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Genome-wide search for determinants of survival in 1,926 patients with advanced colorectal cancer with follow-up in over 22,000 patients

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ABSTRACT

Background

While genome-wide association studies (GWAS) have identified germline variants influencing colorectal cancer (CRC) risk, there has been limited examination of the possible role of inherited variation as a determinant of patient outcome.

Patients and methods

We performed a GWAS for overall survival (OS) in 1,926 patients with advanced CRC from the COIN and COIN-B clinical trials. For SNPs showing an association with OS ($P < 1.0 \times 10^{-5}$), we conducted sensitivity analyses based on time from diagnosis to death and sought independent replications in 5,675 patients from the Study of Colorectal Cancer in Scotland (SOCCS) and 16,964 patients from the International Survival Analysis in Colorectal cancer Consortium (ISACC). We analysed the Human Protein Atlas to determine if *ERBB4* expression was associated with survival in 438 patients with colon adenocarcinomas.

Results

The most significant SNP associated with OS was rs79612564 in *ERBB4* (Hazard Ratio [HR]=1.24, 95% confidence Interval [CI]=1.16-1.32, $P = 1.9 \times 10^{-7}$). SNPs at 17 loci had suggestive associations for OS and all had similar effects on time from diagnosis to death. No lead SNPs were independently replicated in the meta-analysis of all patients from SOCCS and ISACC. However, rs79612564 was significant in stage IV patients from SOCCS ($P = 2.1 \times 10^{-2}$) but not ISACC ($P = 0.89$); and SOCCS combined with COIN and COIN-B, attained genome wide significance

($P=1.7 \times 10^{-8}$). Patients with high *ERBB4* expression in their colon adenocarcinomas had worse survival (HR=1.50, 95% CI=1.1-1.9, $P=4.6 \times 10^{-2}$).

Conclusions

Genetic and expression data support a potential role for rs79612564 in the receptor tyrosine kinase *ERBB4* as a predictive biomarker of survival.

INTRODUCTION

Clinical stage, which combines depth of tumour invasion, nodal status and distant metastasis (1), is currently the only routinely used marker of survival from colorectal cancer (CRC). Other factors thought to influence patient prognosis include lifestyle (2,3), systemic inflammatory response (4), immunologic microenvironment (5) and the patient's germline and the tumour's somatic profile (6,7). The search for inherited prognostic factors has primarily focussed on candidate genes and single nucleotide polymorphisms (SNPs) that function in pharmacological pathways (8,9), influence tumour progression (10) or alter disease risk (11-16). However, apart from rs9929218 in *CDH1*, most reported SNP associations have not been independently replicated (17).

Genome-wide association studies (GWAS) have been used successfully to identify 83 CRC-susceptibility alleles in the European population (18,19). To-date, the application of GWAS-based strategies for the identification of alleles influencing survival from CRC has been limited. SNPs near to *ELOVL5* and *DCC* have been associated with survival in a restricted discovery analysis but not replicated in follow-up (20) and SNPs in *FHIT*, *EPHB1* and *MIR7515* have been associated with time to metastasis but await independent replication (21). Here, we report a GWAS of survival in 1,926 patients with advanced CRC from two clinical trials with follow-up of promising SNP-associations in over 22,000 CRC patients from clinical trial and population-based studies.

MATERIALS AND METHODS

Discovery GWAS

2,671 unrelated patients with metastatic or locally advanced colorectal adenocarcinoma were recruited into the MRC clinical trials COIN (NCT00182715) (22) and COIN-B (NCT00640081) (23). COIN patients were randomised 1:1:1 to receive continuous oxaliplatin and fluoropyrimidine chemotherapy, continuous chemotherapy with cetuximab, or intermittent chemotherapy. COIN-B patients were randomised 1:1 to receive intermittent chemotherapy and cetuximab or intermittent chemotherapy and continuous cetuximab (**Supplementary Figure S1**). Patients from COIN and COIN-B were combined for survival analyses since there was no evidence of heterogeneity in overall survival (OS; time from trial randomisation to death or end of trial) between patients when analysed by trial ($P=0.49$), trial arm ($P=0.40$; Cochran Q test: $p=1.0$, I^2 test: $P=0.74$), type of chemotherapy received ($P=0.60$), or cetuximab use ($P=0.41$). Blood DNA samples were prepared from 2,244 patients all of whom gave fully informed consent for bowel cancer research (approved by REC [04/MRE06/60]).

