Clinical Cancer Research

Comprehensive Genomic Landscapes in Early and Later Onset Colorectal Cancer

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Abstract

Purpose: The incidence rates of colorectal cancers are increasing in young adults. The objective of this study was to investigate genomic differences between tumor samples collected from younger and older patients with colorectal cancer.

Experimental Design: DNA was extracted from 18,218 clinical specimens, followed by hybridization capture of 3,769 exons from 403 cancer-related genes and 47 introns of 19 genes commonly rearranged in cancer. Genomic alterations (GA) were determined, and association with patient age and microsatellite stable/microsatellite instability high (MSS/MSI-H) status established.

Results: Overall genomic alteration rates in the younger (<40) and older (≥50) cohorts were similar in the majority of the genes analyzed. Gene alteration rates in the microsatellite stable (MSS) younger and older cohorts were largely similar, with several notable differences. In particular, *TP53* (FDR < 0.01) and *CTNNB1* (FDR = 0.01) alterations were more common in younger patients with colorectal cancer, and *APC* (FDR < 0.01), *KRAS* (FDR < 0.01), *BRAF* (FDR < 0.01), and *FAM123B* (FDR < 0.01) were more commonly altered in older patients with colorectal cancer. In the MSI-H cohort, the majority of genes showed similar rate of alterations in all age groups, but with significant differences seen in *APC* (FDR < 0.01), *BRAF* (FDR < 0.01), and KRAS (FDR < 0.01).

Conclusions: Tumors from younger and older patients with colorectal cancer demonstrated similar overall rates of genomic alteration. However, differences were noted in several genes relevant to biology and response to therapy. Further study will need to be conducted to determine whether the differences in gene alteration rates can be leveraged to provide personalized therapies for young patients with early-onset sporadic colorectal cancer.

Introduction

Colorectal cancer is the third most common cancer in men and the second most common in women worldwide (10.0% and 9.2% of total, respectively), and global incidence is estimated at 1.4 million cases annually, with 694,000 deaths (1). In 2019, there will be an estimated 145,600 new diagnoses of colorectal cancer and an estimated 51,020 deaths from this disease in the United States (2). Death rates from colorectal cancer have been declining in the United States since 1992, with an annual decline of 2.6% for males and 3% for females (3).

In contrast to the downturns among screeningaged individuals, colorectal cancer incidence rates in adults aged <50 years rose by 1.6% from 2000 to 2013, for an overall increase of 22% (from 5.9 to 7.2 per 100,000; ref. 4). This increase has been driven by increasing incidence of distal colon cancer and rectal cancer, which has been increasing 3.2% annually from 1974 to 2013 in adults age 20–29 years (5, 6). Patients younger than 50 years of age are not routinely screened for colorectal cancer and are at risk for delayed diagnosis and more advanced stage of disease at the time of diagnosis. A retrospective review found a significantly higher proportion of stage III–IV tumors in young adults (69.3%) compared with older adults (46.4%; refs. 7, 8). There is also evidence that patients diagnosed with colorectal cancer before the age of 50 have had worsened progression-free survival and overall survival compared with older patients (9, 10).

Patients with early-onset colorectal cancer present with unique challenges, as younger patients may have young children, early career goals, financial toxicity, and concerns such as fertility preservation that are not as prevalent in older patients (11). Clinically, patients with early-onset colorectal cancer may present differently than older-onset colorectal cancer with prolonged hematochezia, multiple office visits, and delayed time from onset of symptoms to diagnosis (12). These issues emphasize the importance of specifically investigating underlying biological differences in younger versus older patients with colorectal cancer (9).

