The Role of Cytokine Gene Polymorphisms in Colorectal Cancer and Their Interaction with Aspirin Use in the Northeast of Scotland

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Abstract

The reduced risk of colorectal cancer associated with cyclooxygenase enzyme inhibitors, such as aspirin and other nonsteroidal anti-inflammatory drugs, strongly suggests that chronic inflammation is a key mediator in the development of colorectal cancer. This complements recent molecular evidence demonstrating an association between a number of proinflammatory genetic polymorphisms and risk of colorectal cancer. We assessed polymorphisms in the *IL-1, IL-10, TNF-A*, and *TGF-B* genes in a population-based case-control study of colorectal cancer cases (n = 264) and frequency-matched controls (n = 408) in the Northeast of Scotland and analyzed their interaction with regular aspirin use. There was no evidence of a relation between any of the individual polymorphisms, or pairs of polymorphisms, and risk of colorectal cancer. There was a significant interaction between the *IL-10-592* C/A polymorphism and aspirin use ($P_{interaction}$ = 0.03). Carriers of the variant *IL-10-592* (A) allele, who produce less of the anti-inflammatory cytokine interleukin-10, had a statistically significant 50% reduced risk of colorectal cancer when taking regular aspirin (odds ratio, 0.5; 95% confidence interval, 0.25-0.97), whereas risk was not reduced in carriers of the A allele who did not use aspirin, or among aspirin users with the CC genotype. It is possible that carriers of the mutant *IL-10-592* allele are more likely to derive anti-inflammatory and chemopreventive benefits from aspirin in the presence of a lower production of their own endogenous anti-inflammatory interleukin-10. These results suggest that host genetics may play a role in predicting response to chemopreventive strategies. Confirmation of these findings in other populations is required. (Cancer Epidemiol Biomarkers Prev 2005;14(7):1613–8)

Introduction

The Northeast of Scotland has a relatively high incidence of colorectal cancer, a disease that remains one of the leading causes of cancer morbidity and mortality in the Western world (1). As resources are increasingly directed toward disease prevention, strategies for identifying and targeting high-risk individuals are important. Regular use of nonsteroidal anti-inflammatory drugs (NSAID), such as aspirin, has been shown to reduce the incidence of and mortality from colorectal cancer by as much as 50% and has thus become an attractive option for chemoprevention (2, 3). However, with the complications related to long-term NSAID use, it is particularly important to identify those for whom the benefits outweigh the risk.

The beneficial association between NSAID use and decreased risk of colorectal cancer also provided further evidence to suggest a role for chronic inflammation in the pathogenesis of sporadic colorectal cancer. In the case of chronic inflammation in the gastric mucosa, it is known that a proinflammatory cytokine gene profile increases the risk of malignant progression (4). A recent study has shown that common polymorphisms in a number of inflammatory genes are also associated with sporadic colorectal cancer risk (5). This was the first report to suggest that genes encoding interleukin (IL)-6, IL-8, and proliferator-activated receptor-γ are important in relation to inflammation-related risk of sporadic colorectal cancer. It is possible that genetic variants in other genes controlling the inflammatory response will influence risk of colorectal cancer and, moreover, that they will modify the effects of other factors, such as NSAIDs, that act on this response.

IL-1β and tumor necrosis factor α (TNFα) are prototypical proinflammatory cytokines that have a number of functional polymorphisms. The *IL-1B-31* T to C single nucleotide polymorphism (SNP) and the *TNF-A-308* G to A SNP have been shown to be functionally significant with the C allele of *IL-1B-31* and the A allele of *TNF A-308* being associated with increased production of their respective cytokines (6, 7). In *Helicobacter pylori*–induced gastritis, these proinflammatory alleles are associated with an increased risk of gastric cancer (4).