We genotyped DNA samples using Affymetrix Axiom Arrays according to the manufacturer's recommendations (Affymetrix, Santa Clara, CA 95051, USA) at the King Faisal Specialist Hospital and Research Center, Saudi Arabia (under IRB approval 2110033) (24). After quality control (QC), 1,950 patient samples remained for analyses, 2 of whom had no data on survival and were excluded ($n=1,948$, **Supplementary Figure S1**). Prediction of untyped SNPs was carried out using IMPUTE2 v2.3.0 (25) based on data from the 1000 Genomes Project as reference

(26,27). In line with current GWAS guidelines (28,29), we excluded SNPs with minor allele frequencies (MAFs) <5%, or that had poor imputation scores (INFO score <0.8, n=29 million), missingness >0.02 (n=3.5 million) or Hardy Weinberg Equilibrium (HWE) exact test (30) $P < 1.0 \times 10^{-6}$ (n=47). 2.9 million SNPs remained after QC. rs79612564 in *ERBB4* was independently genotyped by KASPar technology (LGC, Teddington, Middlesex, UK).

Statistical analysis

Somatic and clinicopathological factors available in COIN and COIN-B (trial, trial arm, cetuximab status, sex, age, *KRAS* status, *BRAF* status, *NRAS* status, MSI status, *PIK3CA* status, World Health Organisation [WHO] performance status, resection status of the primary tumour, site of primary tumour, surface area, white blood cell [WBC] count, alkaline phosphatase level, platelet count, chemotherapy regimen, chemotherapy dose, radiotherapy, number of metastatic sites, liver metastases, lung metastases, nodal metastases, peritoneal metastases, other metastases, time to metastases, synchronous or metachronous metastases, creatinine clearance, glomerular filtration rate and carcinoembryonic antigen [CEA] level) were analysed for their effects on OS using either linear and logistic models (**Supplementary Table S1**). For those shown to be prognostic after Bonferroni correction ($P < 1.6 \times 10^{-3}$, n=31 tests), we performed a GWAS for each factor to identify potential SNPs with pleiotropic effects on survival. Lead SNPs at credible independent loci (those with multiple SNPs in the linkage block and that reached the threshold for suggestive significance [$P < 1.0 \times 10^{-5}$]) were tested for their effects on OS.

We carried out a multivariate GWAS of OS under an additive model for patients in COIN and COIN-B using prognostic covariates that were available in the majority of patients (22 patients excluded leaving 1,926 for analysis). The covariates included were WHO performance status, resection status of the primary tumour, WBC count, platelet count, alkaline phosphatase levels, number of metastatic sites, metastases in the liver, site of primary tumour (encoded as 7 binary variables), surface area of primary tumour, time from diagnosis to metastases, and metachronous versus synchronous metastases (**Supplementary Table S1**). For any SNPs that reached suggestive significance we conducted a sensitivity analysis replacing OS (considered left-truncated at randomisation since randomisation is conditional upon survival from diagnosis) with time from diagnosis to death or end of trial using Cox regressions. To test for differences in association between the two measures of survival, for each SNP we calculated differences in beta-coefficients and standard errors to produce a chi-squared distribution with 1 degree of freedom; from this *P*-values were determined.

Gene and gene-set analysis was completed on the summary statistics from the association analysis to identify genes containing significant numbers of highly associated SNPs and significantly enriched gene-sets. The threshold for significance at gene level was $P < 2.5 \times 10^{-6}$, a Bonferroni correction for 20,000 independent tests (31). Correction for multiple testing for gene-set analysis was completed by adjusting *P*-values for the false discovery rate to produce *q*-values (32,33), held to a significance threshold of $q < 0.05$.

Response at 12 weeks was assessed under a univariate dominant model and response was defined as complete or partial response using RECIST 1.0 guidelines and no response was defined as stable or progressive disease.

Bioinformatic analyses

Discordant sex, individual/SNP missingness, heterozygosity, relatedness, principal component analysis, MAF and HWE quality control steps were performed using the *-sex-checks*, *--missing*, *--het*, *--genome*, *--pca* and *--hardy* commands in PLINK 1.9 (<https://www.cog-genomics.org/plink2>) (34) and clumping of GWAS summary statistics into independently associated loci was completed using the *--clump* command. INFO scores were obtained using SNPTTEST v2.5.2 (https://mathgen.stats.ox.ac.uk/genetics_software/snptest/). Linear and logistic SNP association tests were performed in PLINK v2.00a2 (<https://www.cog-genomics.org/plink/2.0/>). Regional association plots were created using LocusZoom (<http://locuszoom.org>). Multivariate OS analyses, genomic inflation factor calculation and manhattan/quantile-quantile plots were performed using the *gwasurvivr* (35), *GenABEL* (36) and *qqman* R (<https://www.r-project.org/>) (37) packages, respectively.

