Common genetic variation and survival after colorectal cancer diagnosis: a genome-wide analysis

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Abstract

Genome-wide association studies have identified several germline single nucleotide polymorphisms (SNPs) significantly associated with colorectal cancer (CRC) incidence. Common germline genetic variation may also be related to CRC survival. We used a discovery-based approach to identify SNPs related to survival outcomes after CRC diagnosis. Genome-wide

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genotyping arrays were conducted for 3494 individuals with invasive CRC enrolled in six prospective cohort studies (median study-specific follow-up = 4.2–8.1 years). In pooled analyses, we used Cox regression to assess SNP-specific associations with CRC-specific and overall survival, with additional analyses stratified by stage at diagnosis. Top findings were followed-up in independent studies. A P value threshold of P < 5 × 10^{-8} in analyses combining discovery and follow-up studies was required for genome-wide significance. Among individuals with distant-metastatic CRC, several SNPs at 6p12.1, nearest the ELOVL5 gene, were statistically significantly associated with poorer survival, with the strongest associations noted for rs209489 (hazard ratio (HR) = 1.8, P = 7.6 × 10^{-10} and HR = 1.8, P = 3.7 × 10^{-4} for CRC-specific and overall survival, respectively). No SNPs were statistically significantly associated with survival among all cases combined or in cases without distant-metastases. SNPs in 6p12.1/ELOVL5 were associated with survival outcomes in individuals with distant-metastatic CRC, and merit further follow-up for functional significance. Findings from this genome-wide association study highlight the potential importance of genetic variation in CRC prognosis and provide clues to genomic regions of potential interest.

### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRC</td>
<td>colorectal cancer</td>
</tr>
<tr>
<td>CPS-II</td>
<td>Cancer Prevention Study II Nutrition cohort</td>
</tr>
<tr>
<td>DALS</td>
<td>Diet, Activity and Lifestyle Study</td>
</tr>
<tr>
<td>DACHS</td>
<td>Darmkrebs: Chancen der Verhütung durch Screening Study</td>
</tr>
<tr>
<td>GWAS</td>
<td>Genome-wide association study</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
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</table>

### Introduction

Advances in colorectal cancer (CRC) early detection and treatment have led to considerable declines in CRC mortality rates (1). Nonetheless, 5-year relative survival for CRC is less than 65% in the United States (2). Although risk factors for incident CRC are relatively well-established, less is known about factors associated with CRC survival. At present, the strongest known predictor of CRC prognosis is stage (2); however, there is considerable heterogeneity in survival among individuals with the same stage at diagnosis (2). To extend our understanding of CRC pathogenesis and potentially direct treatment, there remains a need to identify markers of CRC prognosis. Information on the role of germline genetic factors in CRC prognosis represents an important gap in knowledge in this regard.

### Materials and Methods

#### Discovery study populations

Six cohort studies were included in primary discovery analyses: the Health Professionals Follow-up Study (HPFS) (19), the Nurses’ Health Study (NHS) (20–22), the Physicians’ Health Study (PHS) (23), the Prostate, Lung, Colorectal and Ovarian Screening Trial (PLCO) (24,25), the VITamins And Lifestyle Study (VITAL) (26) and the Women’s Health Initiative (WHI) (27). These studies are included in the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) (28). All studies used a prospective design, with follow-up for incident cancer diagnoses and survival (19–27).

Discovery analyses were restricted to study participants with incident invasive CRC who self-reported European descent, and for whom genotype and survival data were available (N = 3494). Incident cancers were self-reported and confirmed by physician adjudication of medical records (HPFS, NHS, PHS, PLCO, WHI) and/or linkage to cancer registries (VITAL). Two subsets of cases were genotyped in the WHI: WHI1 included colon cancer patients from the WHI observational study diagnosed before September 2005 (4) and WHI2 included non-overlapping CRC patients diagnosed before August 2009. Similarly, two subsets of cases were genotyped in PLCO: PLCO1 included colon cancer patients, and PLCO2 included CRC cases not included in PLCO1. We excluded individuals for whom DNA was collected after CRC diagnosis. All participants provided informed consent for genetic testing. All studies were approved by their respective Institutional Review Boards.

