymphoma, were also excluded.

Identification of consecutive EOCRC patients was undertaken by local teams, utilising electronic patient records and clinicopathological, somatic and germline testing data were collected manually using a standardised coded spreadsheet to allow collation of outcomes. This included patients with colon or rectal cancer of any stage (TNM I–IV). The dataset included histological features such as presence of signet ring cells, degree of differentiation, mucinous features, lymphocyte infiltration, staging, family history of colorectal or other cancers (including other Lynch syndrome associated cancers), mismatch repair testing for possible Lynch syndrome or *MLH1* hypermethylation and subsequent clinically validated NGS somatic and/or germline testing using multi-gene panel tests with associated institutional assessment of pathogenicity. The data were collected by healthcare professionals in each hospital and transferred anonymously for collation and data cleaning. In the UK, the data were collected retrospectively and anonymised via service evaluation with associated ethical approval. For the SECOC cohort, this was a prospective study and therefore patients provided written informed consent as well as consent for research publication. All countries required consent for germline testing to identify pathological variants.

Molecular testing varied by country and institution. In most centres, diagnostic pathways included tumour-based testing such as mismatch repair (MMR) immunohistochemistry and/or microsatellite instability (MSI) analysis. Patients with suspected hereditary cancer underwent germline testing targeting relevant genes, most commonly the MMR genes (*MLH1, MSH2, MSH6, PMS2,* and *EPCAM*), using methods such as DNA sequencing and large rearrangement analysis (e.g., MLPA). In some cohorts, broader next-generation sequencing (NGS) panels were employed to assess therapeutic targets or evaluate hereditary cancer risk. Whole-exome sequencing (WES) was used in select centres as part of universal tumour sequencing approaches. It is not possible to offer exact testing methods on a patient by patient basis from this dataset. Due to institutional variability, we present an example Flowchart 1 illustrating UK guidelines for diagnostic pathways across participating sites [14]. Site-specific variation may reflect national guidelines, local resource availability, or historical evolution in testing strategies.



Flowchart 1 | Molecular testing strategies for Lynch syndrome in people with colorectal cancer.

2.3 | Statistical Analysis

Descriptive and comparative analyses were conducted using Microsoft Excel and R. Summary statistics (including frequencies, medians, and ranges) were calculated to characterise clinical and molecular subgroups. Chi-squared tests were used to evaluate associations between categorical variables. Datasets were stratified by mismatch repair status (e.g., dMMR vs. pMMR), country, and other relevant clinical factors. Statistical significance was determined by p-values, with a significance threshold of $p < 0.05$. Patients from different national cohorts were pooled to enable cross-country comparison, with data validation performed locally.

3 | Results

3.1 | Cohort Description

In total, 893 patients with EOCRC were included from 23 European centres (Table 1). The median age at diagnosis was 42 (range from 14–49) years; 45.7% were female. Regarding CRC location, the majority (66%) were in the left colon and rectum (33.8% and 32.2% respectively) with 22.9% in the right colon (remainder unknown 10.9%). 22.3% were diagnosed with stage IV disease [15, 16]. 4% of patients had evidence of signet ring histology, compared with 1% in reported literature [15]. A record of family history of CRC was provided in 54.2% of patients, of whom 153/484 (31.4%) patients reported a relative with CRC, of which 81/484 (16.7%) had a family history of CRC in a 1st degree relative and 96/484 (19.8%) had a family history of CRC in a 2nd degree relative.

TABLE 1 | Characteristics of the cohort of patients including demographic information, tumour location, stage of disease and family history.

Whole cohort	Number of patients (%)	Number of patients with available results
Country of origin		893
UK	327 (36.6)	
Spain	323 (36.1)	
Italy	210 (23.5)	
Germany	33 (3.6)	
Sex		893
F	408 (45.7)	
M	484 (54.1)	
Not recorded	1 (0.1%)	
Tumour location		893
Rectum	302 (33.8)	
Left	288 (32.2)	
Right	205 (23.0)	
Other	1 (0.2)	Polyposis
Not recorded	97 (10.9)	
Stage at diagnosis		893
I	144 (16.1)	
II	185 (20.7)	
III	334 (37.4)	
IV	198 (22.3)	
Not recorded	32 (3.6)	
Family history of CRC		484
Yes	152 (31.4)	
No	332 (68.6)	
Family relationship CRC		484
1st degree	81 (16.7)	
2nd degree	96 (19.8)	

3.2 | Germline Variants

A minority 210/893 (23.5%) were offered germline testing results (Table 2). Of these, 112/210 (63.3%) had a confirmed germline variant (Figure 1a). Of most of these patients, 94/112 (84.8%) had a pathogenic variant consistent with a diagnosis of Lynch syndrome (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*). Other variants reported included 9 *APC* variants, 9 *MUTYH* variants (heterozygous) and 1 each of *BRCA*, *ATM*, *FANCA* and *CHEK2*, as shown in Figure 1b. The rates of testing varied per country, as shown in Figure 1c. There was disproportionate germline testing in patients with dMMR tumours: 114/148 (77%) patients with dMMR received germline testing compared with 87/732 (11.9%) with pMMR, 9/13 patients with unknown MMR status received germline testing. Among those tested, 17 of 210 patients (8%) with germline testing had Lynch syndrome despite having pMMR tumours. The percentage of the total cohort diagnosed with Lynch syndrome was 10.4% (93/893). The median age of patients with a germline variant was 39 and of those tested was 40 (overall cohort median age 42). Detection of germline variants according to stage is demonstrated in the heatmap in Figure 1d. There were no CMMRD cases described in the cohort.

