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Short Report
Effects of common genetic variants associated with colorectal cancer risk on survival outcomes after diagnosis: A large population-based cohort study

Yazhou He, Evropi Theodoratou, Xue Li, Farhat V.N. Din, Peter Vaughan-Shaw, Victoria Svinti, Susan M. Farrington, Harry Campbell, Malcolm G. Dunlop and Maria Timofeeva

Abstract
Genome-wide association studies have thus far identified 130 genetic variants linked to colorectal cancer (CRC) risk ($r^2 < 0.2$). Given their implication in disease causation, and thus plausible biologically effects on cancer-relevant biological pathways, we investigated whether these variants are associated with CRC prognosis and also whether they might provide predictive value for survival outcome. We conducted the analysis in a well-characterized population-based study of 5,675 patients after CRC diagnosis in Scotland. None of the genetic risk variants were associated with either overall survival (OS) or CRC-specific survival. Next, we combined the variants in a polygenic risk score, but again we observed no association between survival outcome and overall genetic susceptibility to CRC risk—as defined by common genetic variants (OS: hazard ratio = 1.00, 95% confidence interval = 0.96–1.05). Furthermore, we found no incremental increase in the discriminative performance when adding these genetic variants to the baseline CRC-survival predictive model of age, sex and stage at diagnosis. Given that our study is well-powered (>0.88) to detect effects on survival for 74% of the variants, we conclude that effects of common variants associated with CRC risk which have been identified to date are unlikely to have clinically relevant effect on survival outcomes for patients diagnosed with CRC.

Introduction
Globally, colorectal cancer (CRC) is the second leading cause of cancer-related deaths, accounting for 9.2% of all cancer-related deaths (0.8 million CRC deaths in 2018).1 The strongest known predictor of CRC outcome is stage, but even within one stage, there is considerable heterogeneity in survival. Identification of biomarkers of cancer prognosis can inform clinical management and treatment of disease. Evidence of the family concordance for CRC-
Cancer Genetics and Epigenetics

survival (OS) and CRC specific cause of death can be found elsewhere. In order to measure previously published smaller studies examined up to 30 CRC risk genetic loci and detected no or little evidence of associations with survival. Two recent large meta-analyses of genome-wide association studies (GWAS) have identified more than 70 new genetic variants associated with CRC risk. In this analysis, we investigated the association between all previously and newly GWAS-identified common genetic variants and CRC survival.

Materials and Methods

We included 5,675 CRC cases (detailed patient selection in Supporting Information Fig. S1) with genome-wide genotyping data and data on age at diagnosis, sex and American Joint Committee on Cancer (AJCC) stage information from a population-based case-control study (Study of Colorectal Cancer in Scotland, SOCCS; 1999-current). Ethics approval was obtained from the MultiCentre Research Ethics committee for Scotland (approval number MREC/01/0/5) and other committees (presented elsewhere). A total of 130 genetic variants identified by previous GWAS studies have been genotyped or imputed (25/130 variants were directly genotyped). For correlated variants (linkage disequilibrium ≤ 0.80) and rare variants associated with CRC pathogenesis may subsequently affect tumor progression. However, very few studies with sufficient power tested roles of common genetic risk variants in CRC prognosis. Previously published smaller studies examined up to 30 CRC risk genetic loci and detected no or little evidence of associations with survival. Two recent large meta-analyses of genome-wide association studies (GWAS) have identified more than 70 new genetic variants associated with CRC risk. In this analysis, we investigated the association between all previously and newly GWAS-identified common genetic variants and CRC survival.

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All genetic variants were annotated using (i) association with the cis gene expression in colon transverse tissue (n = 246) from the Genotype-Tissue Expression (GTEx) database, (ii) presence of known and predicted regulatory elements in RegulomeDB database and (iii) predicted effect on the structure and function of a protein as implemented in SIFT and PolyPhen-2.
Data availability

The data that support the findings of our study are available upon reasonable request from the corresponding authors. The data are not publicly available due to privacy or ethical restrictions.

Results and Discussion

The basic characteristics of included CRC patients are summarized in Table 1. In total, 1,918 patients (34%) died during follow-up. With 5,675 CRC cases, our study had 88% power to detect a hazard ratio of 1.20 on OS for 97/130 (74%) variants (MAF > 0.15) at the significance level of 0.0005 (a power curve for other effects is shown in Fig. 1). Overall, we observed 14 genetic variants associated with OS and 10 with CSS at nominal statistical significance (p < 0.05) with six variants (rs10994860, rs12143541, rs3217810, rs34405347, rs6065668, rs847208) being associated with both OS and CSS. However, none of the variants remained statistically significant after Bonferroni or FDR correction. The summary results for variants with nominal significance are presented in Table 2.