#### Follow-up study populations

Four independent studies were used for follow-up of discovery-stage findings: the Cancer Prevention Study II Nutrition cohort (CPS-II) (29), the Diet, Activity and Lifestyle Study (DALS) (29), the Darmkrebs: Chancen der Verhütung durch Screening Study (DACHS) (30,31) and the UK Medical Research Council (MRC) combined COIN (32) and COIN-B trials (33). CPS-II, DALS and DACHS are included in GECCO. Study design details for these studies and COIN/COIN-B are published elsewhere (28–33). DALS and DACHS are population-based case-control studies for CRC incidence involving rapid case ascertainment and follow-up for survival; CPS-II is a prospective cohort study, with follow-up for incident cancers and survival; COIN/COIN-B are phase III treatment trials for advanced CRC. All studies were approved by their respective Institutional Review Boards.

#### Ascertainment of survival outcomes

Protocols for assessing survival in the included studies have been described previously (19,22,26,28–30,32–36). Most used active follow-up to ascertain vital status (HPFS, NHS, PHS, PLCO, WHI): dates and cause of death were confirmed via review of death certificates and/or medical records by trained adjudicators. Active follow-up was also used to ascertain survival outcomes in COIN/COIN-B, although information on cause of death was not available. For other studies (VITAL, CPS-II, DACHS, DALS), vital status was ascertained via linkage to the National Death Index, state cancer registries, state death records, or population registers with cause of death verified by death certificates. In all studies, patients alive at the most recent study follow-up or data linkage were censored on that date. In VITAL, individuals who moved outside Washington State were censored at their date of move.

#### Genotyping and quality control

Genotyping details for GECCO studies have been reported previously (34). Genomic DNA was extracted from blood or buccal samples using conventional methods. Genotyping was performed on manufacturer’s protocols for the Illumina HumanMethylation385 and HumanMethylation450 BeadChips.
Supplementary Table 3