Gene and gene-set analyses were performed using MAGMA (38) v1.07b (<https://ctg.cncr.nl/software/magma>). SNPs were annotated to genes (including those 35 kilobases before the genes transcription zone and 10 kilobases after) using the *--annotate* command and the gene location file for hg19: 'NCBI37.3.loc'. SNP *P*-values were assessed with the linkage disequilibrium between them using the *multi=snp-wise* and *--gene-model* commands. This model takes advantage of the sum of the $-\log(P)$ for all SNPs, as well as the top SNP associations within each

gene, to assess the association of their constituent genes. Genes were annotated to sets by gene-ontology terms (39) including experimental evidence, phylogenetically inferred annotation, computational-analysis, author statement, curator statement and electronic annotation (40). A competitive model (*--set-result* command) was used to assess each gene-set's association with OS. Expression quantitative trait loci analysis was completed by searching the Genotype-Tissue Expression project database (<https://gtexportal.org/home/>) (41) for significant associations between any relevant SNPs and gene expression.

The Human Protein Atlas (42) was used to find associations between *ERBB4* expression levels and survival in 438 patients with colon adenocarcinomas (<https://www.proteinatlas.org/ENSG00000178568-ERBB4/pathology/colorectal+cancer/COAD>). RNA-seq data was reported as median number of fragments per kilobase of exon per million reads (FPKM) generated by The Cancer Genome Atlas. Samples were classified as high expression using a threshold of FPKM>0 as per The Human Protein Atlas recommendations (42).

Replication series

Independent replication of lead SNPs at 17 loci showing suggestive evidence of an association with OS in COIN and COIN-B was performed in two independent patient series:

(i) Study of Colorectal Cancer in Scotland

5,675 patients (1,358 CRC specific deaths) of which 784 had stage IV CRC (522 deaths) from the Study of Colorectal Cancer in Scotland (SOCCS; 1999-current

(43,44); ethics approval number MREC/01/0/5 obtained from the MultiCentre Research Ethics committee for Scotland). Information on recruitment, genotyping, QC and criteria for assigning cause of death has been previously documented (45). We considered CRC specific survival, assigned as time from diagnosis to death from CRC and applied a Cox proportional hazards model and corrected for age, sex and AJCC stage.

(ii) International Survival Analysis in Colorectal cancer Consortium (ISACC)

16,964 patients (4,010 deaths) of which 1,847 had stage IV CRC (1,448 deaths) from ISACC which comprised of 15 studies: the Cancer Prevention Study-II (CPS-II), the German Darmkrebs: Chancen der Verhütung durch Screening Study (DACHS), the Diet Activity and Lifestyle Study (DALIS), the Early Detection Research Network (EDRN), the Swedish population of the European Prospective Investigation into Cancer (EPIC), the Health Professionals Follow-up Study (HPFS), the Melbourne Collaborative Cohort Study (MCCS), the Nurses' Health Study (NHS), the N9741 clinical trial, the Physician's Health Study (PHS), the Prostate, Lung, Colorectal, and Ovarian Study (PLCO), the UK Biobank (UKB), the VITamins And Lifestyle Study (VITAL), the Women's Health Initiative (WHI), and four Colon Cancer Family Registry (CCFR) sites: Seattle, Ontario, Australia, and the Mayo Clinic. References for each study are provided in **Supplementary Material**. Study participants included individuals of European genetic ancestry diagnosed with CRC and with available genotyping and CRC-specific survival data. All participants provided informed consent for genetic testing, and all studies were approved by their respective Institutional Review Boards.

Meta-analyses of the follow-up cohorts

Meta-analyses were performed using the inverse variance based method in the METAL software package (46). $P < 0.05$ was considered significant for replication of the findings in the discovery cohort.

RESULTS

We determined the influence of clinicopathological factors and somatic mutation status on OS in 1,948 patients from COIN and COIN-B. We found that *KRAS* and *BRAF* mutation status, MSI status, platelet count, CEA levels, WHO performance status, resection status of the primary tumour, WBC count, alkaline phosphatase levels, number of metastatic sites, metastases in the liver, lymph nodes and peritoneum, site and surface area of the primary tumour, time from diagnosis to metastases and metachronous versus synchronous metastases were all associated with OS after Bonferroni correction (**Supplementary Table S1**). We considered whether SNPs associated with these factors might influence OS and conducted independent GWASs for each factor ($n=16$). One SNP was associated with WBC count (rs142358223 at 16p13.3, beta coefficient [beta]=1.36, standard error [SE]=0.25, $P=3.5 \times 10^{-8}$) and two SNPs with CEA levels (rs17418475 at 1p21.2, beta=932.53, SE=163.05, $P=1.3 \times 10^{-8}$ and rs72870425 at 2q24.2, beta=1196.53, SE=211.27, $P=1.8 \times 10^{-8}$). We tested rs142358223, rs17418475, rs72870425 and 133 lead SNPs from other suggestive loci for their effects on OS, however, none were significant after adjustment for multiple testing ($P < 3.7 \times 10^{-4}$; **Supplementary Table S2**).

