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The histological and molecular characteristics of early-onset colorectal cancer: a systematic review and meta-analysis

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Background: Early-onset colorectal cancer (CRC), defined as diagnosis before age 50, has increased in recent decades. Although more often diagnosed at advanced stage, associations with other histological and molecular markers that impact prognosis and treatment remain to be clarified. We conducted a systematic review and meta-analysis concerning the prevalence of prognostic and predictive tumor markers for early- vs. late-onset CRC, including oncogene mutations, microsatellite instability (MSI), and emerging markers including immune cells and the consensus molecular subtypes.

Methods: We systematically searched PubMed for original research articles published between April 2013–January 2024. Included studies compared the prevalence of tumor markers in early- vs. late-onset CRC. A meta-analysis was completed and summary odds ratios (ORs) with 95% confidence intervals (CIs) were obtained from a random effects model via inverse variance weighting. A sensitivity analysis was completed to restrict the meta-analysis to studies that excluded individuals with Lynch syndrome, a hereditary condition that influences the distribution of tumor markers for early-onset CRC.

Results: In total, 149 articles were identified. Tumors from early-onset CRC are less likely to include mutations in *KRAS* (OR, 95% CI: 0.91, 0.85-0.98), *BRAF* (0.63, 0.51-0.78), *APC* (0.70, 0.58-0.84), and *NRAS* (0.88, 0.78-1.00) but more likely to include mutations in *PTEN* (1.68, 1.04-2.73) and *TP53* (1.34, 1.24-1.45). After limiting to studies that excluded Lynch syndrome, the associations between early-onset CRC and *BRAF* (0.77, 0.64-0.92) and *APC* mutation (0.81, 0.67-0.97) were attenuated, while an inverse association with *PIK3CA* mutation was also observed (0.88, 0.78-0.99). Early-onset tumors are less likely to develop along the CpG Island Methylator Phenotype pathway (0.24, 0.10-0.57), but more likely to possess adverse histological features including high tumor grade (1.20, 1.15-1.25), and mucinous (1.22, 1.16-1.27) or signet ring histology (2.32, 2.08-2.57). A positive association with MSI status (1.31, 1.11-1.56) was also identified. Associations with immune markers and the consensus molecular subtypes are inconsistent.

Discussion: A lower prevalence of mutations in *KRAS* and *BRAF* is consistent with extended survival and superior response to targeted therapies for metastatic disease. Conversely, early-onset CRC is associated with aggressive histological subtypes and *TP53* and *PTEN* mutations, which may serve as therapeutic targets.

KEYWORDS

colorectal cancer, colon cancer, rectal cancer, early-onset, oncogenes, prognosis, molecular characteristics

1 Introduction

Colorectal cancer (CRC) is the second leading cause of cancer mortality in the United States (1). The incidence of CRC has steadily declined since the 1980s, largely attributed to greater uptake of colonoscopy screening by adults aged 50 years and older (2). Concurrently, the incidence of sporadic early-onset CRC, generally defined as CRC diagnosis before age 50 without an underlying hereditary cause, has significantly increased since the mid-1990s (2). Data from the Surveillance, Epidemiology, and End Results (SEER) program reflect a 2-3% annual increase in the incidence of early-onset CRC (3). The elevated incidence of early-onset CRC may be explained by birth cohort effects where more recent birth cohorts have increased prevalence of obesity and type 2 diabetes, lower levels of physical activity, and more often consume western-style diets characterized by lower consumption of fruits and vegetables (4), as well as changes in the composition of the gut microbiome (2). While early-onset CRC may be caused by hereditary conditions defined by germline mutations in DNA mismatch-repair genes (i.e. Lynch syndrome) or in the tumor suppressor *APC* (i.e. familial adenomatous polyposis) (5), these inherited conditions account for a relatively small percentage of early-onset CRC and do not explain the increased prevalence observed in recent decades (2).

CRC is a heterogeneous disease and the clinicopathological and molecular characteristics of tumors may influence prognosis and response to treatment (6). Beyond tumor stage, multiple potential prognostic and predictive markers have been identified, including mutations in oncogenes such as *KRAS*, *BRAF*, *PIK3CA*, and *TP53*, histological subtypes including mucinous and signet ring carcinomas, and the microsatellite instability (MSI) phenotype (7). Further, several novel prognostic markers have recently been identified, including immune markers in the tumor microenvironment (8) and the CRC consensus molecular subtypes (9). It is anticipated that the continued characterization of molecular phenotypes in CRC will augment traditional clinical markers for therapeutic decision making and support the development of targeted approaches to treatment (10).

Given the increasing rate of early-onset CRC, recent publications have highlighted potential differences in the clinicopathological and molecular characteristics of tumors based on age of onset (11–14). However, it is currently unclear whether early-onset CRC is distinct from late-onset disease in terms of molecular characteristics and tumor developmental pathways (15). Understanding the molecular characteristics of early-onset CRC is necessary to guide the development of therapeutic approaches for this condition and to address underlying causes. Therefore, we have completed a systematic review and meta-analysis to comprehensively summarize the evidence linking early-onset CRC to differences in prognostic and predictive tumor markers, including oncogene mutations, histological subtypes, MSI status, as well as anti-tumor immunity and the consensus molecular subtypes.

2 Methods

2.1 Literature review

Articles for this systematic review were identified utilizing a Pubmed search incorporating PRISMA guidelines (16). Given the wide breadth of the topic and the limited number of relevant articles published prior to 2013, the search was limited to peer-reviewed, original research articles published in English from the last 10 years (April 2013 – April 2023), with relevant keywords and medical subject headings included in the title and/or abstract. The literature review was repeated in January 2024 to identify recently published articles. Specific biomarker terms to include in the literature search were identified from prior reviews, and the search terms "biomark*", "mark*", and "character*" were included to capture potentially novel prognostic markers. All search terms included for the literature review are displayed in Supplementary Table S1. Manuscripts were included that reported the prevalence of prognostic biomarkers in

Abbreviations: AACR, American Association for Cancer Research; APC, adenomatous polyposis coli; CI, confidence interval; CIMP, CpG island methylator phenotype; CMS, consensus molecular subtypes; COH, City of Hope (National Medical Center); CRC, colorectal cancer; EGFR, epidermal growth factor receptor; MDACC, MD Anderson Cancer Center; MSI, microsatellite instability; MSKCC, Memorial Sloan Kettering Cancer Center; MSS, microsatellite stable; OR, odds ratio; SEER, Surveillance, Epidemiology, and End Results; TIL, tumor infiltrating lymphocytes.