**ELOVL5**

**ICK**

**TMEM114**

**P**

**P**

**P**

on 16 February 2018

Downloaded from https://academic.oup.com/carcin/article-abstract/37/1/87/2365799

**Technology (LGC Genomics, London, UK).**

**genotyping of these SNPs was conducted using KASPar genotyping tech**

**excluding SNPs that were missing for >50% of included cases.**

**non-distant (local/regional, stages I–III) and distant-metastatic (distant,**

**Epidemiology and End Results (SEER) staging in some studies (i.e. local/**

**principal components of genetic ancestry. We examined the Schoenfeld**

**residuals to identify violations of the proportional hazards assumptions**

**ity. All models included age at diagnosis, sex, study and the first three**

**intervals (CIs) for SNP-specific associations. In analyses of CRC-specific**

**used Cox regression to calculate hazard ratios (HRs) and 95% confidence**

**Statistical analysis for discovery**

**Data were pooled across studies for discovery analyses. Survival time was**

**calculated as the time from diagnosis to death or end of follow-up. We**

**used Cox regression to calculate hazard ratios (HRs) and 95% confidence**

**intervals (CI) for SNP-specific association. In analyses of CRC-specific**

**survival, individuals who died from causes other than CRC were censored at**

**the time of death. SNPs were modelled using a log-additive approach,**

**relating genotype dose (i.e. number of copies of the minor allele) to sur**

**vival outcomes. For imputed SNPs, ‘dosage’ was calculated on a scale from**

**0 to 2 based on imputation probabilities for each genotype (37).**

**We constructed separate models for overall and CRC-specific mortal**

**ity. All models included age at diagnosis, sex, study and the first three**

**principal components of genetic ancestry. We examined the Schoenfeld**

**residuals to identify violations of the proportional hazards assumptions**

**according to these covariates. We also conducted analyses stratified by**

**stage at diagnosis. Because stage was classified according to Surveillance,**

**Epidemiology and End Results (SEER) staging in some studies (i.e. local/**

**regional/distant) and American Joint Committee on Cancer (AJCC) stag**

**ing in others (i.e. I/II/III/IV), we stratified stage on harmonized groupings:**

**non-distant (local/regional), stages I-III and distant-metastatic (distant,**

**stage IV). Genome-wide statistical significance was specified at P < 5 × 10^{-8}**

**based on Wald P values in single-SNP models. We inspected Q-Q plots of**

**~log_{10}-transformed P values and assessed the influence of population**

**stratification by calculating genomic control coefficients (38). Analyses**

**were performed using R 2.15.3.**

**Statistical analysis for follow-up**

**Follow-up of top findings from discovery analyses (P < 5 × 10^{-8}) was carried**

**out in CPS-II, DALS1, DALS2, DACHS1 and DACHS2 (N = 3764), adjusting for**

**age at diagnosis, sex, study sample and the first three principal compo**

**nents of genetic ancestry. Two findings from discovery analyses of overall**

**survival in distant-metastatic cases were followed-up in COIN/COIN-B**

**(N = 2234), with analyses adjusted for treatment arm, chemotherapy regi**

**men, age at randomization, sex and time from diagnosis to randomiza**

**tion. Estimates were combined across discovery and follow-up sets using**

**fixed effects meta-analysis. Among correlated SNPs with pairwise R^2 ≥**

**0.8 in the HaMap CEU population, a representative SNP was selected for**

**inclusion in Table 3.**

**Results**

**Characteristics of the discovery study populations are provided in**

**Table 1. Median follow-up after diagnosis ranged from 4.2 to**

**8.1 years across studies. In total, 1223 (35%) CRC patients in discovery**

**analyses died during follow-up; the proportion who died ranged from 22% (PLCO2) to 62% (FHS). Women accounted for**

**65% of the study population. Approximately 14% were diagnosed with**

**distant-metastatic disease. Characteristics of**

**follow-up study populations are provided in Table 2. Study popu**

**lation attributes, pooled across study phase, are also provided in**

**Supplementary Table 1, available at Carcinogenesis Online.**

**In discovery analyses of all cases combined (Supplementary Table 2; Supplemen**

**tary Figures 1 and 2, available at Carcinogenesis Online), the minor allele at rs11077289 (16p13.2/TMEM114) was**

**associated with more favorable overall survival (HR = 0.8, P = 3.9 × 10^{-7});**

**however, this association was not evident in follow-up (Table 3). No SNPs**

**emerged from analyses in non-distant CRC cases (Supplementary Table 3; Supplemen**

**tary Figures 3 and 4, available at Carcinogenesis Online). In discovery analy**

**ses restricted to distant-metastatic CRC cases (Supplementary Figures 5–8,**

**available at Carcinogenesis Online), the minor alleles at rs17544464 (6p12.1/ELOVL5), rs209489 (6p12.1/ELOVL5) and**

**rs1442089 (18q21.2/DCC) were each associated with a 2.0- to 2.2-**

**fold shorter overall survival (P = 1.7 × 10^{-4} to 4.8 × 10^{-4}); P values**

**were similar after adjusting for inflation factors (results not shown).**

**This association with rs209489 persisted in follow-up (2.2 × 10^{-7})**

**and was statistically significant in analyses of discovery and follow-up study populations combined (P = 3.7 × 10^{-4}).**

**Associations with rs209489 were similar and exceeded genome-wide significance in analyses of CRC-specific survival.**

**Associations with overall survival for rs17544464 and rs1442089 were not evident in follow-up (P = 0.330 and P = 0.910,**

**respectively), due largely to the contribution of COIN/COIN-B in the**

**follow-up set (Figures 1 and 2). There was evidence of considera**

**ble heterogeneity across studies when including COIN/COIN-B in follow-up for these two SNPs (P heterogeneity = 1.3 × 10^{-4}**

**and 3.7 × 10^{-4}); but not when COIN/COIN-B was excluded from follow-up**

**(P heterogeneity = 0.14 and 0.11, respectively). Other SNPs in linkage disequilibrium with or nearby rs17544464 or rs209489**