We carried out a multivariate GWAS for OS in 1,926 patients from COIN and COIN-B using 11 prognostic covariates (**Supplementary Figure S1, Figure 1**). No detectable genomic inflation was observed (1.08). We had >80% power to detect a HR of 1.3 for SNPs with MAFs $\geq 20\%$.

The most significant SNP associated with OS was rs79612564 in *ERBB4* (HR=1.24, 95% CI=1.16-1.32, $P=1.9 \times 10^{-7}$). Median survival for patients in COIN and COIN-B carrying one minor allele was reduced by 46 days and for those homozygous for the minor allele by 81 days (**Supplementary Figure S2, Supplementary Table S3**). rs79612564 was not influenced by cetuximab treatment regardless of *KRAS* status (**Supplementary Figure S3**). The prognostic effect appeared to be independent of *KRAS* status and patients carrying at least one rs79612564 minor allele and *KRAS* mutant CRCs had the greatest effect on survival (HR=1.51, CI=1.29-1.77, $P=3.7 \times 10^{-7}$) (**Supplementary Figure S4**). In terms of response to oxaliplatin and fluoropyrimidine-based chemotherapy, patients carrying one or more minor alleles showed less response (55.5% for heterozygotes and 55.9% for homozygotes) as compared to patients carrying both major alleles (60.2%), although this did not reach statistical significance ($P=0.06$) (**Supplementary Table S4**). rs79612564 was not an eQTL.

rs79612564 had an INFO score of 0.99. We sought independent confirmation of the quality of genotyping and predictive score for this SNP by genotyping rs79612564 directly via KASPar technology. For those samples with both KASPar genotyping

and an imputed genotype, we had >99% (1,687/1,703) genotype concordance (**Supplementary Figure S5**).

In total, we identified SNPs at 17 independent loci with suggestive associations with OS (**Table 1, Figure 1**). We conducted a sensitivity analysis for lead SNPs at all 17 loci replacing OS with an alternative measure of survival - time from diagnosis to death or end of trial. There were no significant differences between the two measures of survival for any of the 17 SNPs ($P=0.46-0.95$). rs6568761 at 6q21 (in a gene desert) passed the threshold for genome wide significance ($P=5.0\times 10^{-8}$) with diagnosis to death (HR=0.88, 95% CI=0.78-0.98, $P=4.5\times 10^{-8}$).

We did not find any significantly associated genes (**Supplementary Table S5**), or gene-sets under competitive analyses (**Supplementary Table S6**) for OS after correction for multiple testing.

Replication analyses

We analysed lead SNPs at all 17 loci in 5,675 patients with CRC from SOCCS and 16,964 patients with CRC from ISACC (**Table 2, Figure 2**). Together, we had >98% power to replicate all 17 SNPs (**Supplementary Table S7**). After meta-analysis, no lead SNPs were independently replicated and only rs1352374 and rs2050337 reached nominal significance in SOCCS (**Table 2**).

We considered whether the lack of replication of the COIN and COIN-B data might be confounded by patients with differing stages of disease in the follow-up cohorts. We therefore tested the 17 lead SNPs in a subset of 784 patients from SOCCS and

1,847 patients from ISACC with stage IV CRC (**Table 3, Figure 3**). We had >80% power to replicate 16 of the SNPs (for rs3103204 we had 62% power) (**Supplementary Table S7**). rs79612564 was significant in stage IV patients from SOCCS ($P=2.1 \times 10^{-2}$) but not in stage IV patients from ISACC ($P=0.89$, **Table 3**). When SOCCS was combined with COIN and COIN-B, rs79612564 reached genome wide significance (HR=1.22, 95% CI=1.15-1.29, $P=1.7 \times 10^{-8}$), but not when ISACC was also included (HR=1.12, 95% CI=1.06-1.17, $P=3.4 \times 10^{-5}$).

rs6983214 was significant in the meta-analysis of stage IV patients from SOCCS and ISACC ($P=1.2 \times 10^{-3}$), however, the direction of effect was opposite to that found in COIN and COIN-B (**Table 3**). rs1352374 reached nominal significance in SOCCS ($P=3.3 \times 10^{-2}$), but not in ISACC. rs2050337 reached nominal significance in the meta-analysis ($P=1.1 \times 10^{-2}$, **Table 3**) with the same direction of effect in all cohorts tested (meta-analysis with COIN and COIN-B included HR=1.13, 95% CI=1.08-1.18, $P=1.6 \times 10^{-6}$).

