Clinical Cancer Research

Analyses of 7,635 Patients with Colorectal Cancer Using Independent Training and Validation Cohorts Show That rs9929218 in *CDH1* Is a Prognostic Marker of Survival

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Abstract

Purpose: Genome-wide association studies have identified numerous loci associated with colorectal cancer risk. Several of these have also been associated with patient survival, although none have been validated. Here, we used large independent training and validation cohorts to identify robust prognostic biomarkers for colorectal cancer.

Experimental Design: In our training phase, we analyzed 20 colorectal cancer-risk SNPs from 14 genome-wide associated loci, for their effects on survival in 2,083 patients with advanced colorectal cancer. A Cox survival model was used, stratified for treatment, adjusted for known prognostic factors, and corrected for multiple testing. Three SNPs were subsequently analyzed in an independent validation cohort of 5,552 colorectal cancer patients. A validated SNP was analyzed by disease stage and response to treatment.

Results: Three variants associated with survival in the training phase; however, only rs9929218 at 16q22 (intron 2 of *CDH1*, encoding E-cadherin) was significant in the validation phase. Patients homozygous for the minor allele (AA genotype) had worse survival (training phase HR, 1.43; 95% confidence intervals; CI, 1.20–1.71, $P = 5.8 \times 10^{-5}$; validation phase HR, 1.18; 95% CI, 1.01–1.37, $P = 3.2 \times 10^{-2}$; combined HR, 1.28; 95% CI, 1.14–1.43, $P = 2.2 \times 10^{-5}$). This effect was independent of known prognostic factors, and was significant amongst patients with stage IV disease ($P = 2.7 \times 10^{-5}$). rs9929218 was also associated with poor response to chemotherapy ($P = 3.9 \times 10^{-4}$).

Conclusions: We demonstrate the potential of common inherited genetic variants to inform patient outcome and show that rs9929218 identifies approximately 8% of colorectal cancer patients with poor prognosis. rs9929218 may affect *CDH1* expression and E-cadherin plays a role in epithelial-to-mesenchymal transition providing a mechanism underlying its prognostic potential. *Clin Cancer Res; 21(15); 3453–61. ©2015 AACR.*

Introduction

Worldwide, over a million people are diagnosed with colorectal cancer each year. Several factors influence survival after diagnosis, but the only routinely used prognostic marker is clinical stage, which combines depth of tumor invasion, nodal status, and distant metastasis (1). Other factors thought to influence prognosis include lifestyle (2, 3), systemic inflammatory response to the tumor (4), the tumor immunologic microenvironment (5), and the tumor's somatic molecular profile (6–9).

The search for inherited factors that affect prognosis has primarily focused on candidate genes that either function within the pharmacologic pathways of the chemotherapeutic agents used in the treatment of colorectal cancer (10, 11) or that influence tumor progression (12). Recently, high-throughput SNP arrays have been used to search for colorectal cancer-susceptibility alleles by genome-wide association studies (GWAS) and, to date, identified 27 genome-wide significant low penetrance loci mapping to 8q24 (13, 14), 18q21 (15, 16), 15q13 (17, 18), 11q23 (16), 10p14 (19), 8q23 (19), 14q22 (20), 16q22 (20), 19q13 (20), 20p12 (20, 21), 1q41 (22), 3q26 (22), 12q13 (22), 20q13 (22), 6p21 (23), 11q13 (23), Xp22 (23), 2q32 (24), 12p13 (21, 25, 26), 5q31 (21), 1q25.3 (24, 25), 10q24 (25), 10q22 (26), 10q25 (26), 11q12

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Translational Relevance

Numerous studies have attempted to identify common inherited variants that affect survival in patients with colorectal cancer. However, none of the proposed prognostic biomarkers have been confirmed, often because the original studies have used small numbers of patients and/or not used independent validation cohorts. We have overcome these limitations and sought robust prognostic biomarkers by analyzing 20 genome-wide significant colorectal cancer-risk alleles in a large training phase cohort (n = 2,083 patients with colorectal cancer), with subsequent validation of positive associations in an independent study group (n = 5,552 patients with colorectal cancer). We found that rs9929218 (intron 2 of *CDH1*, encoding E-cadherin) was robustly associated with survival. Patients homozygous for the minor allele (AA genotype, ~8% of patients) had worse survival, which equated to a median decrease in life expectancy of 4.3 months, and was independent of known prognostic factors. Our findings clearly demonstrate that common germline variants influence life expectancy in patients with colorectal cancer.

(26), 17p13 (26), and 19q13 (26). Studies have suggested that some of these risk alleles may also affect patient survival (27–32); however, none of the survival findings or prognostic biomarkers identified through the candidate gene analyses, have been validated in independent studies (33–35).