3.3 | Somatic Variants

Most patients received MMR testing (98.9% of this cohort; Figure 2a). However, a minority of patients, 359/893 (40.2%) had available somatic NGS testing results to inform treatment (Figure 3a). Standard somatic testing panels include variants that allow access to clinical trials. For example, 144/359 (40.1%) of those patients who have had testing have a *KRAS* mutation; full results are provided in Table 3 and Figure 3b. Some of these mutations provide druggable targets which could provide options for treatment as per ESMO guidelines [16] and via clinical trial eligibility.

3.4 | Characteristics of dMMR Cohort

148/893 patients (16.5%) were diagnosed with dMMR CRCs. Regarding location, the majority of patients had disease in the right colon $n = 71$ (50.0%), left colon $n = 41$ (27.7%), rectum

TABLE 2 | Germline results including number of patients who have had testing and results of NGS germline testing panel of the proportion of tested patients who had dMMR IHC status and important results including germline Lynch syndrome diagnosis.

Germline pathogenic variant (NGS)	Number of patients (%)	Number of patients with available results
Yes	112 (53.3)	210
No	98 (46.7)	
MLH1	41 (19.5)	114 MMRd (54.3)
MSH2	28 (13.3)	87 MMRp (41.4)
MSH6	7 (3.3)	9 unknown (4.3)
PMS2	18 (8.6)	
EPCAM	1 (0.5)	
APC	9 (4.3)	
MUTYH	9 (4.3)	
Other significant result	4 (1.9) *BRCA, ATM, FANCA, CHEK2*	
Lynch syndrome total	93 (45.2)	210
pMMR	17 (18.2)	6 *MLH1*, 2 *MSH2*, 8 *MSH6*, 1 *PMS2*
dMMR	76 (81.7)	

n = 41 (27.7%) and unknown n = 10 (6.8%). We examined the relationship between MMR status and tumour location, which revealed a statistically significant difference in tumour location distribution between dMMR cases (n = 148) and all pMMR cases (n = 735) ($\chi^2(4) = 65.07$, p = 2.488e-13), and suggests that tumour location is not randomly distributed across MMR groups, with dMMR cases more frequently occurring on the right side as shown in Figure 4. In terms of staging 26 patients had stage I disease (17.6%), 42 had stage II disease (28.3%), 58 stage III disease (39.2%), 19 stage IV disease (12.8%), 3 had unknown stage (2.0%) as shown in Figure 5a,b. With regard to the relationship between mismatch repair (MMR) status and cancer stage, this was associated with a highly significant difference in stage distribution between dMMR cases (n = 148) and all MMR cases (n = 893) ($\chi^2(4) = 38.25$, $p < 0.0001$). Notably, dMMR cases had a lower proportion of advanced disease (stage IV) compared to the overall MMR cohort, suggesting differences in disease progression patterns as shown in Figure 5a,b. Seventy-five patients (50.7%) had a somatic variant detected of which only three had a *BRAF* mutation, 2 had a *MLH1* mutation and 1 patient had 2 variants of unknown significance (VUS) in *MSH6*. Ninety-one patients (61.5%) had a germline variant detected, of which 76 (51.4%) were consistent with LS. We observed substantial heterogeneity in molecular testing pathways across countries. Some cohorts applied universal tumour sequencing or routine panel-based testing, while others employed targeted strategies based on clinical suspicion. These variations reflect evolving guidelines and testing availability across time and region. For example, it is not known how many of these patients had tested for *MLH1* promoter hypermethylation, which is now embedded into many different testing pathways for dMMR [14, 17].

3.5 | Patients With pMMR Tumours Who Were Found to Have Lynch Syndrome

Whilst this is a very small proportion of patients in the entire cohort, this should be considered when reviewing the findings. The authors thought however, that this group of patients was interesting and therefore more information is given. Seventeen patients with pMMR tumours were diagnosed with Lynch syndrome on germline testing (Figure 2c), with six *MLH1* pathogenic variant carriers, 2 *MSH2* pathogenic variant carriers, eight *MSH6* pathogenic variant carriers and 1 *PMS2* pathogenic variant carrier. Again, the preponderance was for left sided cancers with 12/17 from the left colon or rectum and 2/17 from the right colon (3 unknown). There was no statistically significant difference in tumour location distribution when comparing pMMR CRC to the entire cohort ($\chi^2(2) = 1.00$, p = 0.607), suggesting that tumour laterality in pMMR Lynch syndrome cases does not differ from the broader CRC population, however, we note the limited numbers. The TNM tumour staging was stage I = 2 patients, stage II = 5 patients, stage III = 7 patients and stage IV = 3 patients, using chi-squared statistics there was a statistically significant difference when compared to the entire cohort ($\chi^2(3) = 49.94$, $p < 0.0001$) suggesting that there is a lower burden of stage IV disease in this group. 4/17 patients had a history of colorectal cancer in a first degree relative, 5 different patients had a history of a possible Lynch associated cancer in a first degree relative, 5 patients in total had a history of CRC in a second degree relative however, 4 of these patients also had a history of CRC in a first degree relative. In 2 of these 17 patients, somatic mutations detected in mismatch repair genes corresponded to the germline pathogenic variant.