Relationship between *ERBB4* expression and survival

We sought additional mechanistic data for a role for *ERBB4* on survival by studying 438 patients with colon adenocarcinomas from the Human Protein Atlas. Patients with high *ERBB4* expression in their tumours had worse survival (Cox-regression HR=1.50, 95% CI=1.10-1.90, $P=4.6 \times 10^{-2}$, **Supplementary Figure S6**).

DISCUSSION

Despite identifying 18 somatic and clinicopathological factors that significantly influenced survival in COIN and COIN-B, we found that SNPs associated with these factors did not themselves affect survival thereby excluding potential pleiotropic effects. To generate a comprehensive genome-wide analysis of survival, we included prognostic factors into our multivariate analyses and observed little genomic inflation supporting the validity of this approach.

The most significant SNP identified was rs79612564 which lies within intron 3 of *ERBB4*, a member of the epidermal growth factor receptor subfamily. We confirmed the quality of the genotyping and imputation for this SNP via an independent assay. Patients carrying the minor allele had an additive effect on survival with a median decrease in life expectancy of approximately 40 days per allele carried in the advanced disease setting. rs79612564 was also significant in stage IV patients from SOCCS and, combined with COIN and COIN-B, reached genome wide significance. Our genetic data was supported by mechanistic data for this gene and we found that patients with high *ERBB4* expression in their colon adenocarcinomas had worse survival. Furthermore, it has previously been shown that *ERBB4* over-expression in experimental systems enhances the survival and growth of cells driven by *Ras* and/or *Wnt* signaling (47).

However, rs79612564 was not replicated in stage IV patients from ISACC, nor in all patients from SOCCS and ISACC combined. This warrants further investigation although it is noteworthy that overexpression and heterodimerization of *ERBB4* and *ERBB2* shows a significant association with late stage colorectal carcinomas (48). Therefore, it is possible that the association for rs79612564 can only be seen in

patients with later stages of disease and survival in these patients is confounded by numerous clinical and pathological prognostic covariates which we accounted for in our GWAS but are, in general, not available in the population based cohorts.

In terms of clinical application, it should be noted that the effect size for rs79612564 is modest and will need to be combined with other prognostic factors to have any role in patient management. For example, our data suggests that this SNP acts independently of *KRAS* mutational status which itself is a prognostic factor. In isolation, rs79612564 has an OR of 1.24 but on a *KRAS* mutant background increases to 1.51. Although this effect size is still modest, it shows the potential for building germline, somatic and clinicopathological factors into a combined prognostic model.

Most of the other loci of interest failed to be replicated or their directions of effect were opposite to those found in our discovery cohort. However, rs2050337 at 10q25.1 reached significance in the stage IV replication meta-analysis with a consistent direction of effect to COIN and COIN-B, and was also significant in all patients from SOCCS. It lies approximately 500Kb upstream of *ADD3* which has been associated with tumor growth and cell migration in breast (49), glioblastoma multiforme (50) and lung cancer (51). However, even combined with COIN and COIN-B, rs2050337 still did not achieve genome-wide significance in patients with stage IV disease suggesting that its effects, if genuine, are modest.

Despite having 1,926 patients with advanced CRC (with a 75% event rate) in our GWAS, we lacked sufficient power to detect common alleles with low effect sizes

(HR<1.3) at genome wide significance levels. Even by considering loci at suggestive significance levels, as we have done, we only had 33% power to detect common alleles with HRs of 1.2. Future studies will therefore have to combine their datasets for meta-analyses to provide sufficient power to identify low impact alleles for survival. For example, to achieve 80% power to detect alleles with HRs of 1.2 and 1.1 would require 4,907 and 18,022 patients with a 75% event rate, respectively.

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AUTHOR CONTRIBUTIONS

JPC obtained funding for and directed this study. The study was designed by CW and JPC. TSM was CI of COIN and provided clinical advice and supported the translational research. RSK managed the COIN and COIN-B trials and facilitated access to the clinical data. NAA oversaw the genotyping of COIN and COIN-B. PJJ and RSH oversaw the imputation and QC. YH and MGD provided data from SOCCS and YL, AIP, QS, SRA, UP, PAN, ATC, LLM, DDB, SG and RKP provided data from ISACC for replication analyses. CW undertook all of the GWAS statistical and meta-

analyses with supervision from VEP and JPC, and with input from MGS. CW and JPC interpreted the data with input from KW, VG, HW and VEP. CW wrote the first draft of the paper with subsequent input from JPC, and all authors provided comments.

DECLARATION OF INTEREST STATEMENT

The authors declare no potential conflicts of interest.