Although etiologies for the increase seen in young adults are yet to be fully elucidated, environmental factors may contribute including changes in lifestyle and dietary patterns. There is

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Translational Relevance

The incidence of early-onset colorectal cancer continues to increase, in contrast to late-onset disease. To determine whether the selective rise in incidence of this "sporadic" early-onset disease reflects a distinct profile of somatic driver mutations, we have compared the genomic landscape of early-onset colorectal cancer to that of later-onset colorectal cancer. Results of this analysis help elucidate differences in molecular carcinogenesis and may impact treatment decision-making.

evidence for an increased prevalence of hereditary risk factors for colorectal cancer including familial adenomatous polyposis (FAP) and Lynch syndrome in early-onset colorectal cancer cases, but these hereditary risk factors do not fully account for the increase seen in younger patients (12–14). Approximately 80% of patients with FAP harbor truncating germline mutations in the Adenomatous Polyposis Coli (*APC*) tumor suppressor gene, with identified mutations including R564X, R876X, Q1045X, 3927-3931delAAAGA, D1822V, and 2601delGA, R923X (15). Inherited mutations in four DNA mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) are the predominant cause of susceptibility to Lynch syndrome (16).

At this time, knowledge regarding the molecular features of sporadic young-onset colorectal cancer is limited, with few studies evaluating the genomic differences in this patient population. In this study, we report a large-scale study investigating comprehensive genomic differences between young-onset and later-onset colorectal cancer in hopes of elucidating further differences between the two groups.

Materials and Methods

Patients

Samples were obtained from 18,218 patients with pathologically confirmed colorectal cancer, who were referred to targeted next-generation sequencing (NGS) assay by their treating physician. Samples were sent either from the original diagnostic samples or from a sample of tumor after recurrence, to identify potentially actionable GA. Multiple samples from the same patient were not allowed in the dataset. Although detailed staging information is not available, the significant majority of patients were diagnosed with advanced (unresectable) disease. This study was conducted and presented according to the most recent REporting recommendations for tumor MARKer prognostic studies (REMARK; ref. 17). All studies were conducted in accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS). All studies were performed after approval by an institutional review board (IRB). Written consent was obtained prior to data collection for this deidentified retrospective analysis.

Targeted NGS

Analysis was performed using a clinical NGS-based assay (FoundationOne, Foundation Medicine Inc.) as described previously (18). The sequencing method was validated on hybridization captured, adaptor ligation-based libraries using DNA extracted from ten formalin-fixed paraffin-embedded (FFPE) sections cut at 5 mm. Comprehensive genomic profiling was performed on hybridization-captured, adaptor ligation-based libraries to a mean specimen median sequencing coverage depth of 688× for all coding exons and selected introns of up to 403 cancer-related genes. GAs (base substitutions, small indels, rearrangements and copy number alterations) were determined. Tumor mutational burden (TMB) data was available for a subset of the overall dataset.

Statistical analysis

Sequence data were evaluated for GAs including point mutations, insertions and deletions, copy number alterations, and select gene fusions/rearrangements, as described previously (18). Custom analysis was conducted as described previously (18), comparing results in younger patients (defined as <40 years) and older patients (defined as ≥50 years) diagnosed with colorectal cancer. For a more in-depth analysis of individual genes, a logistic model of the type alteration status was created for each gene. Alteration status was 1 or 0 for each sample and was determined by whether there was a functional alteration in that gene for that individual. All types of alterations were included for this analysis (amplifications, deletions, truncations, point). The false discovery rate (FDR) method was utilized to correct for multiple testing for (19). Plots were created for all genes significant at FDR = 0.05.

Statistical considerations

Descriptive analyses were performed using frequencies and percentiles. Medians and ranges were reported for continuous variables. Fisher exact test was used for comparison between categorical variables. Statistical significance was defined as $P < 0.05$.

Data and material availability

Summary level data are available by application to Foundation Medicine.

