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Innate immunity gene polymorphisms and the risk of colorectal neoplasia

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Inherited variation in genes that regulate innate immunity and inflammation may contribute to colorectal neoplasia risk. To evaluate this association, we conducted a nested case–control study of 451 colorectal cancer cases, 694 colorectal advanced adenoma cases and 696 controls of European descent within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. A total of 935 tag single-nucleotide polymorphisms (SNPs) in 98 genes were evaluated. Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association with colorectal neoplasia. Sixteen SNPs were associated with colorectal neoplasia risk at $P < 0.01$, but after adjustment for multiple testing, only rs2838732 (*ITGB2*) remained suggestively associated with colorectal neoplasia ($OR_{per\ T\ allele} = 0.68$, 95% CI: 0.57–0.83, $P = 7.7 \times 10^{-5}$, adjusted $P = 0.07$). *ITGB2* codes for the CD18 protein in the integrin beta chain family. The *ITGB2* association was stronger for colorectal cancer ($OR_{per\ T\ allele} = 0.41$, 95% CI: 0.30–0.55, $P = 2.4 \times 10^{-9}$) than for adenoma ($OR_{per\ T\ allele} = 0.84$, 95%CI: 0.69–1.03, $P = 0.08$), but it did not replicate in the validation study. The *ITGB2* rs2838732 association was significantly modified by smoking status (P value for interaction = 0.003). Among never and former smokers, it was inversely associated with colorectal neoplasia ($OR_{per\ T\ allele} = 0.5$, 95% CI: 0.37–0.69 and $OR_{per\ T\ allele} = 0.72$, 95% CI: 0.54–0.95, respectively), but no association was seen among current smokers. Other notable findings were observed for SNPs in *BPI/LBP* and *MYD88*. Although the results need to be replicated, our findings suggest that genetic variation in inflammation-related genes may be related to the risk of colorectal neoplasia.

Introduction

Chronic inflammation is hypothesized to play an important role in the etiology of colorectal cancer and is strongly supported by a number of observations. Patients with inflammatory bowel disease, including ulcerative colitis and Crohn's disease, have a 4- to 20-fold increased risk of developing colorectal cancer (1). A meta-analysis estimated cumulative probabilities of ulcerative colitis patients developing colorectal cancer to be 2% by 10 years, 8% by 20 years and 18% by 30 years (2). The use of non-steroidal anti-inflammatory drugs (NSAIDs) has been consistently associated with a significantly reduced risk of adenoma, a precursor to colorectal cancer (3) and colorectal cancer (4–7) in both randomized trials and observational studies. Further supporting these epidemiologic findings, rats fed diets containing aspirin and then treated with azoxymethane, a carcinogenic neurotoxic chemical compound used to induce colon cancer in animals, had a significantly lower incidence of colon cancer and fewer tumors compared with rats on a control diet (8). Other risk factors for colorectal neoplasia have also been hypothesized to influence colorectal tumorigenesis through inflammatory pathways (1); both smoking, recently classified as having sufficient evidence to cause colorectal cancer by the International Agency for Research on Cancer (IARC) (9,10), and body mass index (BMI) have been observed to increase inflammation *in vitro*, in mouse and in epidemiologic studies (11–13), whereas cruciferous vegetable intake has been shown to (14) decrease inflammation in mice.

Inflammation is a mechanism of the innate immune system, which provides the host's first line of defense against infections in a non-specific manner. Innate immunity has been observed to facilitate the development of colitis-associated colorectal cancer and sporadic colorectal cancer (15,16). For example, ablation of *TLR4* and *MYD88* signaling pathways, both of which are involved in innate immunity, has been observed to reduce tumor growth and invasion based on data from different mouse models (15,17,18). The USA- and European-based case–control studies of genes or selected single-nucleotide polymorphisms (SNPs) involved with inflammatory pathways have observed some significant associations (19–26), although not all have been replicated and most were limited in scope. These studies suggest a role for inflammatory pathways in colorectal cancer and warrant further investigation.

In a nested case–control study within the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial, we comprehensively evaluated the association between 935 SNPs in 98 innate immunity genes and the risk of colorectal neoplasia (colorectal cancer and advanced adenoma combined). Inflammation is believed to play a role in the risk of both adenoma and colorectal cancer, as demonstrated by the inverse association with NSAIDs (3,4). Thus, our primary aim was to estimate the associations of these SNPs with colorectal cancer and advanced adenoma combined, referred to as colorectal neoplasia throughout this article. However, we also evaluated the associations with advanced adenoma and cancer separately as a comparison to see if there were differences by stage of carcinogenesis. As a secondary aim, we evaluated putative gene–environment interactions between the most significant SNPs for colorectal neoplasia and known colorectal cancer risk factors believed to modulate inflammation, such as smoking, BMI and NSAID use.

Abbreviations: BMI, body mass index; BPI, bactericidal permeability-increasing protein; FDR, false discovery rate; GECCO, Genetics and Epidemiology of Colorectal Cancer Consortium; LBP, lipopolysaccharide-binding protein; LD, linkage disequilibrium; NSAID, non-steroidal anti-inflammatory drugs; OR, odds ratio; PLCO, Prostate, Lung, Colorectal and Ovarian; SNP, single-nucleotide polymorphism.