**were also strongly associated with survival among individuals with**

**distant-metastatic CRC in analyses not including COIN/COIN-B**

**Supplementary Table 3, available at Carcinogenesis Online).**

**Discussion**

**In this discovery-based search for common genetic variants associated with**

**CRC prognosis, multiple SNPs at 6p12.1 were identified as significantly associated with distant-metastatic**

**CRC survival: the minor allele at rs209489 was associated with shorter overall and CRC-specific survival at a level of genome-**

**wide significance, and the minor allele at rs17544464 was associ**

**ated with significantly shorter CRC-specific survival. No SNPs**

**were statistically significantly associated with survival among individuals with non-distant CRC or in analyses of all cases**

**combined. To our knowledge, this is the first genome-wide examination of common genetic variation and CRC survival.**

**The loci that emerged from our combined analyses in those with**

**distant-metastatic disease have not previously been described in relation to CRC survival or risk. Most SNPs that**

**were identified as being associated with survival are located in**

**or nearest to the ELOVL5 gene, which encodes a fatty acid elonga**

**ase (ELOVL5). Knockout of ELOVL5 in mouse models appears to result in hepatic steatosis (39). Previous studies have found**

**hepatic steatosis to be an independent risk factor for distant-metastatic CRC (40) and a marker of lower risk of hepatic**

**metastases of CRC (41). Nonetheless, associations between hepatic steatosis and CRC prognosis have been inconsistent**

**(42,43). It is also plausible that noted associations with SNPs at**

**6p12.1 reflect activity of other nearby genes. The coding region**

**for the intestinal cell (MAK-like) kinase (ICK) gene is located within 200kb downstream of the tagged region for rs209489. ICK**
Table 1. Characteristics of colorectal cancer cases in study populations included in primary discovery analyses

<table>
<thead>
<tr>
<th></th>
<th>Health Professions Follow-up Study</th>
<th>Nurses' Health Study</th>
<th>Physicians' Health Study</th>
<th>Prostate, Lung, Colon and Ovarian Cancer Screening Trial</th>
<th>Vitamins and Lifestyle Study</th>
<th>Women's Health Initiative</th>
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<tbody>
<tr>
<td>Abbreviation</td>
<td>HPFS</td>
<td>NHS</td>
<td>PHS</td>
<td>PLCO1</td>
<td>PLCO2</td>
<td>VITAL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>WHI1</td>
</tr>
<tr>
<td>No. cases</td>
<td>168</td>
<td>296</td>
<td>324</td>
<td>531</td>
<td>478</td>
<td>285</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>180 (34)</td>
<td>103 (22)</td>
<td>117 (41)</td>
</tr>
<tr>
<td>No. deaths, total (% of cases)</td>
<td>82 (49)</td>
<td>118 (40)</td>
<td>200 (62)</td>
<td>108 (60)</td>
<td>77 (75)</td>
<td>115 (72)</td>
</tr>
<tr>
<td>Median follow-up in years (SD)</td>
<td>5.8 (3.7)</td>
<td>6.7 (5.9)</td>
<td>8.1 (7.2)</td>
<td>6.6 (3.4)</td>
<td>4.5 (3.6)</td>
<td>4.9 (2.9)</td>
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<td>% Female</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>43</td>
<td>42</td>
<td>47</td>
</tr>
<tr>
<td>Age at diagnosis, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>WHI2</td>
</tr>
<tr>
<td>&lt;65 years</td>
<td>41 (24)</td>
<td>101 (34)</td>
<td>98 (30)</td>
<td>125 (24)</td>
<td>98 (21)</td>
<td>61 (21)</td>
</tr>
<tr>
<td>65-69</td>
<td>21 (13)</td>
<td>66 (22)</td>
<td>53 (16)</td>
<td>145 (27)</td>
<td>115 (24)</td>
<td>57 (20)</td>
</tr>
<tr>
<td>70-74</td>
<td>38 (23)</td>
<td>63 (21)</td>
<td>55 (17)</td>
<td>161 (30)</td>
<td>131 (27)</td>
<td>96 (34)</td>
</tr>
<tr>
<td>75-79</td>
<td>34 (20)</td>
<td>46 (16)</td>
<td>51 (16)</td>
<td>88 (17)</td>
<td>88 (18)</td>
<td>58 (20)</td>
</tr>
<tr>
<td>≥80 years</td>
<td>34 (20)</td>
<td>20 (7)</td>
<td>67 (21)</td>
<td>12 (2)</td>
<td>46 (10)</td>
<td>13 (5)</td>
</tr>
<tr>
<td>Stage at diagnosis, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>WHI1</td>
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<tr>
<td>I/localized</td>
<td>47 (36)</td>
<td>61 (23)</td>
<td>64 (28)</td>
<td>193 (37)</td>
<td>166 (35)</td>
<td>135 (48)</td>
</tr>
<tr>
<td>II-III/regional</td>
<td>61 (46)</td>
<td>151 (58)</td>
<td>121 (53)</td>
<td>282 (54)</td>
<td>246 (52)</td>
<td>100 (36)</td>
</tr>
<tr>
<td>IV/distant</td>
<td>24 (18)</td>
<td>50 (19)</td>
<td>44 (19)</td>
<td>51 (10)</td>
<td>65 (14)</td>
<td>46 (16)</td>
</tr>
<tr>
<td>Unknown</td>
<td>36</td>
<td>34</td>
<td>95</td>
<td>5</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Tumor site, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>WHI2</td>
</tr>
<tr>
<td>Colon</td>
<td>113 (75)</td>
<td>228 (78)</td>
<td>250 (78)</td>
<td>514 (99)</td>
<td>313 (66)</td>
<td>215 (77)</td>
</tr>
<tr>
<td>Rectum</td>
<td>38 (25)</td>
<td>65 (22)</td>
<td>70 (22)</td>
<td>5 (1)</td>
<td>160 (34)</td>
<td>66 (23)</td>
</tr>
<tr>
<td>Unknown</td>
<td>17</td>
<td>3</td>
<td>4</td>
<td>12</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