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LEGENDS TO FIGURES

Figure 1. Manhattan plot of SNP associations with overall survival (OS) (n=1,926 patients with advanced CRC from COIN and COIN-B). SNPs are ordered by chromosome position and plotted against the $-\log_{10}(P)$ for their association with OS. The red line represents the threshold for genome wide significance ($P=5.0 \times 10^{-8}$) and the blue line is the threshold for suggestive significance ($P=1.0 \times 10^{-5}$). Covariates included: World Health Organisation performance status, resection status of the primary tumour, white blood cell count, platelet count, alkaline phosphatase levels, number of metastatic sites, metastases within or outside of the liver, site of primary tumour, surface area of primary tumour, time from diagnosis to metastases and metachronous versus synchronous metastases.

Figure 2. Forest plots for lead SNPs at 17 loci identified in COIN and COIN-B and the independent replication cohorts (all stages). Sample size, number of events, P -value, Hazard ratio and 95% confidence intervals are listed.

Figure 3. Forest plots for lead SNPs at 17 loci identified in COIN and COIN-B and the independent replication cohorts (stage IV disease). Sample size, number of events, P -value, Hazard ratio and 95% confidence intervals are listed.

TABLES

| SNP | Locus | Minor Allele | Genes | Overall survival | | | Diagnosis to death | | |
|------------|----------|--------------|---|------------------|-----------|----------------------|--------------------|-----------|----------------------------|
| | | | | HR | 95% CI | P | HR | 95% CI | P |
| rs79612564 | 2q34 | C | <i>ERBB4</i> | 1.24 | 1.16-1.32 | 1.9x10 ⁻⁷ | 1.08 | 1.00-1.16 | 4.7x10 ⁻⁵ |
| rs9356458 | 6q27 | A | | 0.82 | 0.75-0.90 | 9.1x10 ⁻⁷ | 0.92 | 0.85-1.00 | 1.1x10 ⁻⁵ |
| rs9744647 | 15q14 | T | <i>C145orf51</i> | 1.29 | 1.18-1.39 | 2.0x10 ⁻⁶ | 1.11 | 1.03-1.20 | 4.3x10 ⁻⁶ |
| rs6568761 | 6q21 | G | | 0.78 | 0.67-0.88 | 2.0x10 ⁻⁶ | 0.88 | 0.78-0.98 | 4.5x10⁻⁸ |
| rs244509 | 5q22.1 | C | <i>CAMK4</i> | 0.81 | 0.73-0.90 | 2.0x10 ⁻⁶ | 0.91 | 0.83-0.99 | 1.0x10 ⁻⁶ |
| rs1400673 | 3p25.1 | G | | 1.35 | 1.23-1.48 | 2.1x10 ⁻⁶ | 1.13 | 1.01-1.25 | 1.4x10 ⁻⁵ |
| rs4653255 | 1p34.3 | A | | 0.84 | 0.76-0.91 | 2.6x10 ⁻⁶ | 0.94 | 0.86-1.02 | 1.1x10 ⁻⁴ |
| rs2473571 | 6p21.1 | G | <i>LRFN2</i> | 1.19 | 1.12-1.27 | 3.1x10 ⁻⁶ | 1.06 | 0.98-1.14 | 3.5x10 ⁻⁴ |
| rs9594035 | 13q31.1 | T | | 0.82 | 0.73-0.90 | 5.4x10 ⁻⁶ | 0.92 | 0.84-1.00 | 5.4x10 ⁻⁶ |
| rs3103204 | 4p13 | T | <i>ATP8A1, SHISA3</i> | 0.76 | 0.64-0.88 | 5.4x10 ⁻⁶ | 0.89 | 0.78-1.01 | 2.5x10 ⁻⁵ |
| rs11605969 | 11q24.1 | T | <i>SORL1</i> | 1.26 | 1.16-1.36 | 6.3x10 ⁻⁶ | 1.08 | 0.98-1.18 | 3.3x10 ⁻⁴ |
| rs4411363 | 13q12.12 | G | <i>TNFRSF19</i> | 1.19 | 1.12-1.27 | 7.8x10 ⁻⁶ | 1.06 | 0.98-1.14 | 1.1x10 ⁻³ |
| rs1352374 | 4p15.2 | C | | 0.82 | 0.73-0.91 | 8.4x10 ⁻⁶ | 0.92 | 0.80-1.03 | 1.8x10 ⁻⁵ |
| rs6983214 | 8q13.1 | T | <i>C8orf44, C8orf44- SGK3, VCPIP1</i> | 0.83 | 0.75-0.91 | 8.8x10 ⁻⁶ | 0.92 | 0.84-1.00 | 4.9x10 ⁻⁶ |
| rs11744800 | 5q33.3 | C | <i>ADAM19</i> | 0.82 | 0.74-0.91 | 8.8x10 ⁻⁶ | 0.93 | 0.85-1.01 | 3.5x10 ⁻⁴ |
| rs2050337 | 10q25.1 | G | | 1.19 | 1.11-1.26 | 9.0x10 ⁻⁶ | 1.07 | 0.99-1.15 | 6.5x10 ⁻⁵ |
| rs7145600 | 14q21.1 | T | | 0.79 | 0.69-0.90 | 9.5x10 ⁻⁶ | 0.91 | 0.81-1.01 | 5.2x10 ⁻⁵ |