Materials and methods

Study population and setting

PLCO Cancer Screening Trial was conducted with the objective of evaluating the effects of screening and early detection on cancer-related mortality. The study population consists of approximately 155 000 men and women between the ages of 55 and 74, who were randomized to receive screening (~77 000) for prostate, lung, colorectal and ovarian cancer or to receive their usual care. The trial was conducted at 10 USA sites enrolling participants between 1993 and 2001 (27). Participants in the screening arm underwent flexible sigmoidoscopy examinations for colorectal cancer at two time points: at enrollment and at either 3 or 5 years postenrollment (28). Participants with suspicious lesions detected by sigmoidoscopy were referred to their primary physician for further evaluation, which included colonoscopy in most

cases. Their subsequent diagnostic work-up up to 12 months after flexible sigmoidoscopy examination was tracked by trained medical record personnel who recorded any pathologically verified cases of colorectal adenoma and cancer from medical and pathologic records. Colorectal cancer cases were also ascertained through an annual questionnaire sent to participants asking about recent cancer diagnoses and death certificates. All reported colorectal cancer cases were pathologically confirmed with medical records. Information about demographic factors and potential risk factors was collected through a risk factor questionnaire administered at baseline; information about diet was collected through a food frequency questionnaire. All participants provided informed consent. The study was approved by the institutional review boards at all 10 centers and the National Cancer Institute.

Adenoma cases and controls were obtained from the screening arm of the trial as described previously (29); colorectal cancer cases came from both arms of the study. Individuals were eligible for this study if they provided a blood or buccal specimen and consented to participate in etiologic studies of cancer and other diseases. Excluded from the study were individuals with a self-reported history of colorectal polyps, ulcerative colitis, Crohn's disease, familial polyposis, Gardner's syndrome or cancer (except basal cell or squamous cell skin cancer) (29). Included in this study were 513 colorectal cancer cases diagnosed after enrollment, 742 cases with at least one advanced (≥1 cm in size, containing villous/tubulovillous characteristics or having high-grade dysplasia or carcinoma *in situ*) left-sided adenoma from the baseline screen and 747 controls who were participants without a polyp in the left-sided colon or rectum at the baseline screen. Adenoma cases and controls were frequency matched on sex and race. One control was later found to be an adenoma case and was dropped from the analysis (29).

In order to minimize the potential for biased results as a result of population stratification, the analysis was limited to Caucasians, which comprise 92% of the study population, and included 696 controls, 451 colorectal cancer cases and 694 colorectal adenoma cases. Six adenoma cases that later developed colorectal cancer were included in both the cancer and the adenoma analyses but only counted once in the combined analysis of cancer and adenoma. Thus, there were a total of 1139 cases in the overall colorectal neoplasia analysis.

Laboratory methods

DNA was extracted from blood samples using QIAamp DNA Blood Midi or Maxi Kits and from buccal cells using phenol chloroform extraction. The colorectal adenoma and cancer cases and controls were genotyped at the NCI Core Genotyping Facility (Gaithersburg, MD) using an oligo pool assay (OPA) on the Illumina GoldenGate platform. Tag SNPs were selected for candidate genes involved in innate immunity based on the HapMap CEU population using the Carlson method (30) as implemented in Tagzilla. For each gene, tag SNPs were selected including 20 kb upstream and 10 kb downstream of the gene, assuming an $r^2 > 0.8$, minor allele frequency > 5% and a design score ≥0.4.

A total of 1034 SNPs in 98 genes belonging to the following pathways were genotyped (Supplementary Table 1, available at *Carcinogenesis* Online): pattern recognition molecules and antimicrobials; integrins and receptors; complement; response genes and tissue factors. SNPs were excluded from the analysis ($N = 99$) if they failed to meet the following criteria: Hardy–Weinberg proportions among Caucasian controls ($P < 1 \times 10^{-5}$) ($n = 18$), <90% completion rate ($n = 77$), failed validation or displayed poor concordance in HapMap samples ($n = 64$). After exclusions, 935 SNPs remained for analysis. Randomly distributed replicates ($n = 79$) were used to evaluate assay reproducibility and were found to be >95% concordant.

Statistical analysis

The associations of each SNP were evaluated using unconditional multivariate logistic regression models for total colorectal neoplasia (colorectal cancer and adenoma cases combined), and colorectal cancer and adenoma, separately. Adjusting for age and sex, *P* trends based on the log-additive model were estimated for all SNPs using PLINK (version 1.07, Purcell 2007) (31). We explored putative gene–environment interactions for colorectal cancer and advanced adenoma combined in order to maximize the statistical power. We adopted a two-stage approach for testing interactions. In the first stage, the marginal effects of the SNPs and colorectal neoplasia (adenoma and cancer cases combined) were assessed. All SNPs that reached significance threshold $P < 0.01$ in the marginal model were then taken forward for interaction testing

Table I. Characteristic of controls, colorectal neoplasia, cancer and adenoma cases