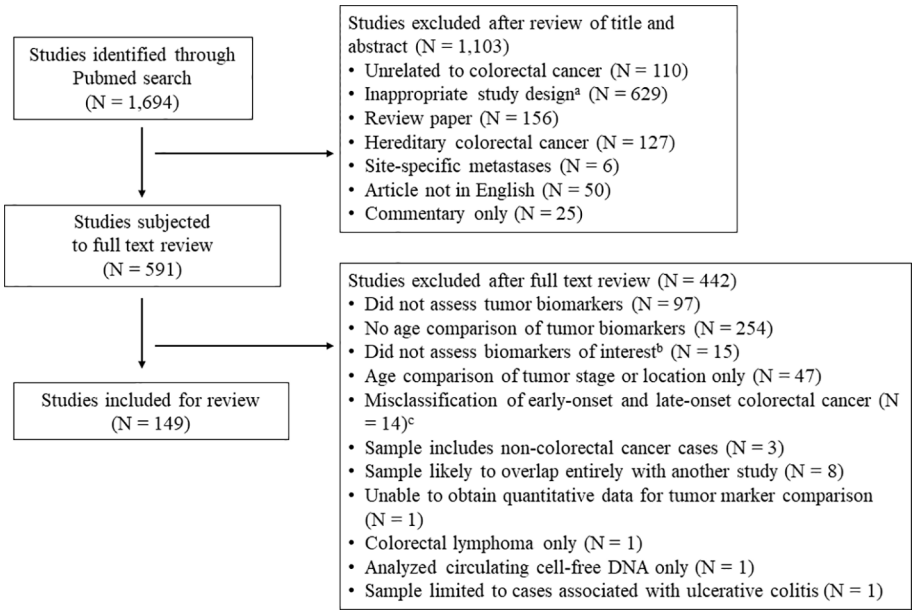

FIGURE 1
Literature review flowchart. [a] Inappropriate study design includes studies concerning colorectal cancer incidence, colonoscopy or other colorectal cancer screening, population level summary statistics for colorectal cancer, and studies of colorectal cancer in model organisms or *in vitro* studies. [b] Markers of interest include oncogene mutations in *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, *PTEN*, *TP53*, *APC*, and *HER2*; histological phenotypes including high-grade tumors and mucinous or signet ring histology; molecular carcinogenesis pathways including microsatellite instability and the CpG island methylator phenotype (CIMP); and novel tumor prognostic phenotypes including immune markers in the tumor microenvironment and the consensus molecular subtypes.[c] Studies where late-onset colorectal cancer was defined as ≥ 40 years at diagnosis (or younger), or early-onset CRC was defined as ≤ 60 years at diagnosis (or older).

CRC tumors separately for early- vs. late-onset disease. Articles were excluded if the prevalence of tumor clinicopathological or molecular biomarkers were not provided for participants with CRC (see Figure 1 flowchart), or if there was no comparison between early- vs. late-onset CRC (or if the comparison was limited to tumor stage or location only). Articles were also excluded that described hereditary CRC only (e.g. Lynch syndrome), site-specific metastases, or included non-CRC cancers in the analysis samples. For the purposes of this analysis, early-onset disease was defined as CRC diagnosed prior to age 50. To avoid misclassification of early- and late-onset CRC, we excluded papers where late-onset CRC was defined as ≥ 40 years at diagnosis or younger, or where early-onset CRC was defined as ≤ 60 years at diagnosis or older. Lastly, to limit sample overlap where possible, we excluded studies if there was evidence of complete overlap in sample and markers reported with a previously published study, or if a study reported the same outcome in a subsample of a previous study.

The systematic review and meta-analysis was limited to the following markers that have been shown associations with CRC survival and/or therapeutic response in CRC: oncogene mutations in *KRAS* (17–20), *NRAS* (17, 21, 22), *BRAF* (17, 19, 23, 24), *PIK3CA* (17, 25, 26), *PTEN* (27, 28), *TP53* (29), *APC* (30, 31), and *HER2* amplifications (32–34); histological phenotypes including high-grade tumors (35, 36) and mucinous (37, 38) or signet ring histology (38, 39); molecular carcinogenesis pathways including MSI (40) and the CpG island methylator phenotype (CIMP) (41); and novel tumor prognostic phenotypes including immune markers (42, 43) in the tumor microenvironment and the consensus molecular subtypes (9, 44). Because it is well-established that early-onset CRC is associated with advanced tumor summary stage at diagnosis and rectal tumor location, these markers are not summarized in this review. The literature review was completed by two authors (T.L. and L.P) independently. Disagreements between reviewers were resolved by further review of the manuscript to determine whether the study included a comparison of tumor markers of interest between early- and late-onset CRC. The final decision to include a manuscript was made by the lead author. In total, 1,694 articles were identified from the literature search and 149 were eligible for review (Figure 1). For each study, the potential for bias was evaluated by the lead author using the Newcastle-Ottawa Scale adapted for cross-sectional studies (45). Pre-registration of the systematic review protocol was not performed.

2.2 Meta-analysis

From each eligible study, the number of mutant and wild-type tumors for each marker in early- and late-onset CRC was extracted by the lead author. Data extraction was completed in duplicate, and the results from the two extractions were compared to identify any errors or inconsistencies in the sample sizes, which were subsequently revised after further review of the original article. If these data were not available from the manuscript, sample sizes were requested from the corresponding author. One study was excluded for which we were unable to obtain the necessary sample