Stratified analyses by stage, sex and tumor site did not identify any statistically significant associations after multiple-testing correction either (Supporting Information Tables S2–S4). With regard to overall genetic susceptibility to CRC, no statistically significant association was observed between the PRS and OS or CSS (Table 2). The LASSO regression model selected six variants for OS in addition to age at diagnosis, sex and AJCC stage to minimize prediction error in the train set. However, nearly no incremental predictive improvement was observed compared to the model without genetic variants in the test set (C-statistic for OS: 0.73282 vs. 0.73277, U-statistic test: p = 0.322). Similar results were found for CSS (Supporting information Table S5).

We used a concept of statistical significance as decision criteria to define if the risk variants have an effect on survival. This concept has been criticized in the literature as subjective and commonly misused.24,25 We additionally looked into

Table 1. Basic characteristics of included CRC cases

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>SOCCS CRC cases (n = 5,675)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (years)¹</td>
<td>64.5 (54.6–71.6)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3,235 (57.0%)</td>
</tr>
<tr>
<td>Female</td>
<td>2,440 (43.0%)</td>
</tr>
<tr>
<td>AJCC stage</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1,005 (17.7%)</td>
</tr>
<tr>
<td>II</td>
<td>1,891 (33.3%)</td>
</tr>
<tr>
<td>III</td>
<td>1,995 (35.2%)</td>
</tr>
<tr>
<td>IV</td>
<td>784 (13.8%)</td>
</tr>
<tr>
<td>Site</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>3,392 (59.8%)</td>
</tr>
<tr>
<td>Rectum</td>
<td>2,201 (38.8%)</td>
</tr>
<tr>
<td>Colon and rectum</td>
<td>16 (0.3%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>66 (1.2%)</td>
</tr>
<tr>
<td>Follow-up time (years)¹</td>
<td>5.09 (2.43–11.42)</td>
</tr>
<tr>
<td>No. of all-cause deaths</td>
<td>1,918 (33.8%)</td>
</tr>
<tr>
<td>No. of CRC-related deaths</td>
<td>1,358 (23.9%)</td>
</tr>
</tbody>
</table>

¹Median and quartiles in parenthesis.

Abbreviations: AJCC, American Joint Committee on Cancer; CRC, colorectal cancer; SOCCS, Study of Colorectal Cancer in Scotland.

Figure 1. Power curve for overall and CRC-specific survival using additive model. Abbreviations: CRC, colorectal cancer; MAF, minor allele frequency. [Color figure can be viewed at wileyonlinelibrary.com]
Similarly, previous studies identified some CRC-risk variants that might be associated with survival after CRC diagnosis, these findings were not immune to false-positive results from multiple testing. A widely studied variant, rs9929218 lies in the intron of CDH1 gene encoding E-cadherin, the loss of function of which can cause tumor progression and metastasis.  Previous studies reported that the CRC-risk decreasing allele (A) is statistically significantly associated with poor survival outcomes. However, in contrast, we observed a potentially favorable effect (though not statistically significant after multiple-testing correction) of the A allele on OS in our study (i.e., the direction of CRC risk and prognosis are consistent in our study). Smith et al. reported that the A allele is significantly associated with poor response to chemotherapy, implying a
possible gene x therapy interaction for this variant. However, our study is limited by data on response to chemotherapy being unavailable so that this could not be explored further. Notably, there has been other evidence showing that rs9929218 may modify CRC susceptibility by interacting with other factors such as height and alcohol consumption. Investigation of possible gene–environment interactions should be considered in future efforts with large sample sizes to further dissect the prognostic effect of this variant in CRC patients. Consistently, when looking for overall direction of effects among 124 tested variants we noted little evidence of potentially detrimental effects of CRC risk on survival with only 41 and 47% of risk variants showing association with poor (HR > 1) OS and CSS in our study.

Previously we have shown that SOCCS study is representative of British and Scottish populations and cases from SOCCS cluster tightly with population-based controls from SOCCS and Generation Scotland. The allele frequencies of SOCCS cluster tightly with population-based controls from British and Scottish populations and cases from our study.

In conclusion, our study finds that overall genetic susceptibility to CRC captured by known CRC risk variants is not statistically significantly associated with survival outcomes of CRC. However, possible roles of each variant in CRC progression remain to be explored. Our study indicated that the heritable variation of patient survival may have distinct genetic determinants from CRC susceptibility. The previous GWAS on CRC survival with 3,494 cases identified no variants at genome-wide significance (p < 5E–8), meriting future collaborative efforts of aggregating larger CRC cohorts to illuminate genetic structure of survival outcomes for CRC patients.

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References

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