Characteristic	Category	Controls	*n* = 696	Neoplasm	*n* = 1139[a]	Cancer	*n* = 451	Adenoma	*n* = 694
Sex	Female[b]	216	31.0%	395	34.7%	191	42.4%	206	29.7%
	Male	480	69.0%	744	65.3%	260	57.6%	488	70.3%
	P value[c]				0.108		<0.0001		0.584
Age (years)	59 or less	312	44.8%	280	24.6%	53	11.8%	227	32.7%
	60–64	188	27.0%	317	27.8%	104	23.1%	213	30.7%
	65–69	134	19.3%	282	24.8%	128	28.4%	156	22.5%
	70–74	62	8.9%	260	22.8%	166	36.8%	98	14.1%
	P value[c]				<0.0001		<0.0001		<0.0001
Family history	Yes	60	8.6%	118	12.2%	33	11.7%	86	12.4%
	No	636	91.4%	853	87.8%	250	88.3%	608	87.6%
	Missing			168		168			
	P value[c]				0.021		0.141		0.022
Aspirin/ibuprofen[b]	Neither taken regularly	271	38.9%	467	41.1%	180	40.1%	287	41.4%
	Asprin only	204	29.3%	364	32.0%	139	31.0%	228	32.9%
	Ibuprofen only	86	12.4%	129	11.3%	56	12.5%	75	10.8%
	Both taken regularly	135	19.4%	177	15.6%	74	16.5%	104	15.0%
	P value[c]				0.133		0.656		0.087
BMI (kg/m^2)[b]	>18.5–25	192	27.9%	297	26.3%	126	28.4%	173	25.0%
	>25–30	324	47.0%	522	46.2%	210	47.3%	316	45.7%
	>30	173	25.1%	311	27.5%	108	24.3%	203	29.3%
	P value[c]				0.497		0.953		0.176
Smoking history[b]	Never smoked	286	41.1%	416	36.6%	182	40.4%	236	34.1%
	Former smoker	326	46.8%	539	47.4%	206	45.7%	337	48.6%
	Current smoker	45	6.5%	134	11.8%	46	10.2%	88	12.7%
	Ever smoked a pipe or cigar only	39	5.6%	49	4.3%	17	3.8%	32	4.6%
	P value[c]				0.001		0.078		0.0002
Cruciferous vegetables (frequency per day)	0–0.2	240	35.3%	343	37.4%	93	35.6%	250	37.7%
	>0.2–0.4	188	27.6%	266	29.0%	87	33.3%	183	27.6%
	>0.4	252	37.1%	309	33.7%	81	31.0%	230	34.7%
	Missing	16		221		190		31	
	P value[c]				0.370		0.134		0.588

[a]Six adenoma cases that later developed colorectal cancer were included in each of the cancer and adenoma analyses but only counted once in the combined analysis of neoplasia.
[b]Missing data from 10 or fewer individuals in each group.
[c]Chi-square *P* value compares each case group with the controls.

Table II. Associations[a] between SNPs with *P* trend < 0.01 for the risk of colorectal neoplasia, cancer and adenoma in the PLCO Cancer Screening Trial