sizes from each group (46). When necessary, sample sizes for separate age groups were combined to create a single category for early-onset and late-onset CRC. For most studies, age 45 or 50 at diagnosis was utilized as the threshold to distinguish early- vs. late-onset CRC, although occasionally other classifications were employed (see Supplementary S2). For each study, sample characteristics including overall sample size, country, tumor stage, sex, or other distinguishing features were also extracted. For each marker, an odds ratio (OR) and 95% confidence interval (CI) were calculated using a standard equation (47). For mutations in oncogenes *KRAS, NRAS, BRAF, PIK3CA, PTEN, TP53*, and *APC*, as well as MSI status and histological subtypes, meta-analyses were completed to compare the prevalence in tumors from early- vs. late-onset CRC. Due to the wide variety of immune markers that have been reported, a meta-analysis was not attempted for the comparison of immune phenotypes in the tumor microenvironment. For each marker that was meta-analyzed, a pooled OR with 95% CI was obtained from a random effects model via inverse variance weighting. The random effects model was selected *a priori*, as between-study heterogeneity is plausible given variability in the definition of early-onset CRC, as well as differences in tumor location, race, nationality and stage between studies. The random effects meta-analysis is capable of providing unbiased estimates in the presence of heterogeneity and will generally provide more conservative estimates than the fixed-effects model (which assumes no between-study heterogeneity) (48). Heterogeneity was determined via the Cochrane's Q statistic and the I^2 statistic. Significant heterogeneity was defined as P <.05 for Cochrane's Q or $I^2 \geq 50\%$. To determine whether the meta-analysis estimates were influenced by a single study, a 'leave-one-out' sensitivity analysis was conducted for each marker. Because Lynch syndrome may influence the prevalence of tumor markers for individuals with early-onset CRC, a second sensitivity analysis was completed to limit the analysis to studies that specifically excluded individuals with Lynch syndrome or family history of CRC, or that restricted the sample to microsatellite stable tumors. All statistical tests were two-sided, with statistical significance defined using a threshold of P <.05. All meta-analyses were completed using Review Manager 5.4.1 from Cochrane.

3 Results

In total, 149 articles were reviewed that compared the prevalence of clinicopathological tumor markers in early- vs. late-onset CRC. All meta-analysis results are summarized in Table 1. Sample characteristics and references for all included studies are presented in Supplementary Table S2. Results of the bias assessment utilizing the Newcastle-Ottawa Scale are presented in Supplementary Table S4.

3.1 Oncogene mutations

The number of studies identified for the following markers is as follows: *KRAS* mutation (49); *BRAF* mutation (49); *NRAS* mutation

TABLE 1 Summary of meta-analysis results showing associations between early-onset colorectal cancer and the prevalence of tumor markers, compared to late-onset colorectal cancer.

	All studies (N = 150)			Studies that excluded individuals with Lynch syndrome [a] (N = 50)		
Marker	Number of studies	OR (95% CI)	P-value	Number of studies	OR (95% CI)	P-value
KRAS mutation	54	0.91 (0.85-0.98)	.01	19	0.87 (0.80-0.95)	.002
BRAF mutation	54	0.63 (0.51-0.78)	<.001	17	0.77 (0.64-0.92)	.004
NRAS mutation	20	0.88 (0.78-1.00)	.06	6	0.89 (0.70-1.13)	.33
APC mutation	19	0.70 (0.58-0.84)	<.001	7	0.81 (0.67-0.97)	.02
TP53 mutation	20	1.34 (1.24-1.45)	<.001	8	1.40 (1.32-1.48)	<.001
PTEN mutation	8	1.68 (1.04-2.73)	.04	3	2.81 (0.56-14.18)	.21
PIK3CA mutation	21	0.95 (0.86-1.05)	.29	8	0.88 (0.78-0.99)	.03
HER2 amplification	4	1.64 (0.86-3.14)	.13	0	N/A	N/A
CIMP status	10	0.24 (0.10-0.57)	.001	7	0.41 (0.21-0.79)	.007
MSI status	64	1.31 (1.11-1.56)	.002	20	1.37 (0.91-2.07)	.13
High tumor grade	87	1.20 (1.15-1.25)	<.001	33	1.34 (1.15-1.57)	<.001
Mucinous histology	57	1.22 (1.16-1.27)	<.001	20	1.51 (1.23-1.84)	<.001
Signet ring histology	44	2.32 (2.08-2.57)	<.001	9	2.70 (1.77-4.14)	<.001

Data presented as odds ratio (95% confidence interval).
CI, confidence interval; CIMP, CpG island methylator phenotype; MSI, microsatellite instability; OR, odds ratio.
[a]Includes studies that excluded individuals with Lynch syndrome, all hereditary syndromes, microsatellite instability, or family history of colorectal cancer.
N/A indicates not applicable.