Transforming growth factor-β (TGF-β) is an important immunoregulatory cytokine within the gastrointestinal tract and this is shown in *TGF-B* gene knockout mice, which proceed to develop uncontrolled gastrointestinal inflammation (8). The −800 (G to A) and −509 (C to T) SNPs of the *TGF-B1* gene are in linkage disequilibrium and the −509 T allele has been related to a higher plasma concentration of the cytokine and a higher level of transcriptional activity than the −509 C allele (9). A gene dose effect has been observed for the T allele so that individuals homozygous for −509 T/T were found to have higher plasma concentrations of TGF-β1 than heterozygous C/T and homozygous C/C individuals (9). It could be postulated that having the −509 T allele is protective against colorectal cancer. There are no published reports of this SNP in relation to colorectal cancer.

IL-10 is a Th2 anti-inflammatory cytokine that plays a crucial role in modulating gastrointestinal tract inflammation (10). The IL-10–deficient mouse develops atrophic gastritis, chronic enterocolitis, and ultimately colorectal cancer (11). Promoter SNPs exist at positions −1082 (G/A), −819 (C/T), and −592 (C/A) of the *IL-10* gene on chromosome 1. The −819 and −592 loci are in total linkage disequilibrium and together the three loci form three haplotypes (GCC, ACC, and ATA)

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that have been associated with greater or lower IL-10 production. The −1082 *IL-10* A allele has been associated with the production of reduced levels of the protein in stimulated cells with the ATA haplotype leading to low IL-10 expression and the GCC haplotype high IL-10 expression (12). However, several contradictory studies have been published and the functional consequences of these haplotypes remains uncertain (13-15). Gibson et al. (16) identified additional SNPs in the IL-10 promoter in Europeans and found that distal SNP haplotypes also associate with quantitative IL-10 production. The low ATA *IL-10* haplotype is associated with an increased risk of gastric adenocarcinoma and this may be related to the role of IL-10 as an anti-inflammatory cytokine that down-regulates IL-1β, TNF-α, IFN-γ, and other proinflammatory cytokines (4). It is presently unknown whether the ATA *IL-10* haplotype is also a risk factor for colorectal cancer.

Our aims were thus to evaluate the role of these proinflammatory cytokine gene polymorphisms, namely *IL-1B* (−31 T/C), *TNF-A* (−308 G/A), *TGF-B* (−509 C/T), and *IL-10* (−592 C/A, −1082 G/A) in colorectal cancer and to investigate their interaction with aspirin use in a population with a relatively high incidence of the disease.

Subjects and Methods

Study Population. The subjects in this study came from a previous population-based case-control study investigating polymorphisms in the methylenetetrahydrofolate reductase (*MTHFR*) and xenobiotic metabolizing genes in colorectal cancer in the Northeast of Scotland (17). Eligible cases were Grampian health board residents with incident, histologically confirmed, invasive tumors of the colon or rectum diagnosed between September 1998 and February 2000. Cases were identified from the database of the pathology laboratory that reviews all histology samples taken from Grampian Health Board residents. Population-based controls were selected from the Grampian Community Health Index, a comprehensive list of everyone registered with a general practitioner. They were frequency matched with cases on age at diagnosis and sex. Controls who declined to participate were replaced.

Subjects were approached by mail and asked to provide a mouthwash DNA sample and to complete a semiquantitative food frequency questionnaire (which had previously been validated in the local population; ref. 18). Part two of the questionnaire collected information on risk factors for colorectal cancer, including smoking habits, alcohol intake, physical activity, and family history of inflammatory bowel disease or colorectal cancer. Data was also collected to enable subjects to be stratified into social class based on occupation, according to the Registrar General's social scale (Appendix 1). With regard to NSAID use, subjects were asked whether they were presently taking, or had ever taken, aspirin regularly, which was defined as every day for a month or more. Two hundred and sixty-four of 423 eligible cases (62%) and 408 of 670 eligible controls (61%) either provided a DNA sample or completed questionnaires, or both. All subjects gave informed written consent and the study was approved by the Joint Ethics Committee of Grampian Health Board and Aberdeen University.