Here, we sought robust biomarkers of patient survival by analyzing 20 genome-wide significant colorectal cancer-susceptibility SNPs in a large training phase cohort, with subsequent validation of positive associations in an independent study group.

Materials and Methods

Samples

Training phase. We prepared blood DNA samples from unrelated patients with advanced (stage IV) colorectal cancer from the MRC clinical trial COIN (NCT00182715; ref. 36). All patients had either previous or current histologically confirmed primary adenocarcinomas of the colon or rectum, together with clinical or radiologic evidence of advanced and/or metastatic disease, or had histologically/cytologically confirmed metastatic adenocarcinomas, together with clinical and/or radiologic evidence of a colorectal primary tumor. Patients were randomized 1:1:1 to receive continuous oxaliplatin and fluoropyrimidine chemotherapy (Arm A), continuous chemotherapy plus cetuximab (Arm B), or intermittent chemotherapy (Arm C). All patients gave informed consent for their samples to be used for bowel cancer research (approved by REC; 04/MRE06/60).

Validation phase. The validation phase consisted of samples from several different trials or prospective cohort studies. COINB is a MRC-funded phase II trial assessing cetuximab efficacy in intermittent oxaliplatin-fluoropyrimidine chemotherapy of advanced colorectal cancer (NCT00640081; ref. 37). FOCUS2 is a trial for patients with unpretreated advanced colorectal cancer judged unfit for full-dose combination chemotherapy (NCT00070213). FOCUS3 is a trial determining the feasibility of molecular selection of therapy using *KRAS*, *BRAF*, and topoisomerase-1 in advanced colorectal cancer (NCT00975897). PICCOLO is a trial of the treatment for fluorouracil-resistant advanced colorectal cancer (NCT00389870; patients from COIN or COINB that were subsequently recruited into PICCOLO were excluded). VICTOR is a trial of rofecoxib as postadjuvant therapy for colorectal cancer (NCT00031863). Six prospective cohort studies from the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO; refs. 24, 38) were also included: the Health Professionals Follow-up Study (HPFS), the Nurses' Health Study (NHS), the Physicians' Health Study (PHS), the VITamins And Lifestyle Study (VITAL), the Women's Health Initiative (WHI), and the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO; see Supplementary Information for references). All of these studies used a prospective design, with follow-up for incident cancer diagnoses and survival outcomes. Cases of incident colorectal cancer arising in these studies were identified from self-report and confirmed by their medical records (HPFS, NHS, PHS, PLCO, WHI) and/or linkage to cancer registries (VITAL). Two subsets of cases were genotyped in WHI: WHI1 included colon cancer patients diagnosed before September 2005 and WHI2 included unrelated colorectal cancer patients diagnosed before August 2009. Two subsets of cases were also genotyped in PLCO: PLCO1 included colon cancer patients and PLCO2 included unrelated colorectal cancer cases. All participants provided informed consent for genetic testing, and all studies were approved by their respective Institutional Review Boards. Protocols for assessing survival in the GECCO studies have been described previously (see Supplementary Information for references).

Genotyping

Training phase. Genotyping of 15 colorectal cancer risk alleles (rs6691170 and rs6687758 at 1q41, rs10936599 at 3q26, rs4444235 and rs1957636 at 14q22, rs9929218 at 16q22, rs10411210 at 19q13, rs961253 at 20p12, rs10795668 at 10p14, rs3802842 at 11q23, rs4925386 at 20q13, rs4939827 at 18q21, rs16892766 at 8q23, rs4779584 at 15q13, and rs6983267 at 8q24) was performed by Illumina's Fast-Track Genotyping Service using their high-throughput BeadArray technology. rs4925386 failed genotyping. For the remaining 14 SNPs, the genotyping concordance rate for duplicate samples (n = 110) was 100% (1,540/1,540 genotypes), GenTrain scores ranged from 0.6814 to 0.9500 and the overall genotype success rate was 99.44% (28,868/29,032 genotypes were called successfully). Genotyping of rs4925386 at 20q13, rs4813802 at 20p12, and rs16969681 and rs11632715 at 15q13 was carried out by LGC genomics using their KASPar technology with a genotype success rate of 99.17% (8,253/8,322 genotypes called successfully) and concordance rate for duplicate samples (n = 94) of 100% (376/376). Genotyping of rs11169552 and rs7136702 at 12q13 was carried out by Geneservice using TaqMan assays (Applied Biosystems) with a genotype success rate of 95.66% (3,966/4,146 genotypes called successfully) and concordance rate for duplicate samples (n = 94) of 100% (188/188).