*aAll platforms were Illumina assays.
Table 2. Characteristics of colorectal cancer cases in study populations included in follow-up analyses

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>MRC COIN and COIN B</th>
<th>Cancer Prevention Study II</th>
<th>Darmkrebs: Chancen der Verhütung durch Screening Study</th>
<th>Diet, Activity and Lifestyle Study</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>COIN/COIN-B</td>
<td>CPS-II</td>
<td>(Subset 1)</td>
<td>(Subset 1)</td>
</tr>
<tr>
<td>Genotyping platform*</td>
<td>KASPar (targeted)</td>
<td>Custom Affymetrix Axiom array</td>
<td>DACHS1</td>
<td>DALS1</td>
</tr>
<tr>
<td>No. cases</td>
<td>2234</td>
<td>523</td>
<td>300K</td>
<td>550K/610K</td>
</tr>
<tr>
<td>No. deaths, total (% of cases)</td>
<td>1612 (72)</td>
<td>113 (22)</td>
<td>1705</td>
<td>730K</td>
</tr>
<tr>
<td>No. deaths, CRC (% of deaths)</td>
<td>Not available</td>
<td>84 (74)</td>
<td>573 (34)</td>
<td>97 (23)</td>
</tr>
<tr>
<td>Median follow-up in years (SD)</td>
<td>2.4 (2.2)</td>
<td>2.8 (2.0)</td>
<td>414 (72)</td>
<td>71 (73)</td>
</tr>
<tr>
<td>% Female</td>
<td>34</td>
<td>50</td>
<td>41</td>
<td>38</td>
</tr>
<tr>
<td>Age at diagnosis, N (%)</td>
<td></td>
<td></td>
<td>288 (41)</td>
<td>161 (39)</td>
</tr>
<tr>
<td>&lt;65 years</td>
<td>1296 (58)</td>
<td>12 (2)</td>
<td>589 (34)</td>
<td>149 (35)</td>
</tr>
<tr>
<td>65–69</td>
<td>456 (20)</td>
<td>79 (15)</td>
<td>318 (19)</td>
<td>66 (16)</td>
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<td>70–74</td>
<td>339 (15)</td>
<td>138 (26)</td>
<td>288 (17)</td>
<td>78 (19)</td>
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<tr>
<td>75–79</td>
<td>127 (6)</td>
<td>172 (33)</td>
<td>260 (15)</td>
<td>61 (14)</td>
</tr>
<tr>
<td>≥80 years</td>
<td>14 (1)</td>
<td>122 (23)</td>
<td>250 (15)</td>
<td>66 (16)</td>
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<tr>
<td>Stage at diagnosis, N (%)</td>
<td></td>
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<td>260 (40)</td>
<td>128 (35)</td>
</tr>
<tr>
<td>I/localized</td>
<td>0 (0)</td>
<td>229 (46)</td>
<td>412 (24)</td>
<td>101 (24)</td>
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<tr>
<td>II–III/regional</td>
<td>0 (0)</td>
<td>223 (44)</td>
<td>1051 (62)</td>
<td>260 (63)</td>
</tr>
<tr>
<td>IV/distant</td>
<td>2234 (100)</td>
<td>51 (10)</td>
<td>238 (14)</td>
<td>55 (13)</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>20</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Tumor site, N (%)</td>
<td></td>
<td></td>
<td>64 (10)</td>
<td>27 (7)</td>
</tr>
<tr>
<td>Colon</td>
<td>1017 (46)</td>
<td>417 (81)</td>
<td>1042 (61)</td>
<td>234 (56)</td>
</tr>
<tr>
<td>Rectum</td>
<td>1216 (54)</td>
<td>101 (19)</td>
<td>663 (39)</td>
<td>186 (44)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