SNP Selection. We assessed a total of five SNPs: *IL-1B* (−31 T/C), *TNF-A* (−308 G/A), *TGF-B* (−509 C/T), and *IL-10* (−592 C/A, −1082 G/A). As the −819 *IL-10* SNP is in total linkage disequilibrium with −592 SNP, we omitted it from analysis. The SNPs have all been shown to confer functional changes in their respective genes and have been positively associated with a number of other diseases in previous case-control studies (19). Moreover, all the genes have relevance to inflammation and carcinogenesis, particularly within the gastrointestinal tract.

SNP Genotyping. DNA was extracted from exfoliated buccal cells using a protocol adapted from the Elucigene CF12 kit (Zeneca Diagnostics, Abingdon, United Kingdom). The only modification was the final step, which involved transferring supernatant to a 1.5 mL microfuge tube. SNPs were discriminated by 5′ nuclease PCR assays (TaqMan). Primers (Operon Technologies, Inc., Alameda, CA) and probes (Applied Biosystems, Foster City, CA) for all except *TGF-B* were those designed and used successfully in previous studies (4, 20). Reactions were run in 96-well plates and read in an ABI 7700 sequence detection system (Applied Biosystems). The sequences of the primers and probes used in the TaqMan assays are reported in Appendix 2.

Data Analysis. The Pearson χ^2 test was used to assess, for each polymorphism, whether the frequencies in the control subjects were in Hardy-Weinberg equilibrium. For each individual polymorphism, logistic regression was used to compute genotype odds ratios (OR) and 95% confidence intervals (95% CI) using STATA 7.0 (StataCorp, 2001), adjusting for age at diagnosis (for controls; grouped as <55, 55-64, 64-74, and 75 years and older) and sex. Homozygosity for the common allele was set as the reference category. Analyses were done for all colorectal cancers combined and for colon and rectal tumors separately. To explore possible heterogeneity in effects by age or sex, the analyses of all colorectal tumors were repeated by stratifying by sex and age (<65, ≥65).

Polymorphisms were also combined into pairs of proinflammatory or anti-inflammatory cytokines, e.g., *IL-10-1082* and *IL-10-592* were analyzed together, as were *TNF-A* and *IL-1B*. This was to investigate whether joint effects or a proinflammatory profile increased ORs. For each pair of polymorphisms, subjects were classified as homozygous wild-type (for both polymorphisms), single heterozygous (heterozygous for one of the polymorphisms, but not for both), double heterozygous (heterozygous for both polymorphisms), or homozygous variant (homozygous for both of the polymorphisms).

It is possible that the polymorphisms of interest might be associated with risk factors for colorectal cancer in the population at risk for the disease. Therefore, several risk factors for colorectal cancer were considered as potential confounders of the relationship between each of the polymorphisms and colorectal cancer; these were body mass index, physical activity, social class, alcohol intake, total energy intake, and smoking status. Each was fitted in turn to the age-sex–adjusted logistic model. None made a statistically significant contribution. Therefore, the results are adjusted for age and sex only.

Joint effects of genotype and regular use of aspirin were explored by computing ORs for combinations of genotype and aspirin use. For these analyses, subjects who were heterozygous or homozygous for the variant were analyzed together, and the reference category comprised subjects who were homozygous for the common allele and had never used aspirin regularly. The change in deviance (−2 × log likelihood) between the main effects model and the model that included an interaction term was computed as a test for interaction.