Region	Chr	SNP	Genotype	Controls	Neoplasm			Cancer			Adenoma		
					Cases	OR (95% CI)	*P* trend	Cases	OR (95% CI)	*P* trend	Cases	OR (95% CI)	*P* trend
ITGB2	21	rs2838732	CC	457	842	1.00 (reference)	7.7E-05	364	1.00 (reference)	2.4E-09[b,c]	483	1.00 (reference)	8.5E-02
			TC	215	265	0.66 (0.53, 0.83)		74	0.41 (0.30, 0.57)		192	0.82 (0.65, 1.04)	
			TT	21	22	0.54 (0.29, 1.00)		4	0.16 (0.05, 0.50)		18	0.76 (0.40, 1.46)	
HGF	7b	rs5745687	CC	633	970	1.00 (reference)	1.3E-03	380	1.00 (reference)	1.9E-02	596	1.00 (reference)	3.5E-03
			TC	59	161	1.81 (1.31, 2.51)		66	1.80 (1.18, 2.74)		95	1.77 (1.25, 2.50)	
			TT	3	4	0.68 (0.14, 3.18)		1	0.48 (0.04, 5.52)		3	0.91 (0.18, 4.60)	
ICAM1/	19	rs3093032	CC	544	805	1.00 (reference)	1.4E-03	299	1.00 (reference)	1.1E-04[b,d]	510	1.00 (reference)	3.4E-02
ICAM4/			TC	141	306	1.46 (1.15, 1.84)		140	1.91 (1.41, 2.60)		168	1.28 (0.99, 1.66)	
ICAM5			TT	11	26	1.56 (0.75, 3.23)		10	1.55 (0.60, 3.98)		16	1.55 (0.71, 3.40)	
NFKB1	4	rs4648006	CC	599	1037	1.00 (reference)	1.8E-03	415	1.00 (reference)	2.8E-03	627	1.00 (reference)	2.4E-02
			TC	94	98	0.58 (0.43, 0.80)		36	0.53 (0.34, 0.82)		63	0.63 (0.45, 0.88)	
			TT	3	4	1.04 (0.23, 4.78)		0	<0.001 <0.001, >999.999		4	1.46 (0.32, 6.62)	
SERPINB2	18	rs1916661	GG	420	751	1.00 (reference)	1.9E-03	288	1.00 (reference)	3.7E-02	469	1.00 (reference)	3.0E-03
			TG	244	351	0.75 (0.61, 0.93)		149	0.81 (0.61, 1.08)		202	0.72 (0.57, 0.91)	
			TT	32	37	0.59 (0.36, 0.98)		14	0.53 (0.26, 1.07)		23	0.64 (0.36, 1.11)	
BPI/LBP	20	rs1780617	AA	503	880	1.00 (reference)	3.3E-03	345	1.00 (reference)	2.5E-02	539	1.00 (reference)	7.1E-03
			GA	176	237	0.73 (0.58, 0.92)		95	0.71 (0.52, 0.97)		144	0.74 (0.57, 0.95)	
			GG	17	19	0.60 (0.30, 1.20)		9	0.63 (0.25, 1.57)		10	0.54 (0.24, 1.20)	
MYD88	3	rs6796045	AA	606	930	1.00 (reference)	3.4E-03	350	1.00 (reference)	1.0E-04[b,e]	584	1.00 (reference)	8.5E-02
			TA	86	188	1.52 (1.14, 2.01)		84	1.95 (1.35, 2.82)		106	1.34 (0.99, 1.83)	
			TT	4	12	1.69 (0.52, 5.50)		8	3.57 (0.89, 14.39)		4	1.03 (0.25, 4.23)	
BPI/LBP	20	rs5743533	GG	210	322	1.00 (reference)	5.8E-03	130	1.00 (reference)	4.2E-02	194	1.00 (reference)	1.2E-02
			AG	368	538	0.96 (0.77, 1.21)		211	0.93 (0.68, 1.26)		331	0.98 (0.77, 1.26)	
			AA	117	271	1.57 (1.18, 2.09)		108	1.57 (1.07, 2.30)		163	1.55 (1.14, 2.12)	
DEFA3	8	rs4332159	AA	616	984	1.00 (reference)	6.8E-03	387	1.00 (reference)	7.4E-05[b,f]	603	1.00 (reference)	2.6E-01
			GA	77	103	0.86 (0.62, 1.19)		17	0.34 (0.19, 0.62)		86	1.14 (0.82, 1.59)	
			GG	2	50	13.55 (3.27, 56.25)		45	34.38 (8.11, 145.82)		5	2.49 (0.47, 13.08)	
TRAM1	8	rs13271014	AA	573	880	1.00 (reference)	7.1E-03	346	1.00 (reference)	1.2E-02	539	1.00 (reference)	2.9E-02
			GA	119	237	1.34 (1.04, 1.72)		88	1.32 (0.94, 1.85)		150	1.35 (1.03, 1.77)	
			GG	4	17	2.43 (0.80, 7.43)		12	4.42 (1.25, 15.62)		5	1.41 (0.37, 5.35)	
MCP	1	rs4844390	AA	454	683	1.00 (reference)	7.2E-03	261	1.00 (reference)	6.0E-03	423	1.00 (reference)	2.3E-02
			GA	213	383	1.23 (0.99, 1.52)		163	1.45 (1.09, 1.92)		225	1.16 (0.92, 1.47)	
			GG	29	72	1.68 (1.06, 2.65)		26	1.68 (0.90, 3.15)		46	1.72 (1.06, 2.81)	
ITGB2	21	rs440555	GG	262	336	1.00 (reference)	7.7E-03	105	1.00 (reference)	2.8E-05[b,g]	234	1.00 (reference)	4.9E-01
			AG	319	572	1.34 (1.08, 1.67)		218	1.46 (1.07, 2.00)		354	1.25 (0.99, 1.58)	
			AA	115	225	1.41 (1.06, 1.87)		122	2.24 (1.54, 3.27)		106	1.02 (0.74, 1.41)	
KLK1/	19	rs2659056	TT	411	580	1.00 (reference)	7.8E-03	235	1.00 (reference)	1.1E-01	348	1.00 (reference)	1.1E-02
KLK15			CT	235	481	1.44 (1.17, 1.77)		183	1.32 (1.00, 1.74)		301	1.50 (1.20, 1.88)	
			CC	48	77	1.17 (0.79, 1.74)		33	1.17 (0.69, 1.99)		44	1.11 (0.72, 1.72)	
FCGR2A	1	rs12142755	AA	340	470	1.00 (reference)	9.0E-03	180	1.00 (reference)	6.9E-02	295	1.00 (reference)	1.8E-02
			GA	281	520	1.31 (1.06, 1.61)		213	1.31 (0.99, 1.74)		307	1.26 (1.00, 1.58)	
			GG	74	142	1.37 (0.99, 1.89)		51	1.33 (0.85, 2.07)		92	1.41 (0.99, 1.99)	
TRAM1	8	rs2622653	GG	521	785	1.00 (reference)	9.2E-03	315	1.00 (reference)	1.2E-02	474	1.00 (reference)	2.3E-02
			AG	163	331	1.36 (1.08, 1.70)		122	1.35 (0.99, 1.83)		211	1.40 (1.10, 1.78)	
			AA	12	22	1.33 (0.64, 2.77)		13	2.40 (0.98, 5.89)		9	0.90 (0.37, 2.17)	

Table II. *Continued*

Region	Chr	SNP	Genotype	Controls	Neoplasm			Cancer			Adenoma		
					Cases	OR (95% CI)	*P* trend	Cases	OR (95% CI)	*P* trend	Cases	OR (95% CI)	*P* trend
SELP	1	rs3917854	CC	352	506	1.00 (reference)	9.4E-03	198	1.00 (reference)	1.5E-02	310	1.00 (reference)	2.3E-02
			TC	289	517	1.28 (1.04, 1.57)		205	1.41 (1.07, 1.87)		315	1.27 (1.01, 1.58)	
			TT	55	116	1.41 (0.98, 2.01)		48	1.48 (0.92, 2.36)		69	1.38 (0.94, 2.04)	