Table 1. Lead SNPs from independent loci that reached suggestive significance in multivariate analysis of overall survival in COIN and COIN-B. Cytogenic band, minor allele, *P*-value, hazard ratio and 95% confidence intervals are shown for overall survival (time from trial

recruitment to death or end of study) and time from diagnosis to death or end of trial. Only rs6568761 reached the threshold for genome-wide significance ($P < 5.0 \times 10^{-8}$, in bold). Genes overlapping with the SNPs attributed to each locus are listed.

| SNP | Independent replication | | | | | | | | |
|------------|--|-----------|--|-----------|----------------------------|---|-----------|----------|----------|
| | COIN and COIN-B 1,926 patients (1,435 deaths) | | SOCCS 5,675 patients (1,358 deaths) | | | ISACC 16,964 patients (4,010 deaths) | | | Meta |
| | HR | 95% CI | HR | 95% CI | <i>P</i> | HR | 95% CI | <i>P</i> | <i>P</i> |
| rs79612564 | 1.24 | 1.16-1.32 | 1.06 | 0.98-1.15 | 0.15 | 1.01 | 0.96-1.05 | 0.77 | 0.34 |
| rs9356458 | 0.82 | 0.75-0.90 | 1.03 | 0.95-1.11 | 0.44 | 1.00 | 0.95-1.04 | 0.87 | 0.82 |
| rs9744647 | 1.29 | 1.18-1.39 | 1.02 | 0.90-1.14 | 0.70 | 1.02 | 0.96-1.09 | 0.45 | 0.73 |
| rs6568761 | 0.78 | 0.67-0.88 | 0.99 | 0.88-1.09 | 0.60 | 1.01 | 0.95-1.06 | 0.86 | 0.97 |
| rs244509 | 0.81 | 0.73-0.90 | 1.08 | 0.99-1.16 | 0.10 | 1.01 | 0.96-1.06 | 0.81 | 0.30 |
| rs1400673 | 1.35 | 1.23-1.48 | 0.98 | 0.84-1.12 | 0.78 | 1.00 | 0.92-1.07 | 0.97 | 0.87 |
| rs4653255 | 0.84 | 0.76-0.91 | 0.97 | 0.89-1.04 | 0.37 | 0.99 | 0.95-1.04 | 0.75 | 0.47 |
| rs2473571 | 1.19 | 1.12-1.27 | 1.01 | 0.93-1.09 | 0.76 | 0.99 | 0.95-1.04 | 0.76 | 0.91 |
| rs9594035 | 0.82 | 0.73-0.90 | 0.99 | 0.90-1.08 | 0.87 | 1.01 | 0.96-1.06 | 0.61 | 0.72 |
| rs3103204 | 0.76 | 0.64-0.88 | 0.98 | 0.86-1.10 | 0.75 | 0.99 | 0.93-1.06 | 0.79 | 0.70 |
| rs11605969 | 1.26 | 1.16-1.36 | 0.98 | 0.88-1.09 | 0.71 | 1.02 | 0.95-1.08 | 0.63 | 0.82 |
| rs4411363 | 1.19 | 1.12-1.27 | 0.99 | 0.91-1.07 | 0.84 | 1.01 | 0.96-1.05 | 0.72 | 0.84 |
| rs1352374 | 0.82 | 0.73-0.91 | 0.89 | 0.80-0.98 | 1.5x10⁻² | 1.01 | 0.96-1.06 | 0.62 | 0.58 |
| rs6983214 | 0.83 | 0.75-0.91 | 1.07 | 0.98-1.15 | 0.13 | 1.00 | 0.95-1.05 | 0.91 | 0.39 |
| rs11744800 | 0.82 | 0.74-0.91 | 1.04 | 0.96-1.13 | 0.33 | 0.98 | 0.93-1.03 | 0.36 | 0.75 |
| rs2050337 | 1.19 | 1.11-1.26 | 1.09 | 1.02-1.17 | 2.4x10⁻² | 1.01 | 0.97-1.06 | 0.60 | 0.11 |
| rs7145600 | 0.79 | 0.69-0.90 | 1.01 | 0.91-1.11 | 0.81 | 1.01 | 0.95-1.07 | 0.79 | 0.72 |