4 | Discussion

In this study, we analysed real-world data from patients diagnosed with EOCRC in 23 European centres in 4 countries. While MMR testing was available in most participating units, the rates of subsequent molecular testing were suboptimal. Only 40.3% of patients received somatic testing results, and only 23% had documented germline testing. The data are primarily observational and retrospective and indications for testing evolved across different centres and countries during the time period. This variability reflects standard data collection practices across different healthcare systems. Selection of consecutively diagnosed patients helps to mitigate reporting bias and highlight clinically relevant gaps in routine testing and data availability. Our findings support the need to unify criteria and universal tumours sequencing across Europe.

Geographical disparities influence access to speciality care, eligibility for testing and treatment options, ultimately impacting patient outcomes. The data we present here is from centres who are motivated in addressing this global problem and likely represent services that are more advanced than the average institution.

MMR immunohistochemistry (IHC) is commonly used across European centres as the index test of mismatch repair function. This test is relatively inexpensive and an effective method for

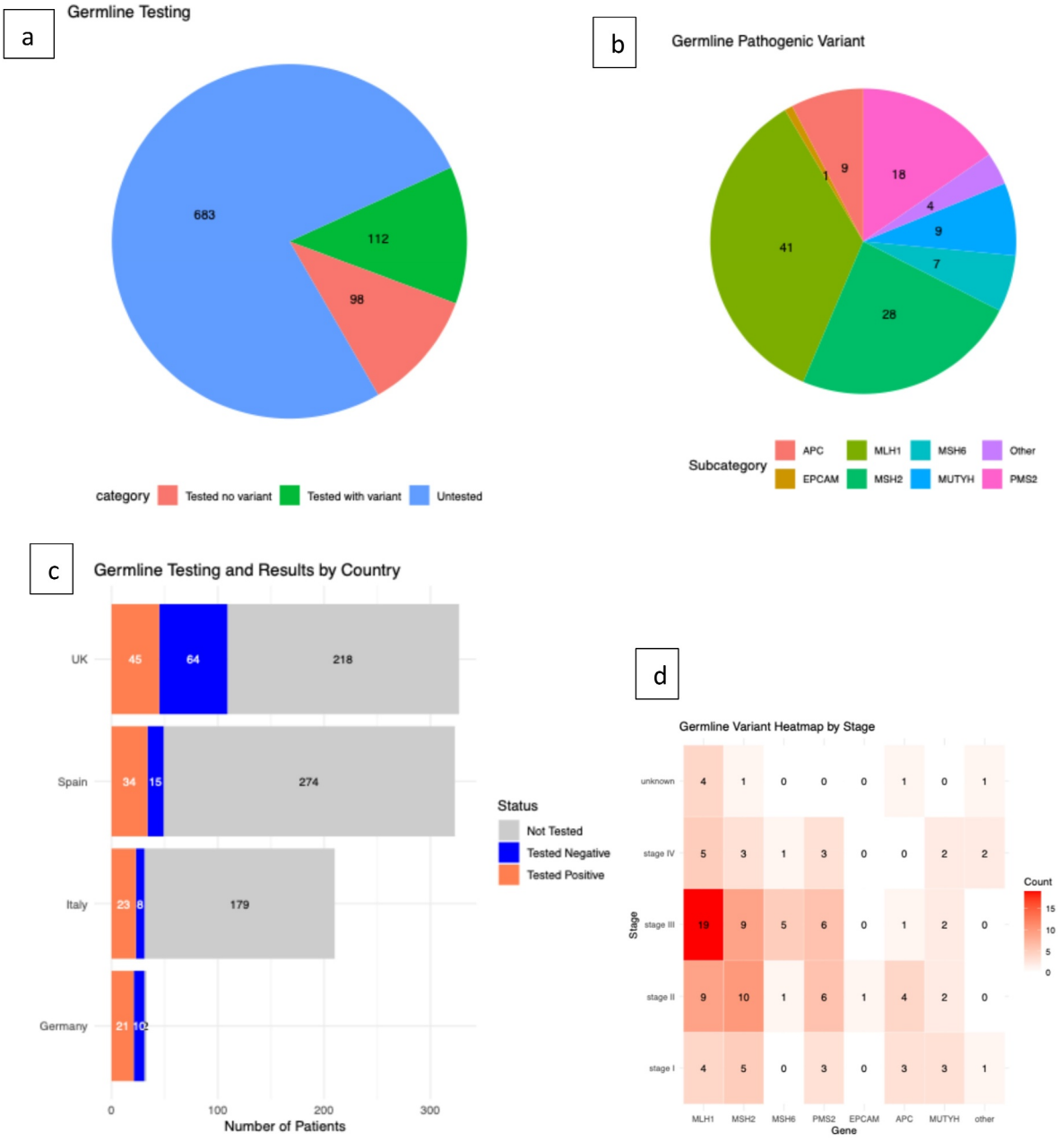


FIGURE 1 | (a) Number of patients receiving germline testing and variants detected. Of the entire cohort of patients 893, a total of 210 received germline testing, of whom 112 tested positive for a pathogenic variant, 98 tested negative and 683 were not tested. (b) Germline variants detected. (c) Germline testing and results per country. (d) Germline variant heatmap per stage.