Validation phase. rs16892766, rs9929218, and rs10795668 were genotyped in patients from COINB, FOCUS2, FOCUS3, and PICCOLO by LGC genomics (KASPar technology). In VICTOR, genotyping was carried out on Illumina HumanHap300 arrays and rs9929218 was directly genotyped, rs16892766 was imputed, and rs706771 was genotyped as a proxy for rs10795668 (R^2 = 0.965, D′ = 1). All three SNPs were genotyped in cases from HPFS,

Table 1. Clinical trial and population-based cohorts analyzed in this study

	Training phase	Validation phase												
	COIN	COINB	FOCUS2	FOCUS3	PICCOLO	VICTOR	HPFS	NHS	PHS	PLCO1	PLCO2	VITAL	WHI1	WHI2
Cases with rs9929218 genotype, *n*	2,078[a]	196	337	172	334	918	259	355	278	531	478	281	450	963
GG	1,061	106	170	83	170	485	128	186	134	273	261	141	217	471
GA	853	73	143	75	137	361	109	132	123	217	173	112	190	399
AA	164	17	24	14	27	72	22	37	21	41	44	28	43	93
Total deaths (% of cases)	1,557 (75)	99 (51)	301 (89)	78 (45)	312 (93)	108 (12)	124 (48)	145 (41)	128 (46)	180 (34)	103 (22)	94 (33)	165 (37)	310 (32)
GG	783 (74)	58 (55)	153 (90)	32 (39)	159 (94)	56 (12)	65 (51)	71 (38)	67 (50)	84 (31)	62 (24)	42 (30)	77 (35)	146 (31)
GA	634 (74)	30 (41)	124 (87)	38 (51)	128 (93)	41 (11)	47 (43)	64 (48)	50 (41)	79 (36)	34 (20)	37 (33)	69 (36)	133 (33)
AA	140 (85)	11 (65)	24 (100)	8 (57)	25 (93)	11 (15)	12 (55)	10 (27)	11 (52)	17 (41)	7 (16)	15 (54)	19 (44)	31 (33)
Median follow-up (SD)	2.4 (2.2)	2.0 (4.4)	3.7 (n/a)[b]	1.0 (0.8)	3.0 (3.1)	5.3 (1.4)	5.0 (3.8)	5.4 (4.9)	9.3 (7.4)	6.7 (3.4)	3.4 (3.6)	3.6 (2.3)	5.2 (3.5)	2.9 (3.4)
% Female	34	42	37	37	34	65	0	100	0	43	43	47	100	100
Age at diagnosis, *n* (%)														
<65 years	1,203 (58)	115 (59)	39 (12)	110 (64)	Not collected	Not collected	55 (21)	115 (32)	91 (33)	125 (24)	98 (21)	51 (18)	87 (19)	149 (16)
65–69	422 (20)	35 (18)	54 (16)	32 (19)			32 (13)	75 (25)	42 (15)	145 (27)	115 (24)	59 (20)	87 (19)	205 (21)
70–74	318 (15)	31 (16)	104 (31)	17 (10)			55 (21)	78 (22)	37 (13)	161 (30)	131 (27)	90 (31)	133 (30)	248 (26)
75–79	124 (6)	10 (5)	94 (28)	13 (8)			53 (21)	60 (17)	43 (16)	88 (17)	88 (18)	67 (23)	96 (21)	199 (21)
≥80 years	9 (<1)	5 (3)	46 (14)	0 (0)			62 (24)	27 (8)	65 (23)	12 (2)	46 (10)	14 (5)	47 (10)	162 (17)
Missing	2 (<1)	0 (0)	0 (0)	0 (0)			2 (1)	0 (0)	0 (0)	0 (0)	0 (0)	8 (3)	0 (0)	0 (0)
Mean (SD)	62.0 (9.6)	61.7 (10.4)	72.7 (7.1)	60.9 (10.0)			72.3 (8.7)	68.5 (7.7)	71.3 (9.8)	69 (5.9)	70 (6.6)	70.4 (6.5)	70.9 (7.1)	72.1 (7.2)
Stage (%)														
I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (1)	72 (28)	78 (22)	57 (21)	193 (36)	166 (35)	105 (37)	126 (28)	293 (30)
II–III	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	913 (99)	89 (34)	183 (52)	108 (39)	282 (53)	246 (52)	126 (45)	252 (56)	493 (51)
IV	2,078 (100)	196 (100)	337 (100)	172 (100)	334 (100)	0 (0)	33 (13)	54 (15)	24 (9)	51 (10)	65 (14)	46 (16)	66 (15)	123 (13)
Unknown	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	65 (25)	40 (11)	89 (32)	5 (1)	1 (<1)	4 (1)	6 (1)	54 (6)
Tumor site, *n* (%)														
Colon[c]	1,103 (53)	124 (63)	240 (71)	83 (48)	225 (64)	574 (63)	173 (67)	273 (77)	195 (70)	514 (97)	314 (66)	211 (75)	436 (97)	678 (70)
Rectum[d]	951 (46)	71 (36)	94 (28)	86 (50)	121 (34)	344 (37)	54 (21)	73 (21)	55 (20)	5 (1)	159 (33)	64 (23)	11 (2)	232 (24)
Unknown	24 (1)	1 (1)	3 (1)	3 (2)	7 (2)	0 (0)	32 (12)	9 (3)	28 (10)	12 (2)	5 (1)	6 (2)	3 (1)	53 (6)

NOTE: Data are provided for those samples with an rs9929218 genotype.