Results

DNA was extracted from 18,218 formalin-fixed, paraffin-embedded clinical specimens from patients with colorectal cancer. The age distribution for the cohort is shown in Fig. 1, and the alteration rate across all cases is shown in Fig. 2. There were a total of 1,420 patients under the age of 40, 3,248 between 40 and 49, and 13,550 age 50 and older. In accord with prior studies of colorectal cancer (20, 21), the most frequent alterations were observed in *APC* (76.8%), *TP53* (75.9%), *KRAS* (50.5%), *PIK3CA* (17.8%), and *SMAD4* (14.9%). Notable fusion events which may

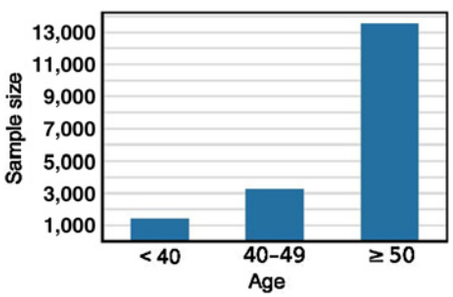

Figure 1.
Age distribution of the entire cohort.

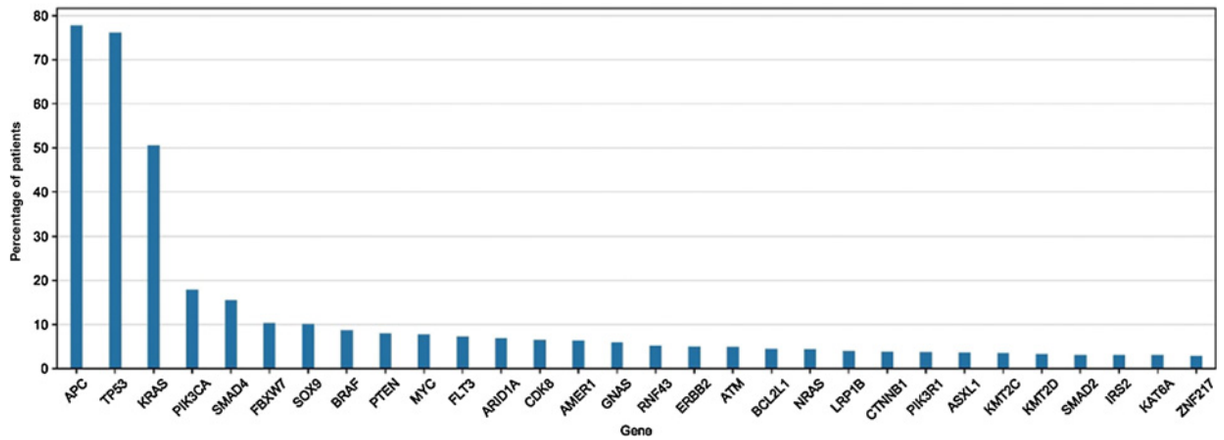

Figure 2.
Across all colorectal cases, the most frequent alterations were in APC (76.8%), TP53 (75.9%), KRAS (50.5%), PIK3CA (17.8%), and SMAD4 (14.9%).

confer sensitivity to targeted therapy, were detected at extremely low rates (<1%) in *ALK*, *BRAF*, *FGFR1*, *FGFR3*, *RET*, and *ROS1*, as has been reported previously (Supplementary Table S1; ref. 22).

When comparing the <40 cohort to the ≥50 cohort, gene alteration rates in the microsatellite stable (MSS) younger and older cohorts were largely similar for the majority of the genes analyzed. However, several notable differences were seen between the MSS < 40 and ≥50 age cohorts. *TP53* ($P < 0.01$, FDR < 0.01) and *CTNNB1* ($P < 0.001$, FDR < 0.01) alterations were found to be more common in tumor samples from patients < 40 years old diagnosed with colorectal cancer. In contrast, *APC* ($P < 0.01$, FDR < 0.01), *KRAS* ($P < 0.001$, FDR < 0.01), *BRAF* ($P < 0.001$, FDR < 0.01), *PIK3CA* ($P < 0.001$, FDR < 0.01), and *FAM123B* ($P < 0.001$, FDR < 0.01) were more commonly altered in tumor samples from patients ≥ 50 years old with colorectal cancer (Table 1; Fig. 3). In the MSI-H cohort, several notable differences between the two age cohorts were also observed, with statistically significant increases in alteration rates observed in early-onset colorectal cancer in *APC* ($P < 0.001$, FDR < 0.01) and *KRAS* ($P < 0.001$, FDR < 0.01), and statistically significant increases in alteration rates in later-onset colorectal cancer in *BRAF* ($P < 0.001$, FDR < 0.01) and *MLH1* ($P < 0.001$, FDR < 0.01). However, similar to the MSS cohort, other genes of interest showed very few differences.