Chr, chromosome.
[a]All models adjusted for age and sex. *P* values reported in the table are not adjusted for multiple testing.
[b]FDR-adjusted *P* value is significant (<0.05).
[c]FDR-adjusted $P = 2.3 \times 10^{-6}$.
[d]FDR-adjusted $P = 1.7 \times 10^{-2}$.
[e]FDR-adjusted $P = 1.7 \times 10^{-2}$.
[f]FDR-adjusted $P = 1.7 \times 10^{-2}$.
[g]FDR-adjusted $P = 8.8 \times 10^{-3}$.

with select environmental factors in stage 2. For the interaction testing, we selected risk factors that were consistently associated with colorectal cancer in the literature (3,4,32–35), as well as known to modulate inflammation: BMI (continuous), smoking status (modeled as ordinal, never, former, current), pack-years (continuous), NSAID use (never/any regular use of either ibuprofen or aspirin), ibuprofen use (no regular use/regular use), aspirin use (no regular use/regular use) and cruciferous vegetables (servings per day) were evaluated based on *P* values from likelihood ratio tests. Linkage disequilibrium (D′ and r^2) for the genes with multiple SNPs was estimated among controls using Haploview (36). Haplotypes were estimated using an expectation maximization algorithm and associations were evaluated using the generalized linear model and global score test in HaploStats (37). To account for multiple testing, *P*-trend values for the SNPs and *P*-interaction values for the gene–environment interactions were adjusted for the false discovery rate (FDR) using the method by Benjamini and Hochberg (38) with the multtest procedure in SAS 9.1 (Cary, NC). Unless specifically noted, the *P* values presented are unadjusted for multiple testing. Unless otherwise specified, analyses were conducted using SAS.

A validation of SNPs found to be significantly associated with colorectal neoplasia ($P < 0.01$) in this study was carried out using genome-wide association data from seven case–control studies (based on a variety of genotyping platforms) included in the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) (39). A total of 8392 colorectal cancer cases and 10 946 controls of European descent were included in the validation study. In order to exclude the possibility that replication of results could be due to the overlap of cases, data from PLCO was excluded from this lookup of results. Most of the SNPs of interest were not directly genotyped in GECCO but were imputed using MArkov Chain Haplotyping (40) and the HapMap CEU reference population. The imputation quality (r^2) for the SNPs of interest ranged from 0.47 to 1. Associations for each SNP were estimated using logistic regression, adjusting for age, sex and study, assuming a log-additive model.

Results

As expected, neoplasia cases (colorectal cancer and adenoma cases combined) were older than controls in age ($P < 0.0001$), more likely to have a family history of colorectal cancer ($P = 0.021$) and more likely to be smokers ($P = 0.001$) (Table I). Compared with controls, colorectal cancer cases were more often male ($P = 0.0001$) and older ($P < 0.0001$) in age. Adenoma cases were older than controls ($P < 0.0001$), more likely to have a family history of colorectal cancer ($P = 0.022$) and more likely to be current smokers ($P = 0.0002$). There were no significant differences in BMI in any of the case groups compared with controls.

At an alpha level of <0.01, the risk of colorectal neoplasia was associated with 16 SNPs in 13 genes (Table II), colorectal cancer was associated with 23 SNPs in 17 genes (Supplementary Table 3, available at *Carcinogenesis* Online) and colorectal adenoma was associated with six SNPs in five genes (Supplementary Table 4, available at *Carcinogenesis* Online). As we had the greatest power for the colorectal neoplasia analysis, and most of the top hits for colorectal cancer and adenoma were encompassed in the most significant findings in the neoplasia analysis, further analyses were focused on colorectal neoplasia. After adjustment for multiple comparisons, only one SNP, *ITGB2* rs2838732, remained suggestively associated with the risk of colorectal neoplasia at an FDR level of 10% (Supplementary Table 2, available at *Carcinogenesis* Online). *ITGB2* rs2838732 was associated with a decreased risk of colorectal neoplasia [odds ratio $(OR)_{\text{per T allele}} = 0.68$, 95% confidence interval (CI): 0.57–0.83, $P = 7.7 \times 10^{-5}$]. The association appeared stronger for colorectal cancer ($OR_{\text{per T allele}} = 0.41$, 95% CI: 0.30–0.55, $P = 2.4 \times 10^{-9}$) than adenoma ($OR_{\text{per T allele}} = 0.84$, 95% CI: 0.69–1.03, $P = 0.08$).

Interestingly, two SNPs in the *ITGB2* region (rs2838732 and rs440555) were among the 16 most significant SNPs for colorectal neoplasia ($P = 7.7 \times 10^{-5}$ and $P = 0.0077$, respectively). The SNPs were in weak linkage disequilibrium (LD) ($D' = 0.49$, $r^2 = 0.03$). When both SNPs were included in the same model for colorectal neoplasia, both SNPs remained associated with risk ($P = 0.0004$ and $P = 0.01$), suggesting independent effects of each SNP. A haplotype analysis of SNPs sharing an LD block on *ITGB2* revealed several haplotypes associated with colorectal neoplasia risk but not all appeared to be driven by either rs2838732 or rs440555 (Supplementary Table 5,

available at *Carcinogenesis* Online), suggesting a more complex association between *ITGB2* and the risk of colorectal neoplasia.

Two SNPs in the *BPI/LBP* region, rs5743533 and rs1780617, were also among the most significant SNPs for colorectal neoplasia overall. Both were associated with adenoma and cancer ($P < 0.05$) and were in moderately strong LD ($D' = 0.82$) but weakly correlated ($r^2 = 0.09$). When both SNPs were included in the same model for colorectal neoplasia, both SNPs remained associated with risk of neoplasia (rs1780617, $P = 0.046$; rs5743533, $P = 0.046$), suggesting independent effects of each SNP. In a haplotype analysis of an LD block containing both *BPI/LBP* SNPs, there was a statistically significant association with colorectal neoplasia for one haplotype driven by *BPI/LBP* rs5743533 (OR = 1.81, 95% CI = 1.01–3.22, $P = 0.045$) (Supplementary Table 6, available at *Carcinogenesis* Online).