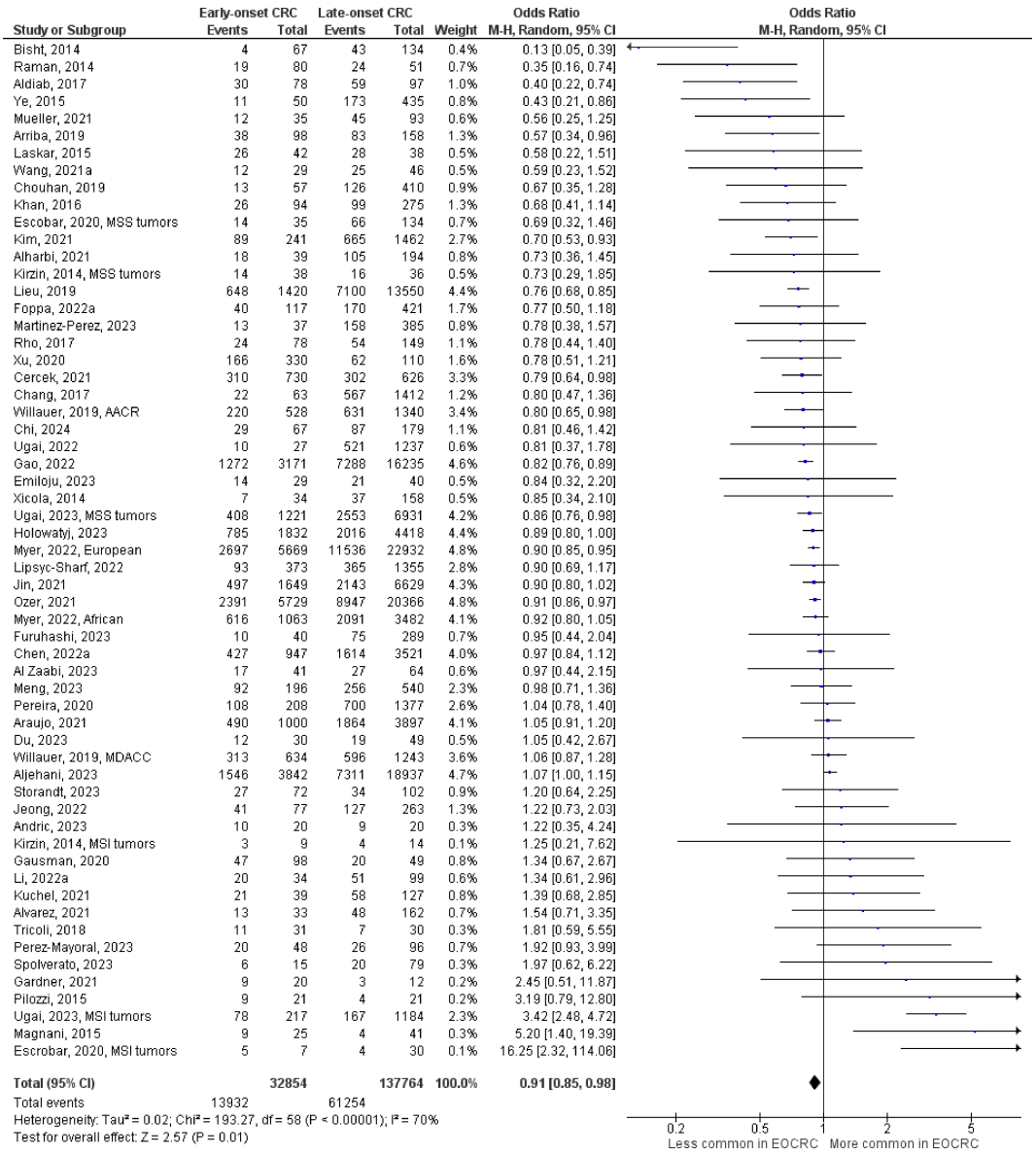

FIGURE 2
Odds ratios for *KRAS* mutation in early-onset CRC. Data presented as odds ratios (95% confidence interval) for *KRAS* mutation in early-onset relative to late-onset colorectal cancer. The pooled odds ratio is obtained via a random effects model using inverse variance weighting. AACR, American Association for Cancer Research; MDACC, MD Anderson Cancer Center; MSI, microsatellite instability; MSS, microsatellite stable.

(20); *PIK3CA* mutation (21); *PTEN* mutation (8); *HER2* amplifications (5); *APC* mutation (19); *TP53* mutation (20). For early-onset CRC, there is evidence for a significantly lower prevalence of mutations in *KRAS* (Figure 2, OR 0.91, 95% CI 0.85-0.98), *BRAF* (Figure 3, OR 0.63, 95% CI 0.51-0.78) and *APC* (Figure 4, OR 0.70, 95% CI 0.58-0.84) compared to late-onset CRC. Early-onset CRC was associated with non-significantly lower prevalence of mutations in *NRAS* (Figure 5, OR 0.88, 95% CI 0.78-1.00, p = .06). Conversely, early-onset CRC is associated with a higher prevalence of mutations in *TP53* (Figure 6, OR 1.34, 95% CI 1.24-1.45) and *PTEN* (Figure 7, OR 1.68, 95% CI 1.04-2.73). There was no significant difference in the prevalence of *PIK3CA* mutations (Supplementary Figure S1, OR 0.95, 95% CI 0.86-1.05), or *HER2* amplifications (Supplementary Figure S2, OR 1.64, 95% CI 0.86-3.14). Significant inter-study heterogeneity was observed for mutations in *KRAS*, *BRAF*, *PTEN*, and *APC*. Hazard ratios for oncogene mutations were stable in the leave-one-out sensitivity analysis (Supplementary Table S3), although the association for *NRAS* and *PTEN* mutations did not always reach statistical significance.

Fifty studies were identified that specifically excluded individuals with Lynch syndrome or family history of CRC, or that restricted the analysis to individuals with microsatellite stable tumors (Table 1; Supplementary Table S2). Compared to the full analysis, the association between early-onset CRC and *BRAF* (OR 0.77, 95% CI 0.64-0.92) and *APC* mutations (OR 0.81, 95% CI 0.67-0.97) were attenuated but remained statistically significant, while the associations with *KRAS*, *NRAS*, and *TP53* mutations were similar. Further, an inverse association between early-onset CRC