identifying patients who require further testing. However, the sensitivity and specificity of this test may not be well-validated in younger patients due to the fact it has historically been validated in the older population, although some more recent publications include a younger cohort [18]. For this age group, it might be more appropriate to use a broader range of tests to evaluate tumour phenotype, including MSI and tumour mutational burden (TMB) analysis. These tests would need to be clinically validated against the current gold-standard methods to determine if multiple methods of testing improved diagnosis in this age group. Globally, MMR and Lynch syndrome practices vary with some centres providing reflex testing with other centres needing to request tests on a patient-by-patient basis. However, without robust service planning, diffusion of responsibility can result in lower levels of testing [7] although our study does not have information on why certain patients did not receive testing.

Our findings align with data suggesting that there are similar overall numbers of patients with dMMR in EOCRC when compared with LOCRC, around 16% [6]; however, the representativeness of our sample must be considered [19]. Germline testing results were available in 23.5% of all patients included in the cohort. This may reflect the lag time of testing for patients to receive genetic testing, focus on immediate care or if patients were too unwell to undergo testing or to attend appointments for discussion about genetic testing. As a result, only 10.4% of the cohort had a diagnosis of Lynch syndrome lower than in the reported literature for this age group [6].

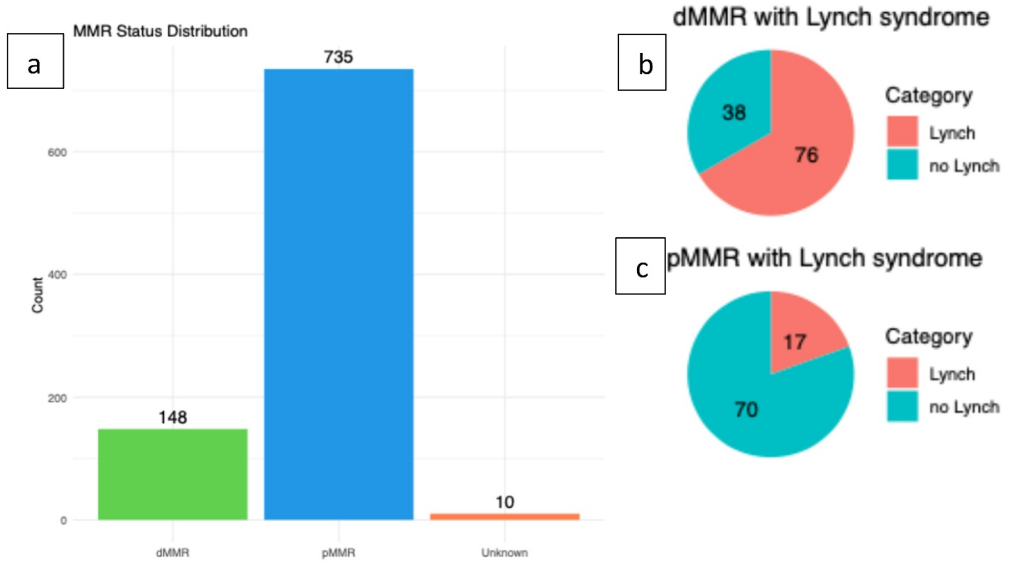


FIGURE 2 | (a) Number of patients tested for mismatch repair (MMR) with immunohistochemistry and result whether dMMR (mismatch repair deficient) or pMMR (proficient). (b) Proportion of patients with dMMR status having Lynch syndrome on germline testing. (c) Proportion of patients with pMMR status having Lynch syndrome on germline testing.