Table 1. Significant alterations and alterations in genes of interest between cohorts using false discovery rate (FDR) in MSS colorectal cancer (CRC) and MSI-H colorectal cancer

Alteration rates in the MSS cohort			
Gene	**Rate observed in under 40 group (%)**	**Rate observed in 50 and over group (%)**	**FDR**
TP53	82.3	76.7	1.56E−05
APC	65.8	79.7	4.84E−26
KRAS	45.6	52.4	1.56E−05
PIK3CA	14.1	17.5	0.002959601
CTNNB1	4	2.7	0.013488987
BRAF	5.2	7.7	0.002067048
FAM123B	2	6.8	1.35E−12
NRAS	3.7	4.6	0.171847712
Alteration rates in the MSI-H cohort			
Gene	**Rate observed in under 40 group (%)**	**Rate observed in 50 and over group (%)**	**FDR**
TP53	28.6	36.2	0.264580718
APC	70.1	34.4	1.51E−08
KRAS	49.4	24.1	2.00E−05
PIK3CA	42.9	30.6	0.066741704
CTNNB1	15.6	8.2	0.080623845
BRAF	5.2	48.8	2.05E−14
FAM123B	16.9	13.1	0.422335612
NRAS	0	1.4	0.605361263

To perform a more detailed analysis of gene alteration rates by patient age, alteration rates were evaluated using age as a continuous variable within the MSS set. In this analysis, several genes were noted to have a statistically significant increase in mutation rate with patient age, including *ASXL1*, *BRAF*, *CEBPA*, *CDKN2A*, *DNMT3A*, *FAM123B*, *RNF43*, *SF3B1*, *SOX9*, and *TET2* (Fig. 4A). Genes showing a decreasing alteration rate with age include *CTNNB1*, *GEN1*, *MYC*, *POLE*, and *TP53* (Fig. 4B). Some specific common oncogenically activating mutations shown to have driver function in colorectal cancer also differed dependent on patient age. A detailed analysis of specific BRAF mutations performed in a smaller subset of patients (10,070 patients) indicated, *BRAF* V600E mutations increased with age, whereas other non-V600E *BRAF* mutations did not (Fig. 5). For *RNF43*, the most common mutation (G659fs*41) showed a substantial increase with age, whereas other mutations in this gene did not (Fig. 5).

Tumor mutational burden (TMB) data were available for 10,070 patients within the overall dataset. Of those with TMB data available, there were a total of 9,615 patients with MSS colorectal cancer and 455 patients with MSI-H colorectal cancer. Interestingly, in the MSI-H cohort, TMB increases with increasing age, with higher TMB scores seen in older patients, and lower TMB scores seen in younger patients (Supplementary Fig. S2). In the MSS subset, there was no difference in TMB by age.