Results

Demographic and other selected characteristics of cases and controls are shown in Table 1. The ratio of males to females was 1.3:1 for cases and 1.06:1 for controls and the majority (71.6%) of cancers arose in the colon. The greatest proportion of tumors was diagnosed in the 65- to 74-year-old age group. The social class distribution of cases and controls was similar with the greatest proportion of cancers arising in the higher social classes (social classes 1-3). The percentage of cases and controls that regularly used aspirin was virtually identical;

Table 1. Characteristics of colorectal cancer patients and control subjects

	Cases, n = 264 (%)*	Controls, n = 408 (%)*
Sex		
Male	56.8	51.5
Female	43.2	48.5
Age at diagnosis (receipt of completed questionnaires for controls)		
<55 y	8.7	24.8
55-64 y	22.7	31.1
65-74 y	34.5	27.2
≥75 y	34.1	16.9
Cancer site		
Colon	71.6	
Rectum	28.4	
Social class†		
1 or 2	36.4	42.6
3 (N/M)	46.3	40.1
4 or 5	17.4	17.3
Regular use of aspirin‡		
No	78.2	78.0
Yes	21.8	22.0
Smoking status at diagnosis (receipt of completed questionnaires for controls)		
Never	45.7	46.4
Ex smoker	38.7	33.2
Current smoker	15.7	20.3
Body mass index§		
<25 kg/m^2	4.5	5.7
25-29 kg/m^2	44.9	37.7
≥30.00 kg/m^2	50.6	56.6
Physical activity‖		
Never	56.5	42.1
<2 times/wk	13.4	18.9
2-3 times/wk	8.0	11.6
>3 times/wk	22.2	27.6
Alcohol intake§		
None	20.4	19.7
≤3.7 g/d	30.8	24.4
3.8-11.5 g/d	23.5	28.6
≥11.6 g/d	25.4	27.4
Total energy intake		
<8,000 kJ/d	27.7	34.6
8,000-9,999 kJ/d	26.9	25.6
10,000-11,999 kJ/d	18.1	19.2
≥12,000 kJ/d	27.3	20.7

*Subjects with missing data excluded on a variable by variable basis.
†For men, own social class; for women who were married or living as married, social class of male husband/partner; for single women, own social class.
‡Ever used every day for ≥1 month.
§Approximately 1 year before diagnosis for cases (and equivalent for controls).
‖Includes working time and nonworking time sessions, of at least 20 minutes, which caused the subject to be out of breath; relates to ~1 year before diagnosis for cases (and equivalent for controls).

among cases, 78% had not used aspirin regularly. There was very little difference between the smoking status of cases and controls and in both groups over 50% subjects were clinically obese with a body mass index of >30. Of cases, 56.5% reported no physical activity as opposed to 42.1% of controls. Alcohol intake and total energy intake figures were similar in both groups and fairly evenly distributed within the categories.

For each polymorphism, the genotype frequencies in controls were found to be in Hardy-Weinberg equilibrium. No statistically significant associations were found among any of the individual cytokine polymorphisms and colorectal cancer risk overall (Table 2). This was also the case when colon and rectal tumors were analyzed separately. The analyses of all colorectal cancers stratified by sex and age group did not reveal any statistically significant associations or striking patterns. Combinations of polymorphisms were analyzed and again ORs were nonsignificant.

We then analyzed the joint effects of genotype and reported regular use of aspirin (Table 3). We found a statistically significant interaction between the *IL-10-592* C/A polymorphism and aspirin use (P = 0.03). Compared to subjects who were homozygous for the common allele and had never used aspirin regularly, those who carried the proinflammatory A allele and who had used aspirin regularly (''ever regular use of aspirin'') had a statistically significant 50% reduction in risk of colorectal cancer (OR, 0.50; 95% CI, 0.25-0.97). In contrast, those who were carriers of the proinflammatory A allele and who had never used aspirin were at increased risk of colorectal cancer (OR, 1.36; 95% CI, 0.92-2.01), although this was not statistically significant. Regular aspirin use was not associated with decreased risk among subjects with the *IL-10-592* C/C genotype. For the other polymorphisms, regular use of aspirin was associated with a lower risk of disease, irrespective of genotype.