[a]Of the 2,083 COIN patients, 5 failed genotyping for rs9929218.

[b]Follow-up never dropped below 50%, so figure represents the median time from patient entry to the cut-off date for analysis.

[c]Colon defined as cecum, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon, and sigmoid colon.

[d]Rectum defined as rectosigmoid junction and rectum.

Table 2. Univariate analyses of overall survival in our training phase cohort

SNP	Locus	Genotyped, *n*	AA	AB	BB	Deaths, *n*	[a]HR (95% CI) AB vs. AA	[a]HR (95% CI) BB vs. AA	χ^2	*P*	Corrected *P*
rs4939827	18q21	2,068	637	1,028	403	1,552	1.00 (0.89–1.12)	1.02 (0.88–1.17)	0.06	0.97	—
rs16892766	8q23	2,079	1,688	378	13	1,557	1.28 (1.13–1.45)	1.26 (0.67–2.35)	15.14	5.2×10^{-4}	1.0×10^{-2}
rs4779584	15q13	2,070	1,245	710	115	1,554	0.97 (0.87–1.08)	0.96 (0.77–1.19)	0.36	0.84	—
rs6983267	8q24	2,065	674	979	412	1,549	1.01 (0.90–1.14)	1.15 (1.00–1.32)	4.41	0.11	—
rs11169552	12q13	2,002	1,086	785	131	1,506	0.91 (0.82–1.01)	0.92 (0.75–1.14)	3.28	0.19	—
rs7136702	12q13	1,964	807	868	289	1,474	1.00 (0.89–1.11)	1.15 (0.98–1.34)	3.63	0.16	—
rs6691170	1q41	2,070	760	1,019	291	1,554	1.01 (0.90–1.12)	0.89 (0.76–1.04)	2.56	0.28	—
rs6687758	1q41	2,066	1,302	666	98	1,551	0.92 (0.83–1.03)	0.97 (0.78–1.22)	2.08	0.35	—
rs10936599	3q26.2	2,070	1,218	739	113	1,554	0.99 (0.89–1.10)	1.09 (0.87–1.36)	0.61	0.74	—
rs4925386	20q13	2,061	973	886	202	1,544	0.92 (0.83–1.02)	0.88 (0.74–1.05)	3.48	0.18	—
rs4444235	14q22	2,066	571	1,008	487	1,552	1.00 (0.89–1.12)	0.92 (0.80–1.05)	1.93	0.38	—
rs9929218	16q22	2,078	1,061	853	164	1,557	1.01 (0.91–1.12)	1.47 (1.23–1.76)	18.79	8.3×10^{-5}	1.7×10^{-3}
rs10411210	19q13	2,070	1,686	360	24	1,554	1.24 (1.09–1.41)	0.94 (0.58–1.52)	10.81	4.5×10^{-3}	0.09
rs961253	20p12	2,069	808	972	289	1,553	1.04 (0.93–1.16)	1.00 (0.85–1.16)	0.65	0.72	—
rs10795668	10p14	1,993	940	868	185	1,491	0.95 (0.86–1.06)	0.70 (0.58–0.85)	12.42	2.0×10^{-3}	4.0×10^{-2}
rs3802842	11q23	2,070	993	870	207	1,554	0.98 (0.88–1.09)	1.13 (0.96–1.34)	2.61	0.27	—
rs1957636	14q22	2,069	656	1,029	384	1,554	0.99 (0.88–1.10)	0.95 (0.82–1.09)	0.59	0.74	—
rs4813802	20p12	2,051	795	958	298	1,543	0.86 (0.77–0.96)	1.01 (0.87–1.18)	9.26	9.8×10^{-3}	0.196
rs16969681	15q13	2,060	1,637	394	29	1,544	1.04 (0.92–1.18)	1.35 (0.92–2.00)	2.61	0.27	—
rs11632715	15q13	2,063	535	1,034	494	1,548	0.86 (0.76–0.97)	0.97 (0.85–1.12)	7.47	2.4×10^{-2}	0.48

NOTE: Analyses used a Cox proportional-hazard model (codominant analyses) with the outcome of overall survival, adjusted for treatment arm and chemotherapy regimen (*P*) and corrected for multiple testing (corrected *P*). rs4939827, rs961253, rs6983267, and rs4444235 have all been previously associated with survival (27–29, 31, 32), but none were validated in our study.