Discussion

The genomic landscape of metastatic colorectal cancer has been described in great detail previously, showing that colon and rectal cancers had similar patterns of genomic alterations (20). However, a comprehensive analysis of genomic alteration rates by age has not been performed and is an area of research interest given the steady increase in young-onset colorectal cancer seen in the

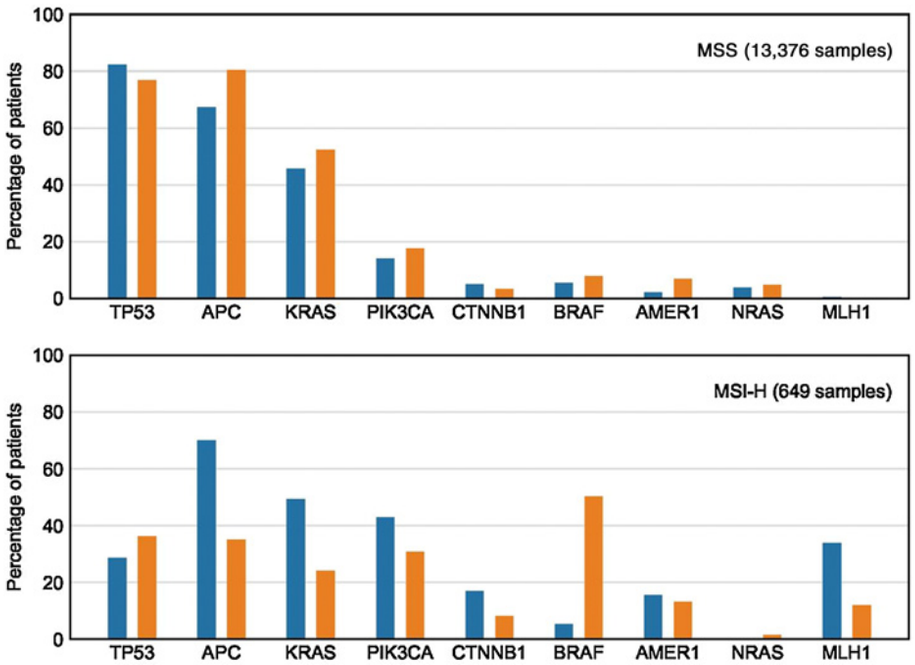

Figure 3.
Alteration rate in genes of interest between early-onset (<40 years - blue) and later-onset (≥50 years - orange) in MSS and MSI-H colorectal cancer (patients between the ages of 40 and 49 are not included in these figures).

United States. This comprehensive analysis of younger and older onset colorectal cancer provides a number of insights into potential genomic differences between the two groups.

Overall, the genomic landscape between younger and older onset colorectal cancer appears similar, with expected rates of alterations in several genes that are critical to the selection of an optimal treatment paradigm for individual patients, including *KRAS*, *NRAS*, and *BRAF* (23). However, several notable differences were seen between the MSS < 40-year-old and the ≥50-year-old cohorts, with *TP53* and *CTNNB1* alterations more frequently seen in younger patients with colorectal cancer, and *APC*, *KRAS*, *BRAF*, *PIK3CA*, and *FAM123B* more commonly altered in older patients with colorectal cancer. Colorectal cancer tumors with *BRAF V600E* mutations have been previously described to be more likely observed in patients aged 70 years or older (24, 25). Another study has suggested that young-onset colorectal cancer have a lower rate of *BRAF* alterations (26). Interestingly, non-V600E *BRAF* mutations showed no differences across age groups, and recently reported data suggest that patients that harbor non-V600E *BRAF* mutations have a distinct and improved prognosis (27). In a previously published study, we performed a detailed characterization of RAS mutational profiles in colon and rectal cancer in young and older patients (28), finding an association of specific mismatch substitutions with age and tumor site. Age- and site-related differences in alteration rates for specific mutations in the other genes reported here have not been described in great detail, and clearly bear greater scrutiny.