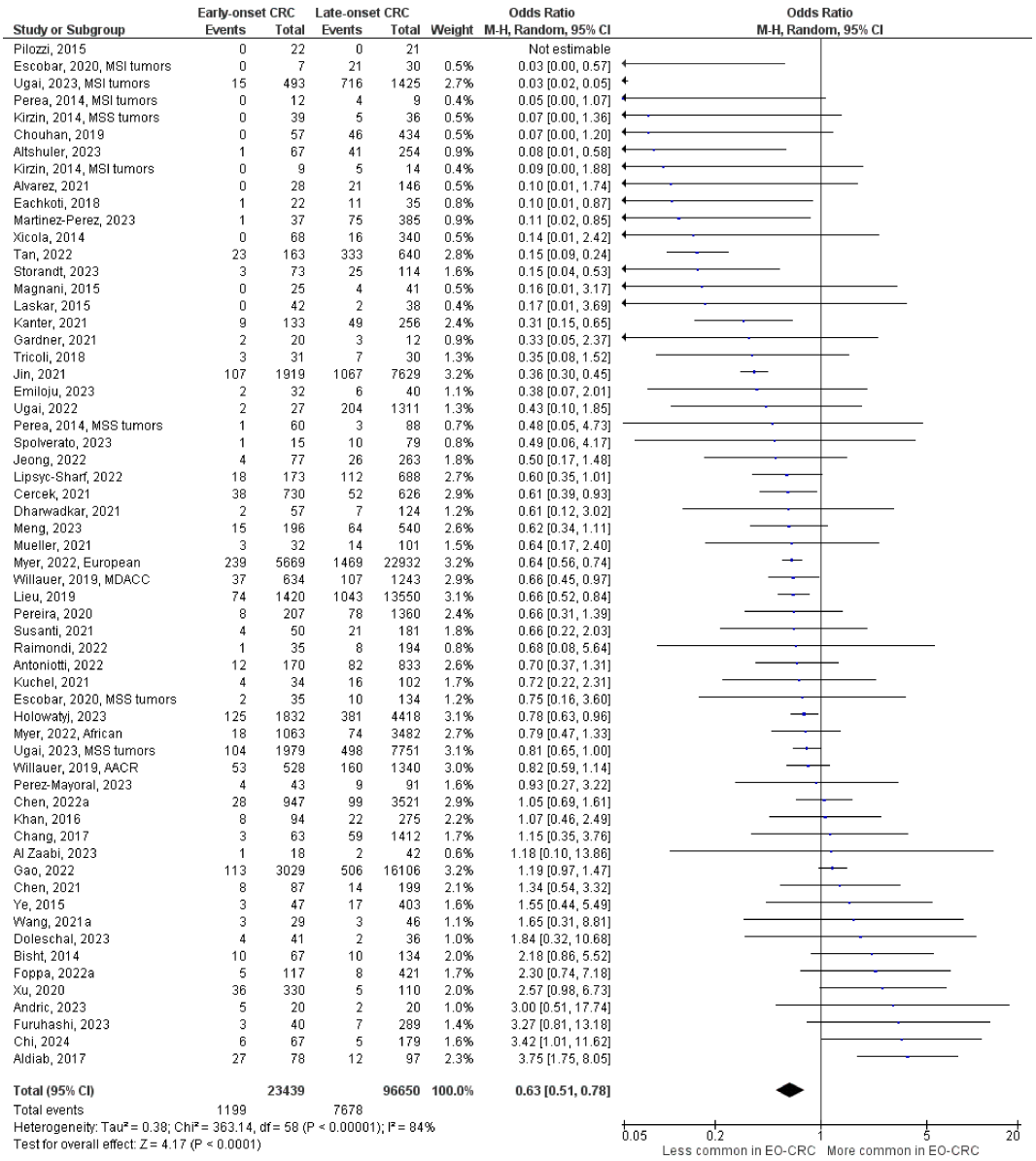

FIGURE 3
Odds ratios for *BRAF* mutation in early-onset CRC. Data presented as odds ratios (95% confidence interval) for *BRAF* mutation in early-onset relative to late-onset colorectal cancer. The pooled odds ratio is obtained via a random effects model using inverse variance weighting. AACR, American Association for Cancer Research; MDACC, MD Anderson Cancer Center; MSI, microsatellite instability; MSS, microsatellite stable.

and *PIK3CA* mutation was also observed (OR 0.88, 95% CI 0.78-0.99).

3.2 Molecular carcinogenesis pathways

There were 10 studies that compared the prevalence of CIMP-high status in early- vs. late-onset CRC, and 64 studies that compared MSI status. Individuals with early-onset CRC had significantly lower odds for CIMP-high tumors compared to individuals with late-onset disease (Supplementary Figure S3, OR 0.24, 0.10-0.57), but significantly higher odds for the MSI phenotype (Supplementary Figure S4, OR 1.31, 1.11-1.56). Significant heterogeneity was observed for both markers. Associations were stable in the leave-one-out sensitivity analysis (Supplementary Table S3), and after limiting the analysis to studies that excluded individuals with Lynch syndrome or family history of CRC (Table 1).

3.3 Histological characteristics

There were 86 studies that compared the prevalence of high-grade tumors (i.e. poorly differentiated or undifferentiated tumors) in early- vs. late-onset CRC, 57 studies that compared the prevalence of mucinous histology (or mucinous characteristics), and 44 studies that reported on signet ring cell carcinomas. In early-onset CRC, there was evidence for a significantly higher prevalence

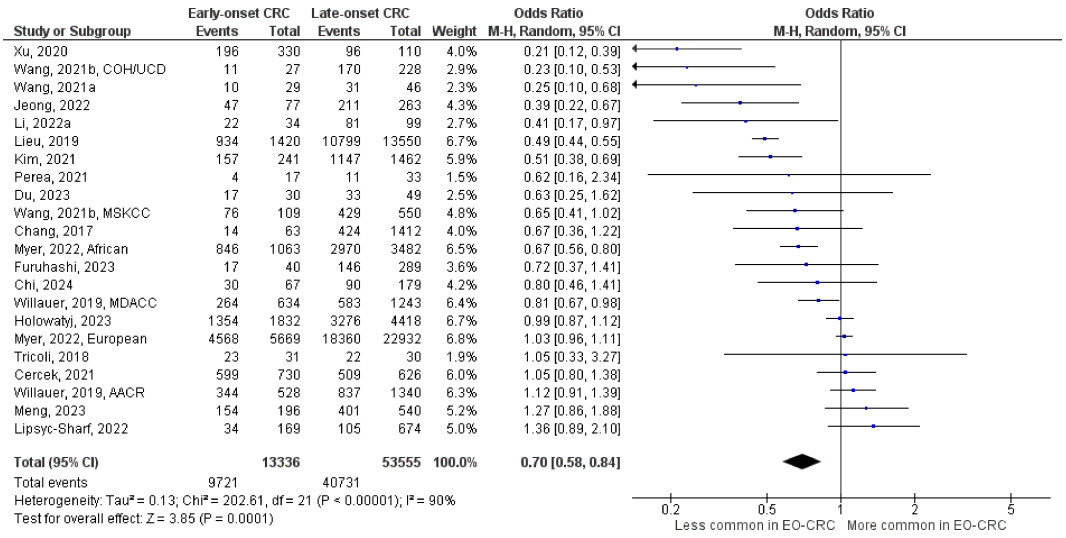

FIGURE 4
Odds ratios for *APC* mutation in early-onset colorectal cancer. Data presented as odds ratios (95% confidence interval) for *APC* mutation in early-onset relative to late-onset colorectal cancer. The pooled odds ratio is obtained via a random effects model using inverse variance weighting. AACR, American Association for Cancer Research; COH, City of Hope National Medical Center; CI, confidence interval; EO-CRC, early-onset colorectal cancer; MDACC, MD Anderson Cancer Center; MSKCC, Memorial Sloan Kettering Cancer Center; UCD, University of California, Davis.

of high-grade (i.e., poorly differentiated) tumors (Supplementary Figure S5, OR 1.20, 95% CI 1.15-1.25), as well as mucinous tumors (Supplementary Figure S6, OR 1.22, 95% CI 1.16-1.27), and signet ring cell carcinomas (Supplementary Figure S7, OR 2.32, 2.08-2.57). Significant inter-study heterogeneity was observed for all histological markers. All associations were stable in the leave-one-out sensitivity analysis (Supplementary Table S3) and after limiting the analysis to studies that excluded individuals with Lynch syndrome or family history of CRC (Table 1).