Discussion

In this population-based case control study, we have investigated associations between the cytokine polymorphisms of *IL-1B-31*, *TNF-A-308*, *IL-10-1082*, *IL-10-592*, *TGF-B-509* and colorectal cancer. In addition, we have studied their interaction with regular aspirin use and risk of colorectal cancer. Whereas a statistically significant association was not found between any of the SNPs and colorectal cancer alone, we observed a significant interaction between the *IL-10-592* genotype and aspirin use. The effect of aspirin on colorectal cancer risk was limited to carriers of the variant A allele; compared with those who were homozygous for the common C allele and had never used aspirin regularly, carriers of the variant A allele who were regular aspirin users had a statistically significant halving in their risk of colorectal cancer (OR, 0.50; 95% CI, 0.25-0.97). This is the first report of such an interaction between a genetic variant in the important anti-inflammatory cytokine IL-10, aspirin, and colorectal cancer. Interestingly, a recent report by Ulrich et al. (21) showed that the risk of colorectal polyps is reduced by NSAID use in individuals with the proinflammatory cyclooxygenase-2 encoding *PTGS2*-765GG (wild type) genotype and possibly 765GC genotypes. These findings, relating to precancerous stages of colorectal cancer, complement our findings in the cancer cases and suggest that subjects with a proinflammatory genetic makeup may benefit most from chemoprevention with aspirin.

It might be postulated that individuals who are genetically prone to producing reduced levels of the anti-inflammatory IL-10 (i.e., carriers of the variant A allele) are more likely to benefit from the anti-inflammatory properties of aspirin. Ideally, we would have liked to examine all three *IL-10-592* genotypes separately rather than combining heterozygotes and homozygotes, but this was not possible due to sample size. In addition, it would have been more relevant to focus on combinations of polymorphisms with aspirin use but this was precluded by the low numbers. We did have information on use of other types of NSAIDs (including ibuprofen, diclofenac, and mefenamic acid) in our subjects. Current or past use of these on a daily basis for at least 1 month was associated with a decreased risk of colorectal cancer overall (22). There was no evidence that the effect was modified by *IL-10-592* genotype.

The cytokine polymorphisms investigated in this study were selected on the basis of functional data relating to their potential role in inflammation and, with the exception of *TGF-B*, their role in the classic example of inflammation-mediated carcinogenesis that is gastric adenocarcinoma. The first proinflammatory cytokine shown to be relevant in the development of *H. pylori*–associated gastric adenocarcinoma was IL-1β (20). The *IL-1B-31* (T/C) polymorphism also involves a TATA sequence in the *IL-1B* promoter that has been shown to affect DNA-protein interactions *in vitro* (20). Thus, the presence of this proinflammatory polymorphism in colorectal cancer cases and controls was a pertinent question

Table 2. Cytokine genotype frequencies in colorectal cancer cases and controls, with adjusted OR and 95% CI values, by cancer site