[a]The codominant model tests for the joint effect of AB versus AA and BB versus AA. *n* values give the numbers of patients with their respective genotypes and for whom survival data were available.

NHS, and PHS using the TaqMan Open Array SNP genotyping platform. For the other GECCO studies, genotyping was performed on Illumina 300/240S (PLCO1), 550K (WHI1), 610K (WHI1, PLCO1), and HumanCytoSNP (VITAL, WHI2, PLCO2) arrays; rs9929218 was directly genotyped on these platforms in all studies, and, rs16892766 and rs10795668 were directly genotyped on the platform used in WHI1 and PLCO1, and imputed (using MACH and HapMap2 Release 24) in WHI2, VITAL, and PLCO2. Note – different genotyping platforms were often used because susceptibility SNPs were identified and assayed at different times by different investigators.

Statistical analyses

All SNPs were tested for their genotypes being consistent with the Hardy Weinberg Equilibrium (HWE) using a Pearson χ^2 test. Linkage disequilibrium (LD) was examined using Haploview version 4.2. For survival analyses of the training phase, we used a Cox survival model with overall survival (time from trial randomization to death) as the primary measure. A codominant model was applied, analyses were stratified for treatment arm and type of fluoropyrimidine used, and *P* values were corrected for multiple testing by Bonferroni correction. Significant SNPs were tested for independence to known prognostic factors using a closed-test procedure multiple fractional polynomial model with $P < 0.05$ and the best-fitting genotype model (dominant or recessive) was identified. For survival analyses in the validation phase, overall survival was used for COINB, FOCUS2, FOCUS3, PICCOLO, and VICTOR, and time from diagnosis to death for HPFS, NHS, PHS, VITAL, WHI, and PLCO. A Cox survival model was fitted to the data from each trial or study separately, and an overall pooled result was calculated using a fixed-effects inverse-variance meta-analysis approach. Heterogeneity was assessed using the Q^2 and I^2 statistics. If the pooled validation data generated a significant result, additional analyses were conducted: (i) a further meta-analysis including the training and validation data together, (ii) a sensitivity analysis replacing time from randomization to death (considered left-truncated at randomization to account for the fact that randomization is conditional upon survival from diagnosis) with time from diagnosis to death—for those trials for which this information was available (COIN, COINB, and FOCUS3; $n = 2{,}446$ patients genotyped with survival data), and, (iii) the effect on 12-week response to chemotherapy in COIN Arms A and C (those arms not confounded by treatment with cetuximab; $n = 1{,}369$ patients genotyped with this data). Response was defined as complete response or partial response at 12 weeks and nonresponse was defined as stable disease or progressive disease.

Results

Training phase

We analyzed blood DNA samples from 2,083 unrelated patients with advanced colorectal cancer from the UK national trial COIN (36). In total, 34% of patients were female with a mean age at diagnosis of 62 years (range 18–84 years, Table 1). We assayed 20 independent, genome-wide significant, colorectal cancer-risk alleles (13, 15–17, 19, 20, 22) representing 14 loci; with a single SNP at nine loci, two SNPs at four loci, and three SNPs at one locus (loci with ≥2 SNPs contain multiple independent risk alleles; refs. 20, 22). Fifteen SNPs were genotyped using the Illumina GoldenGate platform (one failed), four (including a repeat of the failed SNP) were successfully genotyped using KASPar technology and two were successfully genotyped using TaqMan assays. All 20 SNPs, apart from rs7136702 ($P = 0.027$), had genotype distributions consistent with the HWE with no imbalances between the treatment arms or according to the somatic mutation status of the colorectal cancers (42.27%,