3.4 Immune markers

There have been nine studies to investigate age differences in the immune cell populations of CRC tumors, with inconsistent results (49–57). Du et al. reported that Chinese patients with sporadic early-onset CRC showed significantly higher densities of multiple immune cell populations in the tumor microenvironment compared to patients with late-onset disease, including higher levels of B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells (50). By contrast, Ugai et al. reported no significant differences in the populations of T cells, macrophages, and other myeloid cells in participants with early- vs. late-onset CRC from the Nurses' Health Study and Health Professionals Follow-up Study (51). In a small study of 14 tumors utilizing single cell RNA sequencing, Li et al. reported that early-onset CRC was associated with lower levels of effector CD8+ T cells and antigen-presentation in the tumor microenvironment, but higher levels of naïve CD8+ T cells and immunosuppressive regulatory T cells compared to individuals with late-onset disease, suggesting an impaired anti-tumor immune response for early-onset CRC (54). Because MSI status may influence the anti-tumor immune response, recent studies have examined associations between early-onset CRC and tumor lymphocyte populations in samples limited to microsatellite stable tumors, or after careful exclusion of participants with Lynch syndrome (56, 57). In a matched analysis of microsatellite stable tumors, Lu et al. (2023) reported that there was no significant differences between early- and late-onset CRC for the infiltration of 22 different lymphocyte populations in the tumor microenvironment (57). Likewise, Andric et al. found no significant difference for five lymphocyte populations (total T cells, conventional CD4+ and CD8+ T cells, regulatory T cells, and γδ T cells) in a matched sample limited to cases of sporadic CRC (56). Other studies have reported no significant differences between early and late-onset CRC for the density of total tumor infiltrating lymphocytes (53, 55).

3.5 The consensus molecular subtypes

There have been six studies to determine the distribution of consensus molecular subtypes (CMS) for CRC by age at diagnosis (50, 57–61). Utilizing tumor tissues samples from 626 individuals diagnosed with CRC from The Cancer Genome Atlas and MD Anderson Cancer Center, Willauer reported that the CMS1 subtype was more common among patients aged 30-39 years at diagnosis (46%) compared to older participants, while the CMS4 subtype was less common (13%) (58). Conversely, in a smaller study from the Nanjing Colorectal Cancer Cohort, Du et al. reported a higher prevalence of the CMS4 subtype in early- vs. late-onset CRC (36.7% vs. 12.2%, respectively), although the comparison between age groups did not reach statistical significance (50). Recent results, including from a small sample of South Korean participants (59) and additional analyses of The Cancer Genome Atlas (60, 61) did not show any significant association between early-onset tumors and the distribution of consensus molecular subtypes.

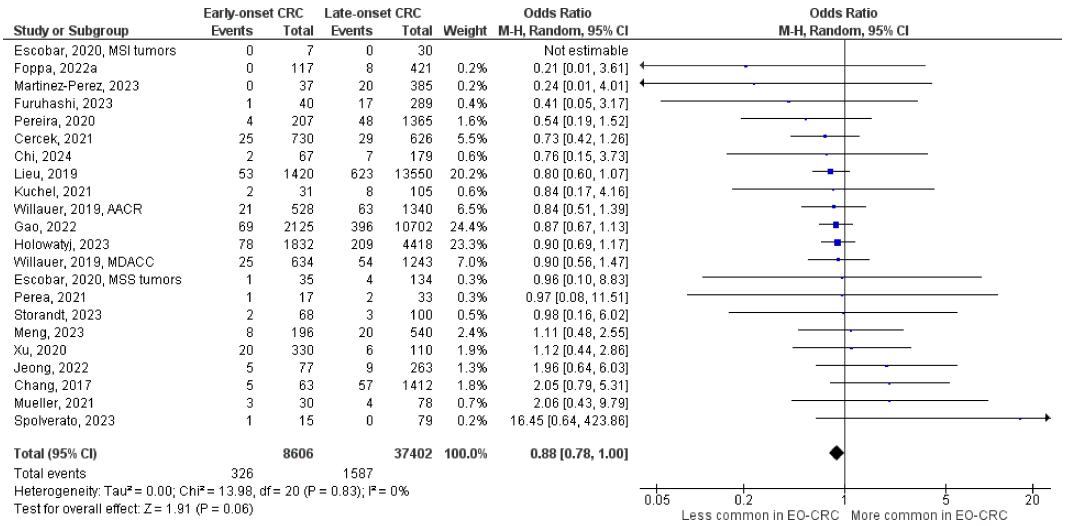

FIGURE 5
Odds ratios for *NRAS* mutation in early-onset colorectal cancer. Data presented as odds ratios (95% confidence interval) for *NRAS* mutation in early-onset relative to late-onset colorectal cancer. The pooled odds ratio is obtained via a random effects model using inverse variance weighting. AACR, American Association for Cancer Research; CI, confidence interval; EO-CRC, early-onset colorectal cancer; MDACC, MD Anderson Cancer Center.

4 Discussion

Sporadic early-onset CRC is a significant public health concern, increasing by 2-3% per year in the U.S. since 1990 (3, 62). Early-onset CRC is more often diagnosed at advanced stages compared to late-onset disease (63, 64). However, there is inconsistent evidence that survival varies between early- and late-onset CRC (65, 66), complicated by reports that younger patients receive more aggressive systemic treatment (67–69). Thus, international guidelines do not endorse separate treatment recommendations for early-onset disease (70). Investigating the associations between early-onset tumors and molecular and histological characteristics, and novel tumor markers including immune cell populations, may help to guide the development of therapies that benefit early-onset CRC. Further, highlighting associations between early-onset CRC and tumor markers may aid in the design of clinical trials for targeted therapies. To the authors' knowledge, this is the first comprehensive systematic review and meta-analysis of tumor prognostic and predictive markers in early-onset CRC. We found that early-onset CRC was associated with a lower prevalence of