*Unless otherwise stated, genotyping platforms were Illumina assays.
Table 3: Single nucleotide polymorphisms identified from discovery analyses as being associated with survival after colorectal cancer diagnosis at significance level P < 5 × 10⁻⁸

<table>
<thead>
<tr>
<th>SNP</th>
<th>Combined follow-up</th>
<th>Discovery + follow-up</th>
<th>Discovery</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>rs11077289</td>
<td>3.5 (3.0–4.0)</td>
<td>3.2 (2.9–3.6)</td>
<td>3.5 (3.0–4.0)</td>
<td>3.2 (2.9–3.6)</td>
</tr>
<tr>
<td>rs17544464</td>
<td>2.2 (1.6–3.0)</td>
<td>2.2 (1.6–3.0)</td>
<td>2.2 (1.6–3.0)</td>
<td>2.2 (1.6–3.0)</td>
</tr>
<tr>
<td>rs1442089</td>
<td>2.2 (1.6–3.0)</td>
<td>2.2 (1.6–3.0)</td>
<td>2.2 (1.6–3.0)</td>
<td>2.2 (1.6–3.0)</td>
</tr>
<tr>
<td>rs209489</td>
<td>2.2 (1.6–3.0)</td>
<td>2.2 (1.6–3.0)</td>
<td>2.2 (1.6–3.0)</td>
<td>2.2 (1.6–3.0)</td>
</tr>
</tbody>
</table>

*All analyses adjusted for age at diagnosis and sex. Study sample and first three principal components were included in the analysis.*

Our results should be interpreted in the context of study limitations. Treatment information was not available for studies encoded a protein kinase that localizes to the intestinal crypt and is thought to be important in epithelial cell proliferation and differentiation (44); knockdown of ICK in CRC cell lines has been shown to induce G₁ cell cycle delay and slow cell growth (45). Other nearby genes include glutathione S-transferases alpha 1–5 (GSTA1, GSTA2, GSTA3, GSTA4, GSTA5). GST polymorphisms have been associated with CRC incidence and survival (46). Thus, although the functional significance of the SNPs at 6p12.1 found here to be associated with CRC survival has not been established, these findings merit further study.