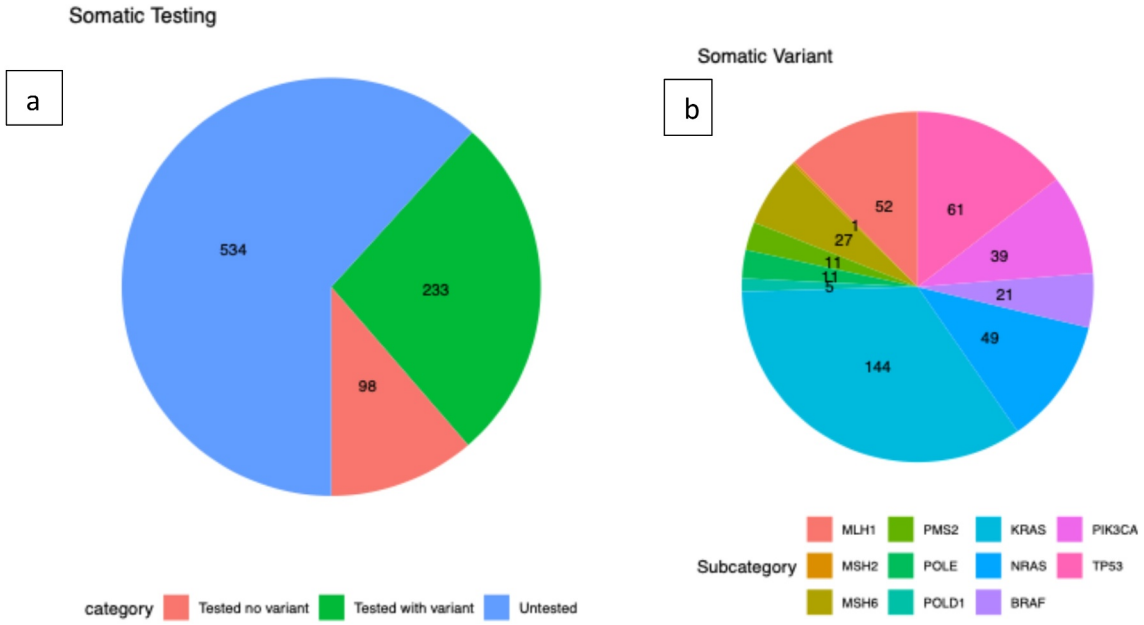


FIGURE 3 | (a) Number of patients receiving somatic (tumour) testing and variants detected. Of the entire cohort of 893, a total of 331 patients received somatic testing. (b) Somatic variants detected.

Our findings also show higher levels of germline testing in patients with dMMR tumours: 114/148 (77%) patients with dMMR received germline testing compared with 87/732 (11.9%) patients with pMMR. Among those tested, 17 of 210 patients (8%) with germline testing had Lynch syndrome despite having pMMR tumours. This discrepancy suggests that IHC alone may miss Lynch syndrome or other genetic causes of colorectal cancer. Some patients with pMMR CRC may be tested in the absence of dMMR, for example based on family history or because of age thresholds in different health systems. However, the proportion of patients who were diagnosed with Lynch syndrome out of the cohort that were tested was 17/87 (19.5%) pointing to potential underdiagnosis [9, 16, 20]. In a larger study by Latham et al., 1.9% of pan-cancer patients with MSI-indeterminate and 0.3% microsatellite-stable had Lynch syndrome [11]. These findings support germline testing for every patient under 50 years of age regardless of MMR status.

Somatic variant testing is useful in prognostication as well as to personalise oncological treatments for patients. The landscape of molecular changes may differ in EOCRC compared with LOCRC and there may be mutations found that allow access to clinical trials. Comprehensive molecular testing improved data collection, enhanced clinical trial access and ensured optimal

TABLE 3 | Tumour characteristics and somatic results including number of patients with available results and pathogenic variants detected and implications on treatment options based on these diagnoses.

Tumour characteristics	Number of patients (%)	Number of patients with available results
Somatic testing		893
dMMR	148 (16.5)	Access to immune checkpoint inhibitors and prognosis in early stage disease
pMMR	735 (82.3)	
Unknown	10 (1.2)	
Somatic pathogenic variant (NGS)		359
Yes	233 (64.9)	NB patients may have more than one mutation
No	126 (35.1)	
MLH1	52 (14.5)	
MSH2	1 (0.3)	
MSH6	27 (7.5)	
PMS2	11 (3.1)	
POLE	11 (3.1)	
POLD1	5 (1.4)	
KRAS	144 (40.1)	RAS/RAS wt left sided tumours suitable to receive EGFR inhibitors targeted treatment–encorafenib/cetuximab for *BRAF V600E* mutations
NRAS	49 (13.6)	
BRAF	21 (5.8)	
PIK3CA	39 (10.9)	
TP53	61 (17.0)	

FIGURE 4 | Tumour location for dMMR tumours $n = 148$ with right colon $n = 71$ (50.0%), left colon $n = 41$ (27.7%), rectum $n = 41$ (27.7%) and unknown $n = 10$ (6.8%) and for pMMR tumours $n = 735$ with 258 (35.4%) rectal disease, 257 (35.0%) in the left colon, 132 (18.0%) in the right colon, 1 (0.1%) in other area and 87 (11.8%) unknown.