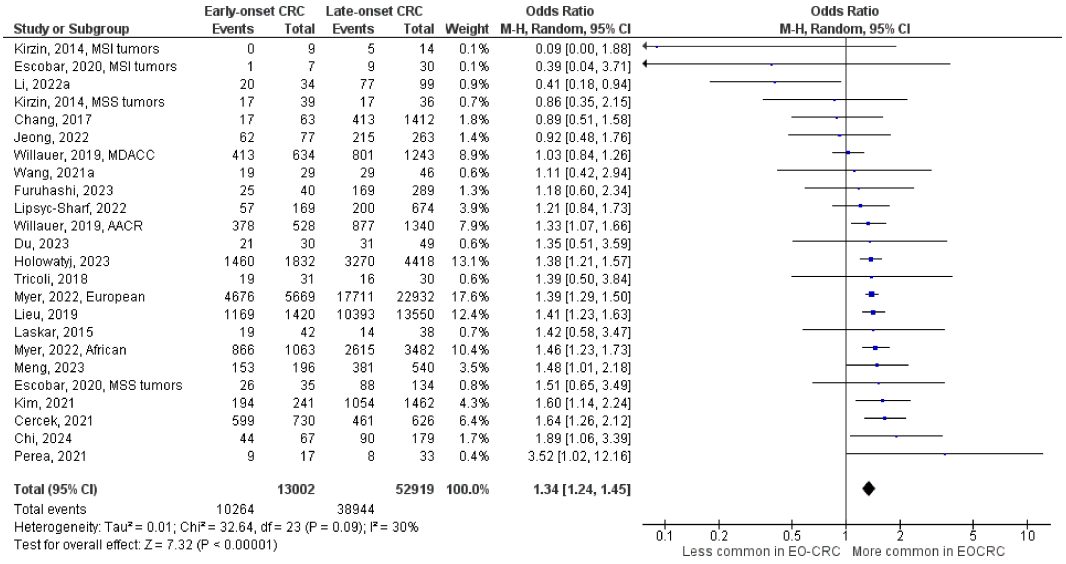

FIGURE 6
Odds ratios for *TP53* mutation in early-onset colorectal cancer. Data presented as odds ratios (95% confidence interval) for *TP53* mutation in early-onset relative to late-onset colorectal cancer. The pooled odds ratio is obtained via a random effects model using inverse variance weighting. AACR, American Association for Cancer Research; CI, confidence interval; EO-CRC, early-onset colorectal cancer; MDACC, MD Anderson Cancer Center; MSI, microsatellite instability; MSS, microsatellite stability.

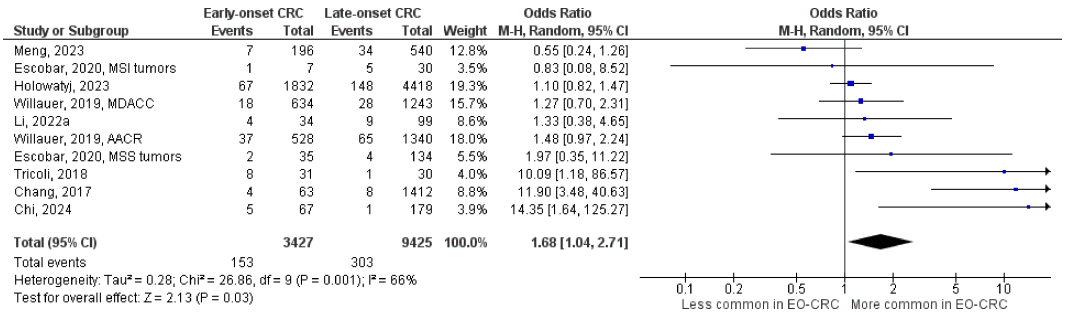

FIGURE 7
Odds ratios for *PTEN* mutation in early-onset colorectal cancer. Data presented as odds ratios (95% confidence interval) for *PTEN* mutation in early-onset relative to late-onset colorectal cancer. The pooled odds ratio is obtained via a random effects model using inverse variance weighting. AACR, American Association for Cancer Research; CI, confidence interval; EO-CRC, early-onset colorectal cancer; MDACC, MD Anderson Cancer Center.

oncogene mutations in *KRAS*, *BRAF*, *NRAS*, and *APC*, but a higher prevalence of *TP53* and *PTEN* mutations and adverse histologic subtypes, with inconsistent associations for immune cell populations and the consensus molecular subtypes.

KRAS, *BRAF*, and *NRAS* encode proteins that act downstream of the epidermal growth factor receptor (EGFR) and activate Mek/Erk signaling (21, 71). Mutations in these oncogenes are negative predictive markers for EGFR inhibition in metastatic CRC (17, 18) and are associated with inferior survival outcomes across tumor stage (19, 20, 23, 72), including for early-onset CRC (73–75). Early-onset CRC is associated with a lower prevalence of mutations in these genes compared to late-onset disease, indicating that individuals with metastatic early-onset CRC may be more likely to benefit from EGFR inhibition. Notably, the association with *NRAS* mutations was not statistically significant, which may be due to the scarcity of this marker (76). Further, the association with *BRAF* mutation was attenuated but still statistically significant in studies that excluded individuals with Lynch syndrome, who are less likely to have *BRAF* mutations compared to sporadic disease (77). Further, this sensitivity analysis revealed an inverse association with *PIK3CA* mutation, which has also been linked to higher risk for mortality and resistance to EGFR inhibition (17, 78). Conversely, early-onset CRC was associated with a *higher* proportion of mutations in tumor suppressor *PTEN*, which encodes a lipid-phosphatase that suppresses the activity of PI3k/Akt/mTOR signaling and interacts with the EGFR pathway (27). Loss of PTEN activity has been linked to resistance to EGFR inhibition in metastatic CRC (79) but is not currently used in clinical decision making. Pharmaceutical therapies to restore normal PTEN activity are under development but have not been evaluated in CRC. Early-onset CRC was associated with a significantly higher prevalence of *TP53* mutations, which cause loss of p53 tumor suppressor activity and pro-tumorigenic gain of function effects that accelerate cell proliferation, angiogenesis, and metastasis (80). *TP53* mutations are found in approximately 60% of tumors and may promote resistance to EGFR inhibitors and chemotherapies that rely on wild type p53 to induce cellular apoptosis (e.g. 5-fluorouracil and Oxaliplatin) (29). Consequently, targeted therapies to restore wild type p53 activity or degrade mutant p53, or to inhibit downstream effector pathways, are currently being investigated in clinical trials (81). Potentially, individuals with early-onset CRC may be more likely to benefit from treatments that inhibit pro-tumorigenic p53 activity and should be targeted for enrollment in these trials.

Early-onset CRC was associated with a lower prevalence of *APC* mutation, a key driver of the canonical adenoma-carcinoma pathway (82). *APC* mutations are present in approximately 80% of CRC tumors (11, 12, 14), and recent evidence indicates that *APC*-mutant tumors are associated with extended overall and progression-free survival compared to wild type (30, 31) (5). Notably, the association with *APC* mutation was attenuated but still statistically significant when limiting the analysis to studies that excluded individuals with Lynch syndrome, or that included microsatellite stable tumors only. Individuals with early-onset CRC had a higher prevalence of MSI, defined by a high density of somatic mutations in short, non-coding sequences caused by defects in DNA mismatch repair (40). MSI is associated with lower risk for overall mortality and distant metastases compared to microsatellite stable tumors, including in early-onset CRC (75). Further, MSI tumors secrete truncated proteins that trigger an anti-tumor immune response (83), and consequently MSI is a positive predictor for response to immune checkpoint inhibitors (83). Our findings therefore highlight the importance of MSI testing for individuals younger than 50, in accordance with clinical guidelines (70). Unexpectedly, the association between early-onset CRC and MSI status was modestly strengthened in studies that excluded individuals with known Lynch syndrome, which causes tumors with MSI (84). Because a significant proportion of individuals with Lynch syndrome may be unaware of the condition (85), it is possible that the exclusion of Lynch syndrome was incomplete in some studies. Early-onset CRC was associated with a lower prevalence of the CpG island methylator phenotype (CIMP), characterized by methylation and inactivation of tumor-suppressor genes (86). Although CIMP has been linked to poor prognosis in multiple studies, it currently has limited value as a prognostic marker due to a lack of standardized assessment and competing effects of MSI and *BRAF* mutation, which are associated with CIMP (41).