The difference in *APC* alteration rates is of significant interest, as mutation of *APC* has been previously described to have an effect on prognosis (29). Colorectal cancer tumors lacking any *APC* mutation carry a worse prognosis than tumors with single *APC* mutations, and given the decreased rates of *APC* alterations in younger patients, this may help explain a potential difference in response to therapy in younger patients with metastatic colorectal cancer as reported previously (10). The lower incidence of *APC* mutations in younger patients is also of interest, given the higher expected incidence of FAP (associated with *APC* alterations) in the younger population. Differences in *WNT* pathway alteration by age is also supported by differences in *RNF43 G659fs*41* alteration rates, with a clear increase seen with increasing age. The RNF43 tumor suppressor gene encodes a transmembrane ubiquitin ligase that ubiquitinates Frizzled receptors and thereby downregulates the surface expression of the WNT receptor; the truncating *G659fs*41* mutation eliminates RNF43 function, elevating activity of WNT effectors (30).

When looking at age as a continuous variable, several genes showed modest decreases in alteration rates with increasing age, including the WNT effector *CTNNB1*, the DNA damage repair genes *GEN1* and *POLE*, and *TP53*, although the alteration rates for these genes, with the exception of *TP53*, are quite low. The increased alteration rates in *CTNNB1* in younger patients support a prior study that implicated the WNT/β-catenin pathway in sporadic early-onset colorectal cancer (31). There has been increasing interest in genes involved in DNA replication or repair (such as *POLE*, *POLD1*, *MSH2*) in colorectal cancer, suggesting that loss of normal DNA repair fidelity may have contributed to increased mutational burden and immune checkpoint blockade response in these tumors. To date, a role for *GEN1* in colorectal cancer has not been reported, although its roles in maintaining genome integrity by promoting repair of Holliday junctions and intensifying the phenotypes associated with early-onset cancers arising from Bloom syndrome are well established (32, 33); our data suggests further investigation would be of interest. However, the overall alteration rates for these genes is extremely low, limiting their predictive value for therapeutic decision-making (34).

Within the MSI-H cohort, several large alteration rate differences were noted in the younger and older cohorts, including differences in the alteration rates in *APC*, *KRAS*, and *BRAF*. Two major causes of defective MMR that can lead to MSI-H are