Table 2. Independent replication of lead SNPs in SOCCS and ISACC. Hazard Ratio, 95% confidence intervals and *P*-value are listed for overall survival (time from trial recruitment to death or end of study) in COIN and COIN-B, and CRC-specific survival (time from diagnosis to death due to CRC) in SOCCS and ISACC. Nominally significant *P*-values are highlighted in bold.

| SNP | Independent replication | | | | | | | | |
|------------|--|-----------|---|-----------|----------------------------|---|-----------|----------------------------|-----------------------------|
| | COIN and COIN-B 1,926 patients (1,435 deaths) | | SOCCS Stage IV 784 patients (522 deaths) | | | ISACC Stage IV 1,847 patients (1,448 deaths) | | | Meta |
| | HR | 95% CI | HR | 95% CI | <i>P</i> | HR | 95% CI | <i>P</i> | <i>P</i> |
| rs79612564 | 1.24 | 1.16-1.32 | 1.17 | 1.04-1.30 | 2.1x10⁻² | 0.99 | 0.92-1.07 | 0.89 | 0.28 |
| rs9356458 | 0.82 | 0.75-0.90 | 1.09 | 0.96-1.21 | 0.19 | - | - | - | - |
| rs9744647 | 1.29 | 1.18-1.39 | 1.01 | 0.81-1.21 | 0.93 | 0.97 | 0.86-1.07 | 0.52 | 0.82 |
| rs6568761 | 0.78 | 0.67-0.88 | 1.02 | 0.86-1.17 | 0.62 | 1.03 | 0.93-1.12 | 0.58 | 0.56 |
| rs244509 | 0.81 | 0.73-0.90 | 1.08 | 0.94-1.21 | 0.30 | 1.00 | 0.92-1.09 | 0.96 | 0.56 |
| rs1400673 | 1.35 | 1.23-1.48 | 1.03 | 0.82-1.24 | 0.78 | 1.08 | 0.96-1.21 | 0.22 | 0.23 |
| rs4653255 | 0.84 | 0.76-0.91 | 1.00 | 0.88-1.12 | 0.97 | 1.04 | 0.96-1.11 | 0.35 | 0.41 |
| rs2473571 | 1.19 | 1.12-1.27 | 0.99 | 0.87-1.11 | 0.86 | 0.97 | 0.90-1.05 | 0.49 | 0.50 |
| rs9594035 | 0.82 | 0.73-0.90 | 0.96 | 0.82-1.10 | 0.57 | 0.96 | 0.88-1.05 | 0.36 | 0.28 |
| rs3103204 | 0.76 | 0.64-0.88 | 0.89 | 0.71-1.07 | 0.19 | 0.93 | 0.82-1.03 | 0.17 | 0.06 |
| rs11605969 | 1.26 | 1.16-1.36 | 1.12 | 0.95-1.29 | 0.18 | 1.05 | 0.95-1.15 | 0.35 | 0.14 |
| rs4411363 | 1.19 | 1.12-1.27 | 1.03 | 0.90-1.16 | 0.65 | 1.02 | 0.94-1.10 | 0.65 | 0.53 |
| rs1352374 | 0.82 | 0.73-0.91 | 0.85 | 0.71-0.99 | 3.3x10⁻² | 1.00 | 0.91-1.08 | 0.99 | 0.59 |
| rs6983214 | 0.83 | 0.75-0.91 | 1.15 | 1.02-1.28 | 3.6x10⁻² | 1.11 | 1.03-1.19 | 1.2x10⁻² | 1.2x10^{-3*} |
| rs11744800 | 0.82 | 0.74-0.91 | 1.03 | 0.89-1.17 | 0.72 | 1.03 | 0.95-1.12 | 0.47 | 0.42 |
| rs2050337 | 1.19 | 1.11-1.26 | 1.08 | 0.96-1.20 | 0.22 | 1.09 | 1.01-1.17 | 2.7x10⁻² | 1.1x10⁻² |
| rs7145600 | 0.79 | 0.69-0.90 | 1.07 | 0.91-1.23 | 0.39 | 0.92 | 0.82-1.02 | 0.09 | 0.32 |

Table 3. Independent replication of lead SNPs in patients from SOCCS and ISACC with Stage IV CRC. Hazard Ratio, 95% confidence intervals and *P*-value are listed for overall survival (time from trial recruitment to death or end of study) in COIN and COIN-B, and CRC-specific survival (time from diagnosis to death due to CRC) in SOCCS and ISACC. Nominally significant *P*-values are highlighted in bold. *Opposite direction of effect to COIN and COIN-B so not validated. Data for rs9356458, nor any proxies were available for stage IV patients from ISACC.