We also found that early-onset CRC is associated with higher odds for tumors with more aggressive histological features, including poorly differentiated tumors, mucinous carcinomas, and signet ring cell carcinomas (38, 87). The association with signet ring features was

especially pronounced (OR [95% CI]: 2.32 [2.08-2.57]). Although signet ring carcinomas comprise only 1% of CRC tumors (39), this feature is present in 2-3% of early-onset tumors. A recent meta-analysis showed that signet ring carcinomas were associated with significantly higher risk for overall mortality and recurrence compared to conventional adenocarcinomas (88). Results were similar for mucinous tumors, which comprise approximately 10-15% of CRCs (89). The associations between histological subtypes and colorectal cancer mortality, especially poorly differentiated tumors and signet ring carcinomas, have been validated in early-onset CRC (90–93). Currently, there are no treatments that specifically target mucinous or signet ring cell carcinomas and treatment guidelines do not distinguish between histological subtypes (70).

The observed associations between early-onset CRC and certain histological and molecular tumor characteristics may be explained in part by differences in tumor location (94). Approximately 30% of early-onset tumors are located in the rectum, versus 20% of late-onset tumors (64, 95). *KRAS, BRAF, PIK3CA*, and *NRAS* mutations are enriched in proximal tumors (96, 97) while *TP53* mutations are enriched in rectal tumors (98). Notably, studies that were limited to individuals with tumors in the distal colon or rectum have not shown a consistent association between early-onset CRC and the presence of oncogene mutations (46, 55, 56, 99–102). For example, a study with more than 1,000 distal and rectal tumors showed no significant age difference in *KRAS*, *BRAF, NRAS*, *PIK3CA*, *TP53*, or *APC* mutations (46). Conversely, in a large-scale analysis with detailed stratification by tumor location, Ugai et al. found that early-onset CRC had a lower prevalence of *BRAF* mutations for all tumor sites except the sigmoid colon and rectum (103). Notably, aggressive histological subtypes are overrepresented in the proximal colon (104), and consequently the association with early-onset CRC is not explained by differences in tumor location.

We found inconsistent evidence linking early-onset CRC to differences in 'novel' tumor prognostic and predictive markers including populations of immune cells in the tumor microenvironment (8). A recent meta-analysis demonstrated that a higher density of tumor infiltrating lymphocytes was associated with reduced overall mortality among 20,015 individuals with CRC (HR [95% CI]: 0.65 [0.54-0.77]) (42), while others have shown that an 'immunoscore' encompassing cytotoxic T cells and CD3+ cells was a superior prognostic marker compared to the tumor stage (105, 106). Currently, the association between early-onset CRC and the anti-tumor immune response has been inconsistent (48–50, 52, 53, 55, 56, 58). Notably, higher rates of MSI in early-onset CRC due to Lynch syndrome may obscure associations with immune markers in sporadic disease, as MSI tumors trigger a robust anti-tumor immune response (83). Studies limited to microsatellite stable tumors or that carefully excluded participants with hereditary syndromes have tended to show no significant differences in immune cell populations between early- and late-onset CRC (51, 56, 57). Likewise, there is currently no consistent evidence that the distribution of consensus molecular subtypes differs between early- and late-onset CRC, with most studies reporting null findings (50, 57, 59–61). The consensus molecular subtypes have shown to be a robust predictor of mortality outcomes independent of tumor stage (107), but to the authors' knowledge have not been validated specifically in early-onset CRC. Further, the identification of novel molecular subtypes in early-onset CRC based on tumor gene expression is an area for future research.

Strengths of this study include the comprehensive nature of the search strategy, as we were able to summarize the evidence for age-related differences in the prevalence of established tumor prognostic markers as well as emerging markers including immune cell populations in the tumor microenvironment and the consensus molecular subtypes. Further, the large number of studies identified for most markers allowed for relatively precise estimates of the association with early-onset CRC. Lastly, to better understand the associations between early-onset CRC and tumor markers in *sporadic* disease, we completed a sensitivity analysis limited to studies that excluded individuals with known Lynch syndrome (or family history of CRC). This analysis is also attended by several limitations. Due to the breadth of the review, our literature search was limited to original research studies published within the last ten years in Pubmed. Consequently, it is possible that a relevant study was missed. However, this is unlikely to be a significant limitation given the paucity of large tumor genomic studies published prior to 2013 and the comprehensive nature of our search strategy. Further, there was evidence for significant heterogeneity in the estimates for most tumor markers, but we were unable to investigate underlying sources of inter-study heterogeneity because the prevalence of tumor prognostic markers was rarely presented in subgroups defined by tumor location, tumor stage, or MSI status. Between-study differences in the definitions of early- and late-onset CRC may also have contributed to heterogeneity, although we excluded studies where misclassification of early-onset CRC was apparent. Lastly, although we attempted to control for bias by performing a sensitivity analysis limited to studies that accounted for Lynch syndrome in the study design, it is possible that residual confounding by hereditary conditions or differences in tumor location may have biased the results.

5 Conclusions

In summary, early-onset CRC was associated with a lower prevalence of mutations in several oncogenes linked to mortality and poor therapeutic response, including *KRAS*, *BRAF*, and *NRAS* compared to individuals with late-onset disease. Conversely, early-onset disease was associated with a higher prevalence of potentially harmful mutations in *TP53* and *PTEN*, as well as aggressive histological subtypes including mucinous and signet ring cell carcinomas. In part, these associations may reflect the higher prevalence of rectal tumors in early-onset CRC and the effect of hereditary syndromes on tumor markers. Given these findings and the alarming rise in the incidence of early-onset CRC, it is essential that clinical trials for targeted therapies enroll sufficient numbers of individuals with early-onset disease to evaluate their efficacy in this subgroup. Additional research is required to clarify the relationships with novel tumor characteristics including immune markers and to identify molecular subtypes specific to early-onset CRC that can inform treatment and prognosis.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

TL: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. LP: Writing – review & editing, Writing – original draft, Investigation, Data curation. SW: Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1349572/full#supplementary-material

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