Interactions between diet/lifestyle exposures and the top 16 SNPS associated with the risk of neoplasia were evaluated, focusing on colorectal cancer risk factors known to play a role in inflammation: BMI, smoking, aspirin, ibuprofen, or either NSAID use, and cruciferous vegetable intake (Table III). Of note, smoking status and/or pack-years significantly modified the association of colorectal neoplasia with *ITGB2* rs2838732 ($P = 0.003$ for both). Any NSAID use and regular ibuprofen use had statistically significant interactions with *MYD88* rs6796045 ($P = 0.013$ and 0.012, respectively). However, only the interactions between *ITGB2* rs2838732 and smoking status and pack-years remained statistically significant after adjustment for multiple comparisons (adjusted $P = 0.032$).

Among the SNPs and exposures with statistically significant interactions ($P < 0.05$), stratified ORs (per allele) by level of exposure are presented in Table IV. Of the more striking differences, *ITGB2* rs2838732 was only associated with a reduced risk of colorectal neoplasia among never ($OR_{per\ T\ allele} = 0.5$, 95% CI: 0.37–0.69, $P = <0.0001$) and former smokers ($OR_{per\ T\ allele} = 0.72$, 95% CI: 0.54–0.95, $P = 0.021$) with no association among current smokers (Table IV). Similar effect modification was observed when *ITGB2* rs2838732 was stratified by pack-years of smoking. The association between *NFKB1* rs4648006 was also modified by smoking status with a reduced risk only observed among never smokers ($OR_{per\ T\ allele} = 0.36$, 95% CI: 0.22–0.60, $P = <0.0001$). *MYD88* rs6796045 was found to be associated with a significant increased risk of colorectal cancer among non-users of NSAIDs ($OR_{per\ T\ allele}$, 95% CI: 1.49–3.86, $P = 0.0003$) but not among regular users of aspirin or ibuprofen.

To validate the primary association findings, we looked up the top 16 SNPs with $P < 0.01$ in GECCO (Table V). At a nominal significance level of 0.05, only two SNPs showed a consistent direction of effect and were significantly associated with colorectal cancer in GECCO: *BPI/LBP* rs5743533 ($OR_{per\ A\ allele} = 1.07$, 95% CI: 1.00–1.13, $P = 0.036$) and *BPI/LBP* rs1780617 ($OR_{per\ G\ allele} = 0.89$, 95% CI: 0.80–0.98, $P = 0.023$). A marginal association with a consistent direction of effect was observed with MYD88 rs6796045 ($OR_{per\ T\ allele} = 1.09$, 95% CI: 0.98–1.22).

Discussion

Our most significant finding for risk of colorectal neoplasia overall was with *ITGB2* rs2838732 ($P = 7.7 \times 10^{-5}$). A second SNP in *ITGB2* (rs440555) and several haplotypes in this region were also associated with colorectal neoplasia, suggesting a more complex relationship with risk. *ITGB2* codes for the CD18 protein in the integrin beta chain family, known for participating in cell adhesion and cell surface-mediated signaling. Defects in this gene lead to leukocyte adhesion deficiency type I, in which neutrophil recruitment to sites of infection is impaired, increasing susceptibility to bacterial infections in the skin or mucosal surfaces (41,42). *ITGB2* rs2838732 was found to interact with smoking and colorectal neoplasia risk with the protective effects of the T allele limited to never and former smokers. Interestingly, in an *in vitro* study, neutrophils from smokers and non-smokers were exposed to cigarette smoke exposure, resulting in a 15–20% increase in CD18 expression in both groups (43), supporting a potential biological mechanism for the smoking interaction we observed with *ITGB2* rs2838732. However, no association was observed between these SNPs and colorectal cancer risk in GECCO.

Two SNPs in the *BPI/LBP* region also were associated with risk of colorectal neoplasia. In terms of direction and statistical significance, both of these SNPs replicated in GECCO, supporting their association with colorectal neoplasia. The bactericidal permeability-increasing protein (BPI) and lipopolysaccharide-binding protein (LBP) are involved in the defense against gram-negative bacterial infections. The two proteins bind with high affinity to lipopolysaccharide, which is expressed by gram-negative bacteria. A candidate SNP study of selected inflammatory-related genes, including polymorphisms in *LBP*, reported significant associations between the GA and GG genotypes of *LBP* rs2232596 and increased risk of colorectal cancer among the Chinese ($N = 479$ cases) (44). Based on the HapMap

Table III. *P* values[a,b] for the interactions between inflammation-related risk factors and top innate immunity SNPs associated with colorectal neoplasia