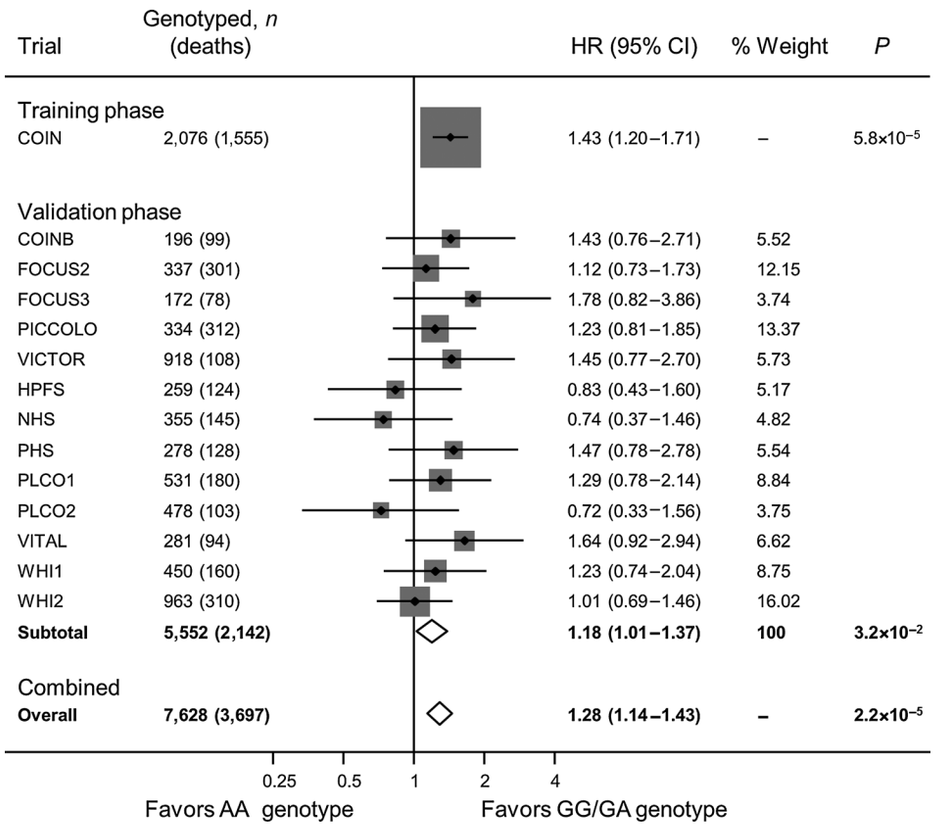

Figure 1.
Forest plot of rs9929218 analyzed for survival in the training phase, validation phase, and all data combined (adjusted for age, sex, and time of diagnosis).

9.01%, and 3.56% of colorectal cancers were *KRAS*, *BRAF*, and *NRAS* mutant, respectively; ref. 39).

Fourteen SNPs did not influence survival under a codominant model (Table 2). Six SNPs were significant in the univariate analyses, of which three (rs16892766 at 8q23, rs9929218 at 16q22, and rs10795668 at 10p14) remained significant after correction for multiple testing (Table 2). We have previously shown that the WHO performance status, number of metastatic sites, white blood cell count, alkaline phosphatase levels, and *KRAS* and *BRAF* mutation status are independent prognostic factors affecting survival in patients from COIN (36). We therefore applied a multivariate model with these factors, together with the best genetic models that fitted the data, and showed that all three SNPs independently influenced survival (Supplementary Table S1).

Validation phase

We used samples from numerous independent trials and cohort studies to provide sufficient power to carry out our validation analyses. In total, we assayed rs16892766, rs9929218, and rs10795668 in 5,552 patients with colorectal cancer (196 from COINB, 337 from FOCUS2, 172 from FOCUS3, 334 from PICCOLO, 918 from VICTOR, 259 from HPFS, 355 from NHS, 278 from PHS, 531 from PLCO1, 478 from PLCO2, 281 from VITAL, 450 from WHI1, and 963 from WHI2; Table 1). No significant heterogeneity was detected in any of the meta-analyses ($I^2 = 0\%$). Only rs9929218 was found to be significantly associated with survival ($P = 2.5 \times 10^{-2}$; Supplementary Table S2).

Further analyses of rs9929218

Patients homozygous for the minor allele of rs9929218 (AA genotype), equating to approximately 8% of patients, showed significantly poorer survival as compared with patients with the AG or GG genotypes (training phase HR = 1.47; 95% CI, 1.24–1.75, $P = 1.4 \times 10^{-5}$ unadjusted, HR, 1.43; 95% CI, 1.20–1.71, $P = 5.8 \times 10^{-5}$ after adjustment for age, sex, and time from diagnosis to randomization; validation phase HR, 1.19; 95% CI,

Table 3. Univariate analysis of rs9929218 on survival according to training phase, validation phase, and combined

Analysis phase	Alleles	Genotyped, *n*	Deaths, *n*	HR (95% CI)	*P*
Training phase	GG/GA	1,913	1,416	1.43 (1.20–1.71)	5.8×10^{-5}
	AA	163	139		
Validation phase	GG/GA	5,069	1,946	1.18 (1.01–1.37)	3.2×10^{-2}
	AA	483	201		
Combined	GG/GA	6,982	3,362	1.28 (1.14–1.43)	2.2×10^{-5}
	AA	646	340		

NOTE: Data are shown for recessive analyses with *P* values adjusted for age, sex, and time of diagnosis. HRs for the validation phase and the combined analysis are pooled effects using fixed-effects inverse-variance meta-analysis.