	Controls, *n* (%)	Colorectal cancer		Colon cancer		Rectal cancer	
		Cases, *n* (%)	OR* (95% CI)	Cases, *n* (%)	OR* (95% CI)	Cases, *n* (%)	OR* (95% CI)
IL-1B-31							
T/T	165 (40.9)	106 (41.1)	1	83 (44.6)	1	23 (31.9)	1
T/C	179 (44.4)	124 (48.1)	1.04 (0.73-1.47)	85 (45.7)	0.88 (0.61-1.31)	39 (54.2)	1.55 (0.89-2.73)
C/C	59 (14.6)	28 (10.9)	0.78 (0.45-1.32)	18 (9.7)	0.62 (0.33-1.15)	10 (13.9)	1.27 (0.56-2.86)
TNF-A-308							
G/G	224 (57.6)	157 (63.8)	1	115 (65.0)	1	42 (60.9)	1
G/A	145 (37.3)	74 (30.1)	0.74 (0.51-1.05)	51 (28.8)	0.71 (0.47-1.07)	23 (33.3)	0.84 (0.48-1.46)
A/A	20 (5.1)	15 (6.1)	1.11 (0.53-2.29)	11 (6.2)	1.14 (0.51-2.56)	4 (5.8)	1.04 (0.33-3.25)
TGF-B-509							
C/C	212 (52.7)	126 (49.4)	1	91 (49.5)	1	35 (49.3)	1
C/T	157 (39.1)	111 (43.5)	1.10 (0.79-1.56)	78 (42.4)	1.06 (0.73-1.57)	33 (46.5)	1.21 (0.72-2.05)
T/T	33 (8.2)	18 (7.1)	0.84 (0.45-1.59)	15 (8.2)	0.97 (0.49-1.94)	3 (4.2)	0.56 (0.16-1.94)
IL-10-1082							
G/G	115 (28.5)	71 (27.6)	1	50 (26.9)	1	21 (29.6)	1
G/A	202 (50.1)	125 (48.6)	0.95 (0.65-1.40)	93 (50.0)	1.01 (0.65-1.56)	32 (45.1)	0.83 (0.46-1.53)
A/A	86 (21.3)	61 (23.7)	1.14 (0.72-1.81)	43 (23.1)	1.13 (0.67-1.90)	18 (25.4)	1.10 (0.55-2.21)
IL-10-592							
C/C	248 (61.5)	151 (58.5)	1	102 (54.8)	1	49 (68.1)	1
C/A	133 (33.0)	99 (38.4)	1.20 (0.85-1.70)	77 (41.4)	1.36 (0.93-1.99)	22 (30.6)	0.85 (0.49-1.47)
A/A	22 (5.5)	8 (3.1)	0.57 (0.24-1.39)	7 (3.8)	0.73 (0.28-1.86)	1 (1.4)	0.21 (0.03-1.66)

*Adjusted for sex and age group (<55, 55-64, 65-74, and 75 years and older).

and one that we are not aware of other investigators having considered.

The second polymorphism that we investigated was *TNF-A-308* G/A. This locus has been the most investigated in association studies of TNF-α and disease (23). Our finding of no association between colorectal cancer and the −308 promoter polymorphism is consistent with that of Park et al. (24). However, the population in the study of Park et al. was Korean and the *TNF-A-308* A allele seems to be present only in 2% to 5% of Asian populations, compared with 15% to 25% of populations in Europe and North America (25). Thus, it is possible that the Korean study lacked power to detect an association. In addition, there exist at least a further 11 promoter region SNPs, so the possibility of an association between colorectal cancer and an alternative promoter polymorphism cannot be ruled out.

It has been estimated that >80% of colorectal tumors have inhibitory mutations in the TGF-β pathway (26). Based on this and the fact that the T (variant) allele of the −509 (C/T) promoter SNP leads to higher plasma concentrations of the cytokine, we were interested to examine the association between colorectal cancer risk and this SNP. To our knowledge, this is the first study investigating TGF-β promoter polymorphisms and colorectal cancer. Our finding of no significant association may be related to the sample size.

Despite considerable evidence demonstrating the importance of IL-10 as an anti-inflammatory cytokine with a pivotal role in the gastrointestinal tract, there are no published studies investigating polymorphisms in the gene and risk of colorectal cancer. In noncardia gastric carcinoma, the adjusted OR for low *IL-10* homozygotes (ATA) was

Table 3. OR and 95% CI values for interactions between individual genotypes and aspirin use and colorectal cancer