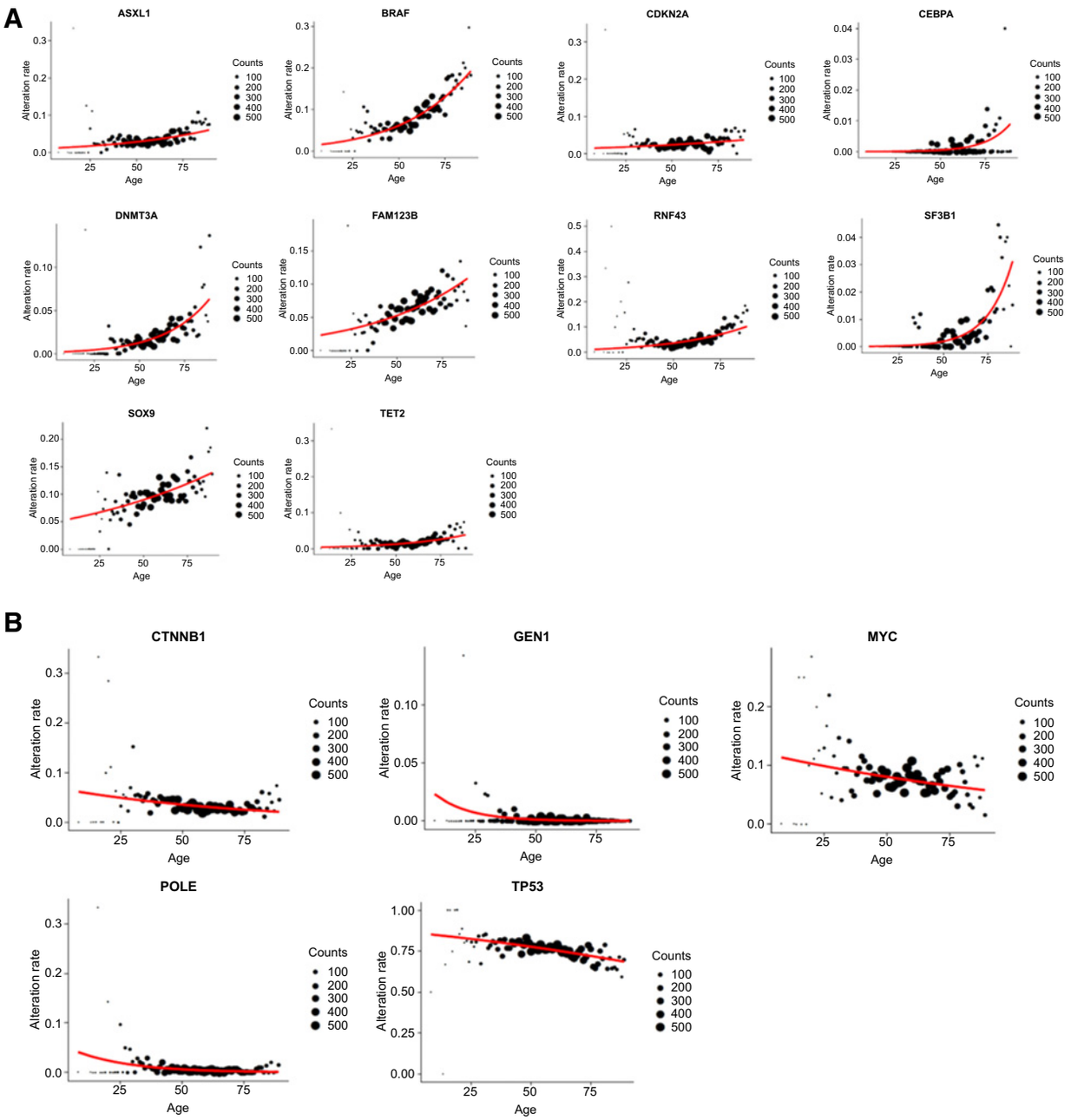

Figure 4.

A, Genes of interest with increasing alteration rates by age (all $P < 0.01$). The dots show the alteration rates at each age (dots are sized according to how many samples there are at that age), and the red line shows the regression fit from the logistic regression model. **B,** Genes of interest with decreasing alteration rates by age (all $P < 0.01$).

acquired hypermethylation of the *MLH1* promoter in sporadic colorectal cancer and germline MMR mutation combined with an acquired inactivation in the remaining functional allele (35). In cases of MSI, the presence of the BRAF V600E hotspot mutation practically excludes the possibility of Lynch syndrome, and the clinical utility of the combination of these two markers is well-established (36). The difference in alteration rates in *BRAF* most likely signifies the increased prevalence of sporadic MSI in the older population and the increased prevalence of inherited MSI in the younger population (37). Interestingly, the overall TMB in the MSI-H cohort appears to increase with increasing age, paralleling results seen in other tumor types (38). Prior studies investigating mutational signatures within cancer show a positive correlation between the age of cancer diagnosis and the number of mutations observed, suggesting that the mutational process may be operative at a constant rate over time leading to increased mutational