Discovery analyses in cases with distant-metastatic CRC also suggested an association between the minor allele at rs1442089 (18q21.2/DCC) and shorter overall survival. DCC (i.e. Deleted in Colorectal Carcinoma) has been implicated in CRC etiology (47), and loss of DCC expression in CRC has been associated with a 2- to 4-fold poorer prognosis (48,49). However, results for rs1442089 were null in follow-up, suggesting our initial findings may have been spurious. Findings in the follow-up population were primarily driven by null results in the large COIN/COIN-B study. There are differences between the discovery study populations and COIN/COIN-B that may have contributed to discrepancies. In particular, the rigorous inclusion/exclusion criteria of the clinical trial setting may have resulted in a study population fundamentally and prognostically different from the population included in the observational studies that comprised the discovery set and the rest of the follow-up sample. Treatment differences may also have contributed. Differing methodologies, however, are unlikely to fully explain observed differences in results. Thus, although it remains possible that rs1442089 (18q21.2/DCC) is associated with prognosis in distant-metastatic CRC, the magnitude of such an association is likely not as strong as noted in our discovery analyses. Similarly, discovery analyses among all cases combined provided suggestive findings for a SNP in TMEM114 (rs11077289) that was not replicated. TMEM114 (transmembrane protein 114) has been implicated in cataract formation (50) but, to our knowledge, has not previously been associated with cancer risk or prognosis.

Previous analyses of genetic variation and CRC survival have taken a candidate approach, evaluating variation in specific pathways, genes, or SNPs based on a priori hypotheses. Several studies have focused on GWAS identified CRC susceptibility SNPs in relation to survival (5,15–18). Using this approach to interrogate 16 CRC susceptibility SNPs in a subset of the cases included in the present analysis, we previously reported a modest association between the minor allele in rs949827 (SMAD7) and poorer CRC survival (P = 0.002) (15). Although results from our previous analysis and other candidate studies have generated suggestive findings, many such findings have not been replicated in subsequent analyses. The limited robustness of findings from prior studies may, in part, reflect the shortcomings of a candidate-based approach; i.e. the pathways, genes and SNPs most relevant to and most robustly associated with CRC survival may be ones without a previously understood role in CRC progression and prognosis.

In the present analysis, we used an agnostic discovery-based approach to search for variants associated with CRC survival. The GWAS approach has successfully identified several CRC susceptibility variants (3–12), most of which were not targets of earlier candidate studies. Based on our current findings, there is reason to suspect that the identified SNPs in the 6p12.1 region fit with this paradigm as loci important to CRC survival that would likely not have been considered through a candidate approach.

Our results should be interpreted in the context of study limitations. Treatment information was not available for studies...
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in discovery analyses; therefore, we were unable to evaluate associations with response to specific treatments. Sample size limitations precluded extensive stratified analyses by other factors (e.g. tumor site). Lastly, one limitation inherent to the GWAS approach is the high likelihood of false-negative findings due to the stringent $P$-value threshold for genome-wide significance. This threshold is set to account for multiple testing and is designed to reduce the number of false-positive findings; however, a consequence of this stringency is that some important SNP-survival associations may have been missed.

The prospective nature of the studies included in discovery analyses constitutes an important strength; DNA specimens were collected prior to CRC diagnosis and, thus, inclusion in the analysis was not influenced by survival time. The included studies employed rigorous follow-up protocols to ensure the completeness of case ascertainment and vital status assessment. The large sample size and long duration of follow-up after diagnosis are also important strengths, as is the replication of findings in a large follow-up sample.

Just as GWAS for CRC risk have provided evidence for inherited susceptibility to CRC, findings from the present analysis support a role of common genetic variation in mediating CRC survival. SNPs at 6p12.1 were robustly associated with survival in individuals with distant-metastatic CRC in discovery and independent follow-up analyses, and merit further follow-up. The fact that the gene nearest to these SNPs, ELOVL5, has not previously been implicated in CRC etiology or progression highlights the utility of the agnostic GWAS approach, although it is
also possible that the identified SNPs reflect the role of another nearby gene (e.g. ICK). Results also highlight the need for independent replication. Future well-powered GWAS with independent follow-up and consideration for stage at diagnosis may yield additional findings that further our understanding of the mechanisms underlying CRC progression.

Supplementary material

Supplementary Tables 1–4 and Supplementary Figures 1–8 can be found at http://carcin.oxfordjournals.org/.

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