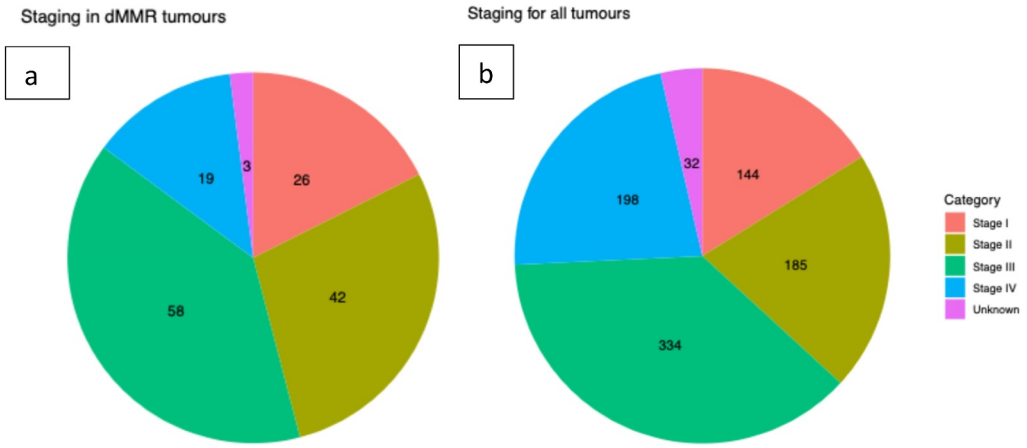


FIGURE 5 | (a) TNM stage for dMMR tumours $n = 148$ with 26 (17.6%) patients stage I disease, 42 (28.4%) with stage II disease, 58 (39.2%) with stage III disease, 19 (12.8%) stage IV disease and 3 (2%) unknown. (b) and for all tumours $n = 893$ with 144 (16.1%) patients having stage I disease, 185 (20.7%) with stage II disease, 334 (37.4%) with stage III disease and 198 (22.1%) with stage IV disease and 32 (3.6%) unknown.

care. Identification of patients as RAS/RAF wildtypes allows access to targeted treatment with EGFR blockade. Standard somatic variant panels often include investigational mutations essential for emerging treatments.

Clinical trials have variable access according to a wide range of factors such as location, recruitment windows and inclusion/exclusion criteria. The digestive cancers Europe website addresses these disparities for patients from EU and EAA countries [21]. Additionally, race- and sex-based molecular differences may offer novel biological insights into disparities in patient outcomes [22]. Accessing these trials can be challenging due to variations in health literacy among patients [23]. The younger EOCRC demographic also amplifies the perceived value of quality-adjusted life years (QALYs) [24].

In the whole CRC population (regardless of age) the proportion of patients diagnosed with stage IV disease is around 10% with lower levels of stage IV cancer in a screen detected population [25, 26]. Our cohort has a higher proportion with advanced disease of 22%, which may reflect multiple adverse factors including a lack of population screening, delays to diagnosis, or more aggressive disease biology in people with EOCRC. However, compared with rates of stage IV disease in a wider population, our recorded rates are lower than previously reported. This may mean our population is not representative of the wider EOCRC population, or may be related to the fact that there is a lot of missing data.

Although this dataset does not capture oncologic and treatment outcomes or survival for patients, it reflects findings from other large clinical trial datasets showing that there are pathogenic variants detected in around 16%–25% of cases with EOCRC [27]. However, when only a small proportion of our cohort had germline testing, it is difficult to make any firm conclusions as to its comparability. This study concluded that EOCRC patients were more likely to complete adjuvant chemotherapy but showed a worse outcome in stage III disease [24]. The IDEA collaboration and other studies suggest that many EOCRC patients receive aggressive treatment without evidence of survival benefit [23, 24].

Family history was documented in only 54% of patients. Of that cohort, only 19.8% of patients had a history of CRC in a 1st degree relative. Family history data is often gathered during oncology clinics, but it is often incomplete, inaccurate, or not collected at all. As a result, prediction models like PREMM [25], which rely on detailed information, may be less effective. This may be due to the clinic's focus on treatment discussions, which are the priority for healthcare professionals and patients at that time in treatment. Using electronic tools or questionnaires could improve the collection and storage of family history data, enabling the development of more accurate and comprehensive datasets for predictive models. There are many suggestions that positive family history could prompt earlier screening in high-risk individuals but with this data not being collected routinely it is difficult to implement this in the real world setting or understand if this is a problem [11, 15, 16, 23, 26].

Identification of inherited syndromes enables personalised surveillance and chemoprevention as well as cascade testing of family members. This allows family members to access preventative screening programmes and explore options for family planning and other measures to reduce risk such as lifestyle management, obesity management, *H. pylori* testing and aspirin use [7], relavent for those diagnosed with Lynch syndrome.

Our findings reveal substantial variability in molecular diagnostic practices for EOCRC across Europe. This likely reflects differences in clinical guidelines, diagnostic infrastructure, and policy frameworks. While the retrospective design and data heterogeneity limit direct comparisons, our study provides valuable insight into real-world testing patterns.

Finally, as molecular testing approaches and indications have evolved over the study period, our findings further support the need for standardised universal tumour sequencing in EOCRC. Despite the limitations—including retrospective design,

inconsistent data completeness, and centre-specific practices—this work highlights clear opportunities to enhance diagnostic pathways and optimise care for individuals with EOCRC [20].