SNP	Gene	BMI (continuous)	Regular NSAID use (yes/no)	Regular ibuprofen use (yes/no)	Regular aspirin use (yes/no)	Smoking status (never/former/current)	Pack-years (continuous)	Cruciferous vegetables (continuous)
rs2838732	ITGB2	0.353	0.776	0.048	0.248	**0.003**[c,d]	**0.003**[c,d]	0.896
rs5745687	HGF	0.551	0.638	0.929	0.616	0.105	0.401	0.302
rs3093032	ICAM1/ICAM4/ICAM5	0.128	0.096	0.309	0.188	0.324	0.499	0.657
rs4648006	NFKB1	1.000	0.162	0.781	0.234	**0.012**	0.233	0.383
rs1916661	SERPINB2	0.856	0.638	**0.037**	0.380	0.726	0.371	0.762
rs1780617	BPI/LBP	0.893	0.164	0.096	0.495	0.212	0.386	0.511
rs6796045	MYD88	0.755	**0.013**	**0.012**	0.091	0.185	0.736	**0.007**
rs5743533	BPI/LBP	0.626	0.560	0.304	0.392	0.353	0.614	0.850
rs4332159	DEFA3	**0.033**	0.385	0.358	0.370	0.850	0.461	0.170
rs13271014	TRAM1	**0.032**	0.896	0.869	0.956	1.000	0.273	0.396
rs4844390	MCP	0.950	0.590	0.805	0.913	0.362	0.277	0.068
rs440555	ITGB2	0.909	0.072	0.830	0.428	0.938	0.754	0.975
rs2659056	KLK1/KLK15	0.288	0.888	0.178	0.929	**0.019**	**0.044**	0.738
rs12142755	FCGR2A	0.561	0.067	0.735	0.168	0.248	0.944	0.909
rs2622653	TRAM1	0.086	0.787	0.632	0.616	0.409	0.551	0.384
rs3917854	SELP	0.850	**0.023**	0.512	0.511	0.964	0.920	0.803

[a]*P* values reported in the table are not adjusted for multiple testing. *P* values <0.05 are in bold.
[b]*P* values are from the likelihood ratio test adjusting for age and sex.
[c]FDR-adjusted *P* value is significant (<0.05).
[d]FDR-adjusted $P = 0.048$.

Table IV. Stratum-specific ORs[a,b] for neoplasia risk by risk factors with statistically significant interactions with SNPs

SNP/gene	Gene	Effect allele	Overall OR (95% CI)	Stratum 1 OR (95% CI)	Stratum 2 OR (95% CI)	Stratum 3 OR (95% CI)	Stratum 4 OR (95% CI)
Regular NSAID use				Neither taken regularly	Either Aspirin or Ibuprofen		
rs6796045	MYD88	T	1.48 (1.14, 1.92)	2.40 (1.49, 3.86)	1.16 (0.85, 1.59)		
rs3917854	SELP	T	1.22 (1.05, 1.43)	0.99 (0.78, 1.26)	1.42 (1.16, 1.73)		
Ibuprofen frequency				Not taken regularly	Taken 1+/week or more		
rs6796045	MYD88	T	1.48 (1.14, 1.92)	1.80 (1.32, 2.44)	0.79 (0.46, 1.35)		
rs1916661	SERPINB2	T	0.76 (0.64, 0.90)	0.70 (0.58, 0.85)	1.18 (0.76, 1.83)		
BMI				0–25	>25–30	>30	
rs4332159	DEFA3	G	1.40 (1.10, 1.80)	1.51 (0.96, 2.40)	1.66 (1.13, 2.44)	0.89 (0.55, 1.43)	
rs13271014	TRAM1	G	1.37 (1.09, 1.73)	0.91 (0.58, 1.40)	1.52 (1.09, 2.13)	1.66 (1.03, 2.69)	
Smoking				Never	Former	Current	
rs2838732	ITGB2	T	0.68 (0.57, 0.83)	0.50 (0.37, 0.69)	0.72 (0.54, 0.95)	1.82 (0.81, 4.11)	
rs4648006	NFKB1	T	0.63 (0.47, 0.84)	0.36 (0.22, 0.60)	0.91 (0.59, 1.40)	0.83 (0.31, 2.26)	
rs2659056	KLK1/ KLK15	C	1.24 (1.06, 1.46)	1.04 (0.81, 1.34)	1.26 (1.00, 1.60)	2.65 (1.37, 5.13)	
Pack-years				No use	0–20	20–40	40+
rs2838732	ITGB2	T	0.68 (0.57, 0.83)	0.50 (0.37, 0.69)	0.61 (0.42, 0.89)	1.13 (0.67, 1.90)	1.02 (0.58, 1.77)
rs2659056	KLK1/ KLK15	C	1.24 (1.06, 1.46)	1.04 (0.81, 1.34)	1.19 (0.86, 1.64)	1.67 (1.09, 2.56)	1.79 (1.12, 2.87)
Cruciferous vegetables				1st quartile	2nd quartile	3rd quartile	4th quartile
rs6796045	MYD88	T	1.48 (1.14, 1.92)	1.77 (0.92, 3.40)	1.29 (0.75, 2.20)	0.96 (0.58, 1.58)	2.26 (1.24, 4.11)

[a]ORs reflect risk of neoplasia per allele.
[b]All models adjusted for age and sex.