	Never regular use of aspirin		Ever regular use of aspirin	
	No.*	OR (95% CI)	No.*	OR (95% CI)
IL-1B-31				
CC	71/118	1	27/41	0.71 (0.39-1.30)
CT/TT	121/188	0.99 (0.67-1.46)	24/44	0.58 (0.31-1.07)
		$P_{interaction}$ = 0.633		
TNF-A-308				
GG	121/169	1	27/49	0.59 (0.34-1.02)
GA/AA	62/128	0.72 (0.48-1.07)	22/32	0.64 (0.34-1.20)
		$P_{interaction}$ = 0.323		
IL-10-592				
CC	108/192	1	35/50	0.92 (0.55-1.56)
CA/AA	84/114	1.36 (0.92-2.01)	16/35	0.50 (0.25-0.97)
		$P_{interaction}$ = 0.032		
IL-10-1082				
GG	51/90	1	15/21	0.79 (0.36-1.73)
GA/AA	140/215	1.05 (0.69-1.61)	36/65	0.63 (0.36-1.12)
		$P_{interaction}$ = 0.554		
TGF-B-509				
CC	94/165	1	26/42	0.66 (0.37-1.19)
CT/TT	95/140	1.04 (0.71-1.52)	25/43	0.68 (0.38-1.23)
		$P_{interaction}$ = 0.997		

NOTE: OR values were adjusted for age group and sex.
*Numbers of cases/controls.

2.5 (1.1-5.7; ref. 4). We did not look at the −819 SNP but as the three promoter polymorphisms (−1082, −819, −592) are in linkage disequilibrium, we were able to look at joint effects of the −1082 and −592 SNPs. The lack of any statistically significant association could again be explained by our sample size. It is also possible that other functional IL-10 polymorphisms reported in the literature may be relevant (16).

In addition to finding no significant associations between the individual polymorphisms and colorectal cancer, the study did not find any significant association between colorectal cancer and combinations of SNPs. Having multiple proinflammatory or anti-inflammatory cytokine polymorphisms has been shown for noncardia gastric cancer to progressively increase the risk of disease (4). The ORs increased from 2.8 to 26.3, with up to four proinflammatory polymorphisms. This suggests that it is the overall balance between proinflammatory and anti-inflammatory cytokine activity that has more impact on cancer risk than variation at a single locus. Furthermore, the fact that the cytokines have a complex and overlapping range of activities that occur at varying time points in inflammation and tumorigenesis makes it difficult to isolate individual polymorphisms. In this study, we did not have the statistical power to investigate combinations of more polymorphisms. Moreover, a statistical framework for analyzing this complex type of interaction is currently lacking (27). Although our numbers of cases and controls were higher than in the few previous studies investigating *TNF* polymorphisms and colorectal cancer, sample size is probably the main limitation of this study (24). This is particularly true for the subgroup analyses of colon and rectal tumors separately and the analyses of interactions. It is also possible that our null findings with regard to the main effects of *IL-1B-31*, *TNF-A-308*, *IL-10-1082*, *IL-10-592*, *TGF-B-509* were due to limited statistical power. Confirmation of our findings, both positive and null, is needed in large population-based studies in other populations. Studies with sufficient sample size to investigate colon and rectal tumors separately would be particularly valuable.

The response rate in cases (62%) and controls (61%) was virtually identical. Whereas these are lower than in a previous case-control study of colorectal cancer and cytokine polymorphisms, our study was population based (5). It has been noted that participation rates in research seem to be declining (28). In this study, other than refusal, the main reasons for nonparticipation among cases were death (n = 37, 8.7% of those eligible) or General Practitioner refusal of permission to contact subjects (n = 15, 3.5%), which was mainly because subjects were deemed to be too ill. For controls, the General Practitioner refused permission to approach 16 individuals and a further 8 had died (3.6% in total). If genotype was associated with survival, either of cases or controls, bias could result from the nonparticipation of deceased, or very ill, subjects. There seems to be no evidence that the cytokine polymorphisms under investigation here are associated with survival. We were able to compare a limited number of sociodemographic characteristics of participants and nonparticipants. Participating cases were slightly younger, more likely to be male, and less likely to have had a previous cancer than cases who declined to take part. There were no significant differences in sex or age of participating and nonparticipating controls. Among cases, a higher proportion of participants (67%) than nonparticipants (56%) were resident in the least-deprived areas of Grampian. A similar but less pronounced pattern was evident among controls. This would be consistent with a modest social class bias in participation. Subjects approached to take part in the study were not aware of the study hypotheses, either with regard to use of aspirin or genotype. As regards aspirin, 22% of controls reported current or past daily use for a period of at least 1 month. Although aspirin is the most frequently dispensed drug in Scotland, data are lacking on the prevalence of regular use in the population (29). Fourteen percent of 10,000 individuals registered with a General Practitioner in the United Kingdom, ages 40 to 79 years in 1994 to 1997, and free from cancer or colorectal adenoma either had a current prescription for aspirin or had had one in the past (30). This did not include use of aspirin obtained over the counter. It is possible, therefore, but not clear, whether participating controls were biased with regard to aspirin use. As regards genotype, the prevalence among controls of the homozygous variant genotype for each SNP was similar to that reported in other populations of European origin and the genotype frequencies conformed with Hardy-Weinberg equilibrium (4, 31). It seems highly unlikely, therefore, that participation would have been biased by genotype.