Author Contributions

Conceptualization: K.J.M., J.P. Data curation: All authors. Formal analysis: P.V.E., K.J.M., J.P. Methodology: K.J.M., J.P. Supervision: K.J.M., J.P. Validation: K.J.M., J.P. Writing – original draft: P.V.E., K.J.M., J.P. Writing – review and editing: P.V.E., K.J.M., J.P.

Ethics Statement

For the GEOCODE consortium, each participating centre/country first received the study protocol and related documentation and then submitted it to their corresponding ethics committee for approval before contributing any data or results. For the SECOC study, we obtained ethical approval from the coordinating centre (documentation attached). As with GEOCODE, all participating centres were required to seek and obtain approval from their local ethics committees before sharing data. In Spain, for the cases included in GEOCODE prior to SECOC, all patients signed an informed consent form covering the analysis of MMR genes and/or other therapy-related genes (e.g., *BRAF*, *KRAS*). All consent forms explicitly stated that results could be used for research and publications. Patients were given the option to decline this use; none chose to do so. In the UK retrospective data was collected using standard service evaluation.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. C. E. Bailey, C. Y. Hu, Y. N. You, et al., "Increasing Disparities in the Age-Related Incidences of Colon and Rectal Cancers in the United States, 1975–2010," *JAMA Surgery* 150, no. 1 (2015): 17, https://doi.org/10.1001/jamasurg.2014.1756.

2. Surveillance Research Program. *SEER*Explorer: An Interactive Website for SEER Cancer Statistics*. [Internet]. Surveillance Research Program (National Cancer Institute, 2024), https://seer.cancer.gov/statistics-network/explorer/..SEER.2024.

3. S. Zhao, S. Wang, P. Pan, et al., "Magnitude, Risk Factors, and Factors Associated With Adenoma Miss Rate of Tandem Colonoscopy: A Systematic Review and Meta-Analysis," *Gastroenterology* 156, no. 6 (May 2019): 1661–1674, https://doi.org/10.1053/j.gastro.2019.01.260.

4. N. Akimoto, T. Ugai, R. Zhong, et al., "Rising Incidence of Early-Onset Colorectal Cancer—A Call to Action," *Nature Reviews Clinical Oncology* 18, no. 4 (April 2021): 230–243, https://doi.org/10.1038/s41571-020-00445-1.

5. I. O. Silla, D. Rueda, Y. Rodríguez, J. L. García, F. De La Cruz Vigo, and J. Perea, "Early-Onset Colorectal Cancer: A Separate Subset of Colorectal Cancer," *World Journal of Gastroenterology* 20, no. 46 (December 2014): 17288–17296, https://doi.org/10.3748/wjg.v20.i46.17288.

6. M. Daca-Alvarez, M. Martí, A. Spinelli, et al., "Familial Component of Early-Onset Colorectal Cancer: Opportunity for Prevention," *British Journal of Surgery* 109, no. 12 (December 2022): 1319–1325, https://doi.org/10.1093/bjs/znac322.

7. K. J. Monahan, N. Ryan, L. Monje-Garcia, et al., "The English National Lynch Syndrome Transformation Project: An NHS Genomic Medicine Service Alliance (GMSA) Programme," *BMJ Oncology* 2, no. 1 (October 2023): e000124, https://doi.org/10.1136/bmjonc-2023-000124.

8. N. J. Samadder, J. F. Valentine, S. Guthery, et al., "Family History Associates With Increased Risk of Colorectal Cancer in Patients With Inflammatory Bowel Diseases," *Clinical Gastroenterology and Hepatology* 17, no. 9 (August 2019): 1807–1813.e1, https://doi.org/10.1016/j.cgh.2018.09.038.

9. S. C. Shah and S. H. Itzkowitz, "Colorectal Cancer in Inflammatory Bowel Disease: Mechanisms and Management," *Gastroenterology* 162, no. 3 (2022): 715–730.e3, https://doi.org/10.1053/j.gastro.2021.10.035.

10. M. A. Jenkins, D. D. Buchanan, J. Lai, et al., "Assessment of a Polygenic Risk Score for Colorectal Cancer to Predict Risk of Lynch Syndrome Colorectal Cancer," *JNCI Cancer Spectrum* 5, no. 2 (April 2021): pkab022, https://doi.org/10.1093/jncics/pkab022.

11. M. Ranganathan, R. E. Sacca, M. Trottier, et al., Prevalence and Clinical Implications of Mismatch Repair-Proficient Colorectal Cancer in Patients With Lynch Syndrome [Internet] (2023).

12. J. Y. Alshenaifi, G. Vetere, G. Maddalena, et al., "Mutational and Co-Mutational Landscape of Early Onset Colorectal Cancer," [Internet] *Biomarkers* 30 (January 2025): 1–13, https://www.tandfonline.com/doi/full/10.1080/1354750X.2024.2447089.

13. G. Mauri, A. Sartore-Bianchi, A. G. Russo, S. Marsoni, A. Bardelli, and S. Siena, "Early-onset Colorectal Cancer in Young Individuals," *Molecular Oncology* 13, no. 2 (2019): 109–131, https://doi.org/10.1002/1878-0261.12417.