Table V. Replication results in seven case–control studies of the GECCO

SNP_Name	Gene	Effect allele	OR	95% CI	*P* trend[a]
rs2838732	ITGB2	T	1.02	(0.92, 1.13)	0.714
rs5745687	HGF	T	0.99	(0.87, 1.12)	0.842
rs3093032	ICAM1/ ICAM4/ ICAM5	T	1.06	(0.95, 1.17)	0.292
rs4648006	NFKB1	T	0.95	(0.84, 1.07)	0.400
rs1916661	SERPINB2	T	0.99	(0.92, 1.07)	0.837
rs1780617	BPI/LBP	G	0.89	(0.80, 0.98)	0.023
rs6796045	MYD88	T	1.09	(0.98, 1.22)	0.107
rs5743533	BPI/LBP	A	1.07	(1.00, 1.13)	0.036
rs4332159	DEFA3	G	0.95	(0.85, 1.07)	0.403
rs13271014	TRAM1	G	0.98	(0.89, 1.08)	0.734
rs4844390	MCP	G	0.99	(0.93, 1.06)	0.846
rs440555	ITGB2	A	0.98	(0.92, 1.03)	0.403
rs2659056	KLK1/ KLK15	C	1.01	(0.93, 1.09)	0.867
rs12142755	FCGR2A	G	0.99	(0.91, 1.07)	0.738
rs2622653	TRAM1	A	1.00	(0.93, 1.08)	0.935
rs3917854	SELP	T	0.96	(0.90, 1.02)	0.206

[a]ORs are the additive genetic model. *P* values reported in this table are not adjusted for multiple testing.

CEU population, *LBP* rs2232596 is in weak LD with rs5743533 (D′ = 0.58, r^2 = 0.23) and the observed association displays a consistent direction of effect with our study. The SNP shares almost no LD with rs1780617 (D′ = 0.05, r^2 = 0); however, the finding does support a role for genetic variation in this region and the risk of colorectal neoplasia.

In our study, we also found carriers of the T allele at *MYD88* rs6796045 to have a significantly increased risk of colorectal neoplasia, which was more pronounced among participants who reported not taking NSAIDs or ibuprofen regularly. Although not statistically significant, an increased risk of colorectal cancer was observed in GECCO for rs6796045 ($OR_{per\ T\ allele}$ = 1.09, 95%CI: 0.98–1.22, *P* = 0.107). *MYD88* codes for a cytosolic adaptor protein that functions as an essential signal transducer in interleukin-1 and Toll-like receptor signaling pathways. Patients with defects in *MYD88* are susceptible to particular bacterial infections (45). Recent findings have shed light on the role of *MYD88* in colorectal cancer development. In a study of APC-mutant mice, susceptible to developing intestinal tumors, researchers found that *MYD88*-deficient mice had lower mortality than *MYD88*-sufficient mice (25% versus 100% at 45 weeks) and the number of polyps and their size were reduced compared with the *MYD88*-sufficient mice (18). They also found a lower expression of genes that promote intestinal tumorigenesis, including *COX-2*, *IL-6* and *TNF*, in *MYD88*-deficient mice compared with *MYD88*-sufficient mice, supporting a role for *MYD88* in spontaneous and carcinogen-induced tumor development and suggesting a possible biological mechanism for the NSAID interaction observed in the current investigation. Consistent with these findings, a Japanese study of 108 colorectal cancer patients found that high (>30% tumors positive for *MYD88*) versus low (30% or less) expression of *MYD88* was independently associated with risk of poor overall survival (OR = 2.3, 95%CI = 1.2–4.3) (46).

Although *NFKB*1 rs4648006 was not significantly associated with colorectal cancer in GECCO (*P* = 0.4), we found some evidence that *NFKB1* rs4648006 may modify the risk of colorectal neoplasia associated with smoking status. *NFKB* signaling is one of the most important pathways for tumor promotion and acts mainly by activating antiapoptotic genes (47). Inappropriate activation of *NFKB* has been associated with a number of inflammatory diseases and cancers, whereas persistent inhibition of *NFKB* leads to inappropriate immune cell development or delayed cell growth (48). In animal models, inactivation of *IKK* genes is important for activating *NFKB* leading to a decreased number of colorectal tumors (49). Cigarette smoke contains tobacco-specific nitrosamine 4-(*N*-Methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK), which has been shown to activate *NFKB* in lung cancer cell lines (50), induce lung cancer in animals and is likely contribute to smoking-related lung cancer (51). In a study of colon cancer cell lines, researchers found increased *NFKB* nuclear translocation and DNA binding activity but decreased expression of IκB-α, an *NFKB* inhibitor, suggesting that NNK can act as a promoter of colon cancer (51).

Our study had several limitations and strengths. Although we tried to maximize our power by combining the adenoma and cancer cases in one analysis, we had limited sample size to assess gene–environment interactions for colorectal neoplasia. Thus, these interaction results should be interpreted as hypothesis generating and need to be replicated. Because we only included Caucasians in our analysis, our results may not be generalizable to other populations. As sigmoidoscopy was used for screening in PLCO, individuals with undetected right-sided adenoma may have been misclassified as controls. However, if the SNPs that we found to be associated with distal adenoma are also associated with proximal adenoma, then our observed associations are likely attenuated. Strengths of this study include the large number of SNPs encompassing many important innate immunity pathways, the inclusion of both colorectal cancers and advanced adenomas to allow examination of different stages of cancer development and detailed information on risk factors. In addition, the use of a standard survey and screening protocol minimized bias across study centers.

In conclusion, we found a number of SNPs in the innate immunity genes, such as *ITGB2*, *MYD88* and *BPI/LBP*, to be associated with colorectal neoplasia. Although only the SNPs in BPI/LBP were replicated in GECCO, several of the findings deserve further study due to the underlying biology. Overall, our findings provide support for the role of inflammation in the risk of colorectal neoplasia.

Supplementary material

Supplementary Tables 1–6 can be found at http://carcin.oxfordjournals.org/

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