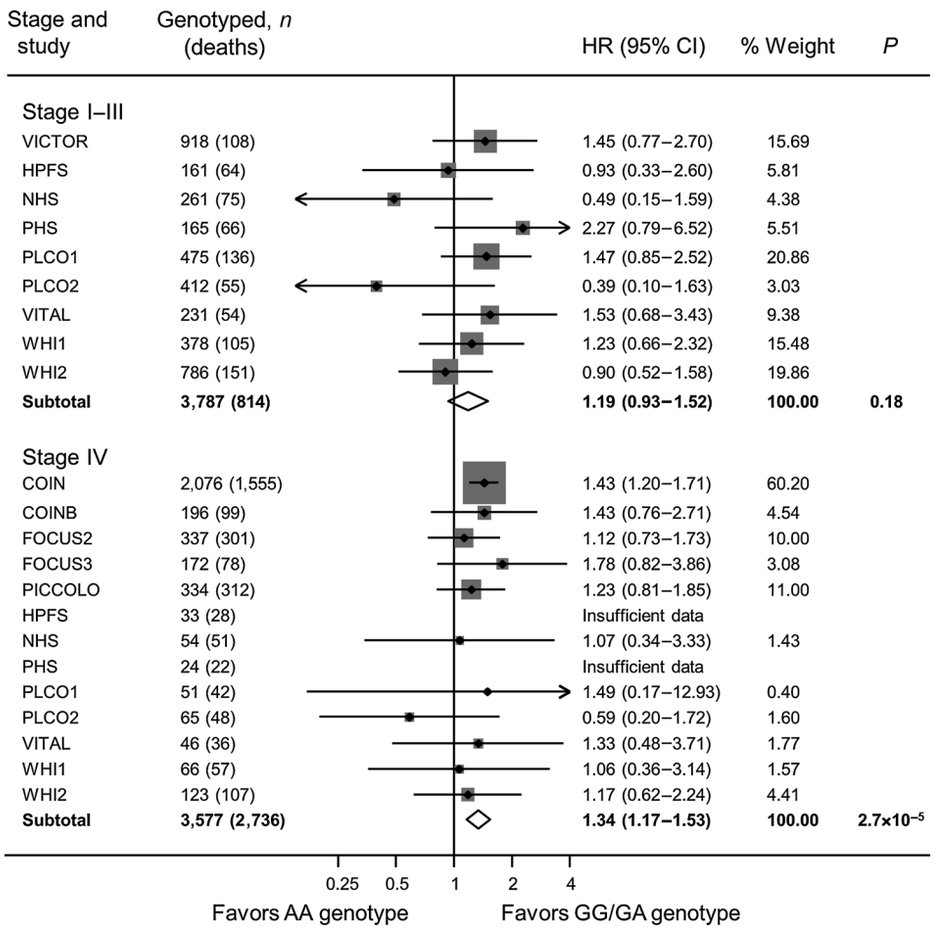

Figure 2.
Forest plot of rs9929218 analyzed for survival and stratified by disease stage (adjusted for age, sex, and time of diagnosis).

1.02–1.38, $P = 2.5 \times 10^{-2}$ unadjusted, HR, 1.18; 95% CI, 1.01–1.37, $P = 3.2 \times 10^{-2}$ adjusted; combined HR, 1.30; 95% CI, 1.16–1.46, $P = 6.1 \times 10^{-6}$ unadjusted, HR, 1.28; 95% CI, 1.14–1.43, $P = 2.2 \times 10^{-5}$ adjusted; Fig. 1 and Table 3). This equated to a median decrease in life expectancy of 4.3 months (based on training phase data). Patients with a single variant allele (AG genotype) had similar survival outcomes to those with a wild-type (GG) genotype (Supplementary Table S3).

We combined the training and validation phase data and analyzed by disease stage. rs9929218 genotype did not deviate from the HWE according to stage (Supplementary Table S4). rs9929218 was not significantly associated with survival amongst patients with stage I–III (premetastatic) disease (HR, 1.19; 95% CI, 0.93–1.52, $P = 0.18$), with little statistical evidence of heterogeneity amongst the individual studies ($P = 0.39$; Fig. 2). In contrast, rs9929218 was highly associated with survival in patients with stage IV (metastatic) colorectal cancer (HR, 1.34; 95% CI, 1.17–1.53, $P = 2.7 \times 10^{-5}$), with no heterogeneity amongst the individual trials and cohorts ($P = 0.91$; Fig. 2). There was, however, no significant difference between the associations of rs9929218 genotype and survival in patients with stage I–III and stage IV disease ($P_{\text{interaction}} = 0.48$).