14. G. M. Cavestro, A. Mannucci, F. Balaguer, et al., "Delphi Initiative for Early-Onset Colorectal Cancer (DIRECt) International Management Guidelines," *Clinical Gastroenterology and Hepatology* 21, no. 3 (March 2023): 581–603, https://doi.org/10.1016/j.cgh.2022.12.006.

15. M. Araghi, M. Arnold, M. J. Rutherford, et al., "Colon and Rectal Cancer Survival in Seven High-Income Countries 2010–2014: Variation by Age and Stage at Diagnosis (The ICBP SURVMARK-2 Project)," [Internet] *Gut* 70, no. 1 (January 2021): 114–126, http://gut.bmj.com/content/70/1/114.abstract.

16. R. Cardoso, F. Guo, T. Heisser, et al., "Overall and Stage-Specific Survival of Patients With Screen-Detected Colorectal Cancer in European Countries: A Population-Based Study in 9 Countries," *Lancet Regional Health—Europe [Internet]* 21 (2022): 100458, https://www.sciencedirect.com/science/article/pii/S2666776222001521.

17. A. Cervantes, R. Adam, S. Roselló, et al., "Metastatic Colorectal Cancer: ESMO Clinical Practice Guideline for Diagnosis, Treatment and Follow-Up," *Annals of Oncology* 34, no. 1 (January 2023): 10–32, https://doi.org/10.1016/j.annonc.2022.10.003.

18. A. N. Archambault, Y. R. Su, J. Jeon, et al., "Cumulative Burden of Colorectal Cancer–Associated Genetic Variants Is More Strongly Associated With Early-Onset vs Late-Onset Cancer," *Gastroenterology* 158, no. 5 (April 2020): 1274–1286, https://doi.org/10.1053/j.gastro.2019.12.012.

19. N. J. Samadder, K. Curtin, L. Pappas, et al., "Risk of Incident Colorectal Cancer and Death After Colonoscopy: A Population-Based Study in Utah," *Clinical Gastroenterology and Hepatology* 14, no. 2 (February 2016): 279–286, https://doi.org/10.1016/j.cgh.2015.08.033.

20. A. N. Holowatyj, W. Wen, T. Gibbs, et al., "Racial/Ethnic and Sex Differences in Somatic Cancer Gene Mutations Among Patients With Early-Onset Colorectal Cancer," *Cancer Discovery* 13, no. 3 (March 2023): 570–579, https://doi.org/10.1158/2159-8290.cd-22-0764.

21. F. N. Mirza, E. Wu, H. F. Abdulrazeq, et al., "The Literacy Barrier in Clinical Trial Consents: A Retrospective Analysis," *eClinicalMedicine* 75 (September 2024): 102814, https://doi.org/10.1016/j.eclinm.2024.102814.

22. A. E. Attema, W. B. F. Brouwer, and J. L. Pinto, "The Role of Perceived Utility of Full Health in Age Weighting," *Value in Health* 25, no. 9 (September 2022): 1559–1565, https://doi.org/10.1016/j.jval.2022.04.1733.

23. E. Fontana, J. Meyers, A. Sobrero, et al., "Early-Onset Colorectal Adenocarcinoma in the IDEA Database: Treatment Adherence, Toxicities, and Outcomes With 3 and 6 Months of Adjuvant Fluoropyrimidine and Oxaliplatin," [Internet] *Journal of Clinical Oncology* 39, no. 36 (2021): 4009–4019, https://doi.org/10.1200/JCO.21.02008.

24. A. Gandini, J. Taieb, H. Blons, J. Netter, P. Laurent-Puig, and C. Gallois, "Early-Onset Colorectal Cancer: From the Laboratory to the Clinic," *Cancer Treatment Reviews* 130 (2024): 102821, https://doi.org/10.1016/j.ctrv.2024.102821.

25. R. C. Mercado, H. Hampel, F. Kastrinos, et al., "Performance of PREMM1,2,6, MMRpredict, and MMRpro in Detecting Lynch Syndrome Among Endometrial Cancer Cases," [Internet] *Genetics in Medicine* 14, no. 7 (2012): 670–680, https://doi.org/10.1038/gim.2012.18.

26. P. J. Kneuertz, G. J. Chang, C. Y. Hu, et al., "Overtreatment of Young Adults With Colon Cancer: More Intense Treatments With Unmatched Survival Gains," [Internet] *JAMA Surgery* 150, no. 5 (May 2015): 402–409, https://doi.org/10.1001/jamasurg.2014.3572.

27. M. E. Mork, Y. N. You, J. Ying, et al., "High Prevalence of Hereditary Cancer Syndromes in Adolescents and Young Adults With Colorectal Cancer," *Journal of Clinical Oncology* 33, no. 31 (November 2015): 3544–3549, https://doi.org/10.1200/jco.2015.61.4503.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.
Supporting Information S1: ueg270112-sup-0001-suppl-data.docx.