In conclusion, while this study did not reveal significant associations between the cytokine polymorphisms of *IL-1B-31*, *TNF-A-308*, *IL-10-1082*, *IL-10-592*, *TGF-B-509* and colorectal cancer, a statistically significant reduction in colorectal cancer risk was shown for carriers of the *IL-10-592* mutant (A) allele who had taken aspirin on a regular basis. These individuals may fall into the category of low IL-10 producers and it is possible that they may, therefore, derive more benefit from the exogenous anti-inflammatory aspirin. Larger studies in other populations are required to investigate this further. Our findings suggest that host genetic factors may play a role in predicting response to chemopreventive agents. In the design of future chemoprevention trials for colorectal and other cancers, the importance of pharmacogenomics should not be underestimated.

Appendix 1. Social class based on occupation

1	Professional occupations
2	Managerial and technical occupations
3N	Skilled nonmanual occupations
3M	Skilled manual occupations
4	Partly skilled occupations
5	Unskilled occupations

Appendix 2. Probes and primers used for SNP genotyping

IL-1B-31 T>C
VIC-CCTCGCTGTTTTTATAGCTTTCAAAAGCAGA-TAMRA-3′
FAM-TCGCTGTTTTTATGGCTTTCAAAAGCAG-TAMRA-3′
Forward primer: 5′-CCCTTTCCTTTAACTTGATTGTGA-3′
Reverse primer: 5′-GGTTTGGTATCTGCCAGTTTCTC-3′
TNF-A-308 G>A
VIC-TGAGGGGCATGAGGACGGG-TAMRA-3′
FAM-AGGGGCATGGGGACGGG-TAMRA-3′
Forward primer: 5′-CCCCAAAAGAAATGGAGGC-3′
Reverse primer: 5′-TCTTCTGGGCCACTGACTGAT-3′
IL-10-592 C>A
VIC-ACCCCGCCTGTACTGTAGGAAGC-TAMRA-3′
FAM-ACCCCGCCTGTCCTGTAGGAAGC-TAMRA-3′
Forward primer: 5′-GGTAAAGGAGCCTGGAACACATC-3′
Reverse primer: 5′-CCAAGCAGCCCTTCCATTT-3′
IL-10-1082 G>A
VIC-AAGGCTTCTTTGGGAAGGGGAAGTAGG-TAMRA-3′
FAM-AAGGCTTCTTTGGGAGGGGGAAGTAG-TAMRA-3′
Forward primer: 5′-CACACACACACAAATCCAAGACAA-3′
Reverse primer: 5′-GCTGGATAGGAGGTCCCTTACTTT-3′
TGF-B-509 C>T
VIC-CCTTCCATCCTTCAGGTGTCCTGTTG-TAMRA-3′
FAM-CCTTCCATCCCTCAGGTGTCCTGTT-TAMRA-3′
Forward primer: 5′-TGCTCAGTAAAGGAGAGCAATTCTTAC-3′
Reverse primer: 5′-GGTAGGAGAAGGAGGGTCTGTCA-3′

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