As a sensitivity analysis, we investigated whether overall survival accurately reflected survival from the time of diagnosis to death. For 2,444 trial patients (from COIN, COINB, and FOCUS3), we had relevant clinical information available and we found little difference in the effect of rs9929218 between the two survival measures (overall survival HR, 1.50; 95% CI, 1.27–1.76, $P = 1.5 \times 10^{-6}$; survival time from diagnosis HR, 1.46; 95% CI, 1.24–1.73, $P = 6.3 \times 10^{-6}$, Supplementary Figure).

We also investigated whether the type and duration of treatment influenced survival, by evaluating rs9929218 according to trial arm in COIN (the largest trial for which we had high-quality clinical data). We did not find significant heterogeneity between the treatment arms ($P = 0.38$) suggesting that treatment did not influence the association between rs9929218 genotype and survival (Supplementary Table S5).

We also sought whether rs9929218 was associated with response to treatment (likely to be correlated with survival). In COIN Arms A and C, treatment was identical for the first 12 weeks apart from the choice of fluoropyrimidine. At 12 weeks, patients from these arms that were homozygous for the rs9929218 minor allele had significantly worse response (36/112 responded, 32%), as compared with patients that were heterozygous or homozygous wild-type (626/1,257 responded, 50%; OR = 0.47; 95% CI, 0.31–0.72, $P = 3.9 \times 10^{-4}$, adjusted for choice of fluoropyrimidine; Table 4).

Discussion

The literature contains many reports of potential common inherited biomarkers of survival for colorectal cancer; however,

Table 4. Prognostic effect of rs9929218 on response to chemotherapy

Outcome	GG/AG *n* (%)	AA *n* (%)	*P*
Response	626 (49.8)	36 (32.1)	$\chi^2 = 12.8$, 1 d.f.
No response	631 (50.2)	76 (67.9)	$P = 3.9 \times 10^{-4}$

NOTE: Patients were from arms A and C of COIN, in which treatment was identical for the first 12 weeks apart from the choice of fluoropyrimidine. *P* value is adjusted for choice of fluoropyrimidine.

most of these have been derived from poorly designed studies, with small numbers of samples and/or no validation of their results. As a consequence, very few of these prognostic biomarkers have been validated by independent groups. To address the critical shortcomings of previous studies, we have carried out an analysis using large independent training and validation phase cohorts as recommended by the REMARK guidelines (40) and produced robust evidence for the first common inherited genetic variant affecting survival in patients with colorectal cancer. As such, this finding represents an important clinical milestone.

Our data suggest that patients homozygous for the minor allele of rs9929218, equating to approximately 8% of patients, have worse survival, with a median decrease in life expectancy of approximately 4 months (in the advanced disease setting). Another study recently reported that the major allele of rs9929218 was associated with improved prognosis (30), providing further support for this variant having a genuine prognostic effect. Although the effect size of rs9929218 identified herein is modest (HR, 1.28; 95% CI, 1.14–1.43), the identification of further prognostic alleles by well-powered GWAS-based approaches may help clinicians model the combined effects of common germline variants together with their somatic mutation profiles to help inform patient outcome. Our study therefore represents a critical first step in this endeavor.

We have shown a clear effect of rs9929218 on survival amongst patients with stage IV disease. However, many of these patients would have received similar therapies raising the possibility that rs9929218 influences survival based upon an interaction with treatment, and we noted that patients carrying both minor alleles had poor response to chemotherapy. However, survival and response are likely to be related and we found similar prognostic effects across all arms of the COIN trial (including in those patients receiving intermittent therapy) and amongst many of the other trials and cohorts used in this study. These data suggest that the prognostic effect may therefore reflect an underlying influence on a biologic process or pathway. rs9929218 lies within intron 2 of *CDH1* encoding E-cadherin, in strong LD with rs16260 (41) in the *CDH1* promoter, which downregulates *CDH1* expression (42). Patients homozygous for the minor allele of rs9929218 would be expected to have reduced E-cadherin expression. E-cadherin functions as a transmembrane glycoprotein that is critical in the establishment and maintenance of intercellular adhesion, cell polarity, and tissue morphology and regeneration (43) and its loss represents a defining feature of the epithelial to mesenchymal transition during metastasis. A clear mechanism therefore exists for the potential prognostic effect of rs9929218 by influencing this process.

Disclosure of Potential Conflicts of Interest

D. Kerr is an employee of and has ownership interest (including patents) in Oxford Cancer Biomarkers, reports receiving commercial research grants for Roche, and is a consultant/advisory board member for Amgen and Bayer. No potential conflicts of interest were disclosed by the other authors.

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Other (chief investigator of the COIN trial from which over 2000 patients contributed samples for this study, was also the lead clinical collaborator with Cheadle and Smith during the time this work was conceived and undertaken): T.S. Maughan
Other (directed study): J.P. Cheadle

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