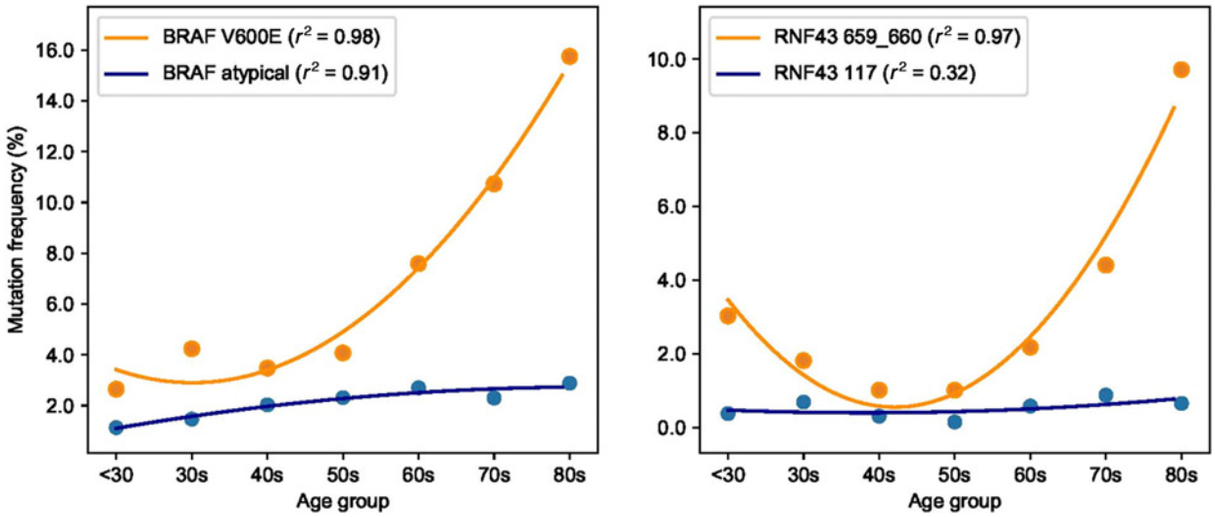

Figure 5.

Differing rates of specific BRAF and RNF43 mutations by age showing a clear distinction between BRAF V600E mutations versus other BRAF mutations as well as a difference in RNF43 659_660 mutations and RNF43 117 mutations.

burden (39, 40). Other reports have suggested that the majority of young-onset colorectal cancer is MSS, and shows a higher rate of CpG island methylator phenotype (CIMP; ref. 26). Future studies will need to investigate the response rates of patients of all ages with MSI-H metastatic colorectal cancer treated with immune checkpoint inhibitors to assess if there is an association with TMB, and if older patients receive additional benefit from these therapies.

Despite the comprehensive analysis and large sample size, there are several limitations of this current dataset. There is emerging awareness that the response of metastatic previously to targeted anti-EGFR inhibitors is impacted by the specific site of origin within the colon. Unfortunately, sidedness data is currently unavailable for this dataset, precluding an analysis into whether different mutational spectrums exist in the left versus right colon (41). Because clinical annotation data are unavailable, the predictive and prognostic effect of these gene alteration rates cannot be studied, although there are significant data on the effect of individual genes in the current literature. In addition, the dataset lacks information on factors such as hypermethylation or mRNA expression; hence, some of the genes may have significantly altered activity due to increased or decreased abundance of transcripts, modifying the estimation of their loss or gain of function as a factor of age.

In conclusion, the genomic landscapes of young-onset and older onset colorectal cancer, while overall similar, display specific differences in genes that have may prognostic implications. Further study will need to be conducted to determine whether the differences in gene alteration rates, particularly within the WNT/β-catenin pathway, can be leveraged to provide personalized therapies for young patients with early-onset sporadic colorectal cancer. This study also highlights the impact of robust next-generation sequencing in the analysis of metastatic colorectal cancer, including the assessment of microsatellite instability as biomarker analysis continues to affect the personalized treatment plans for patients.

Disclosure of Potential Conflicts of Interest

C.H. Lieu is a consultant/advisory board member for Foundation Medicine. J. Newberg and G. Frampton have ownership interests (including patents) in Roche. M. Cooke has ownership interests (including patents) at Foundation Medicine. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.H. Lieu, A. Hemmerich, C. Connelly, G. Frampton, M. Cooke

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.H. Lieu, I.G. Serebriiskii, J. Newberg, A. Hemmerich, C. Connelly, S.G. Eckhardt, G. Frampton, J.E. Meyer

Writing, review, and/or revision of the manuscript: C.H. Lieu, E.A. Golemis, A. Hemmerich, C. Connelly, W.A. Messersmith, C. Eng, S.G. Eckhardt, G. Frampton, J.E. Meyer

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Study supervision: C.H. Lieu

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