

Inflammatory Cytokine Gene Polymorphisms, Nonsteroidal Anti-Inflammatory Drug Use, and Risk of Adenoma Polyp Recurrence in the Polyp Prevention Trial

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Abstract

Background: Pro- and anti-inflammatory cytokine genes may be important in the maintenance and progression of colorectal cancer. It is possible that single-nucleotide polymorphisms in inflammatory genes may play a role in chronic colonic inflammation and development of colorectal adenomas. Furthermore, common variants in cytokine genes may modify the anti-inflammatory effect of nonsteroidal anti-inflammatory drugs (NSAIDs) in the prevention of colorectal cancer.

Methods: We examined the association between cytokine gene polymorphisms and risk of recurrent adenomas among 1,723 participants in the Polyp Prevention Trial. We used logistic regression to calculate odds ratios (OR) for the association between genotype, NSAID use, and risk of adenoma recurrence.

Results: Cytokine gene polymorphisms were not statistically significantly associated with risk of adenoma recurrence in

our study. We observed statistically significant interactions between NSAID use, *IL-10* -1082 G>A genotype, and risk of adenoma recurrence ($P = 0.01$) and multiple adenoma recurrence ($P = 0.01$). Carriers of the *IL-10* -1082 G>A variant allele who were non-NSAID users had a statistically significant decreased risk of multiple adenoma recurrence (OR, 0.43; 95% confidence interval, 0.24-0.77) as well as a nonsignificant 30% decreased risk of any adenoma recurrence. In contrast, NSAID users who were carriers of the *IL-10* -1082 G>A variant allele were at an increased risk of any adenoma recurrence (OR, 1.55; 95% confidence interval, 1.00-2.43).

Conclusion: These findings suggest that individuals who are carriers of the *IL-10* -1082 G>A variant allele may not benefit from the chemoprotective effect of NSAIDs on adenoma polyp recurrence. (Cancer Epidemiol Biomarkers Prev 2006;15(3):494-501)

Introduction

The colorectal adenoma is considered the main precursor lesion of colorectal cancer, and its removal at colonoscopy is thought to reduce colorectal mortality (1). However, the majority of Americans go unscreened and nearly 57% of colorectal cancers are diagnosed with either regional or distant disease (2). Furthermore, it is estimated that 30% to 40% of adults of ages ≥ 60 years have prevalent colorectal adenomatous polyps and individuals with a history of adenoma are at increased risk of colorectal cancer, even with routine colonoscopic exams (3, 4). Identifying modifiable risk factors that affect the development and recurrence of these precancerous lesions is vital for colorectal cancer prevention strategies.

Chronic inflammation is a risk factor for many cancers, including colon cancer, and data from experimental and observational studies suggest that inflammation acts early in

the carcinogenic pathway of colorectal cancer, possibly promoting the progression of colorectal adenomas to adenocarcinoma (5-15). The inflammatory response to cellular stresses, injury and infection, results from increased mucosal production of proinflammatory cytokines (16, 17). Proinflammatory cytokines, such as tumor necrosis factor α and the interleukins (IL-1 β , IL-6, and IL-8), play a key role in angiogenesis, inhibition of apoptosis, and cell proliferation (17-20). These cytokines induce expression of cyclooxygenase 2 (COX-2), one of the key enzymes in the production of prostaglandins (21). COX-2 mRNA and protein are present in both colorectal adenomas and adenocarcinomas, and thus support a role of inflammation early in the carcinogenic pathway of colorectal cancer (5-15).

Further support for an inflammatory role in colon cancer progression comes from recent results of randomized clinical trials investigating the use of aspirin in the prevention of adenoma polyp recurrence (22-24). Similar to the observed findings from 24 case-control studies of nonsteroidal anti-inflammatory drugs (NSAID) and colon cancer, these studies observed a significant decrease in risk for adenoma recurrence among individuals who took aspirin compared with those who took only placebo (11, 22, 23, 25-27). However, the reported reduction in risk of colorectal adenoma and cancer by NSAID use never exceeds 50% (27, 28), suggesting that nonresponders to NSAIDs may attenuate the effect of NSAIDs in the prevention of colorectal cancer. Thus, it is possible that factors

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that differ among individuals, such as dietary or lifestyle characteristics, as well as individual genetic variations in inflammatory genes may modify response to inflammation or to the chemopreventive effect of NSAIDs. Differences in individual lifestyle characteristics and genetic variations may, in turn, modify the association between NSAID use and risk of colorectal cancer.

Single-nucleotide polymorphisms (SNP) in the cytokine genes have been associated with changes in gene expression and may mediate differential expression of cytokine alleles by influencing the binding affinity of transcription factors and the exacerbation of tissue damage or altered cell growth (16, 29). Data show that SNPs in the proinflammatory *IL-1 β* , *IL-6*, and *IL-8* genes and in the anti-inflammatory *IL-10* gene result in changes in biological functions of the inflammation pathway and have been associated with a number of inflammatory diseases, including inflammatory bowel disease, arthritis, and Alzheimer's disease (29-33), as well as a number of cancers including colon, stomach, breast, and liver cancer and melanoma of the skin (34-40).

Recent association studies investigating inflammatory gene polymorphisms and risk of colorectal cancer and adenomas have been mixed (34, 35). Landi et al. (34) reported a significant increased risk of colon cancer among carriers of the *IL-6* -174 C allele and a significant decreased risk of colorectal cancer among individuals with the variant PPAR γ Pro12Ala genotype and among carriers of the *IL-8* -251 A allele (34). Recent data from a case-control study of colon cancer in Scotland did not observe an association between polymorphisms in the *IL-1*, *IL-10*, *TNF- α* , and *TGF- β* genes and colon cancer risk, but they did report a statistically significant interaction between the *IL-10* -592 C/A polymorphism, aspirin use, and risk of colon cancer (35). Finally, a recent report investigating the association between the *COX-2* 765 G>C promoter variant, which is also involved in the inflammation-mediated carcinogenic pathway of colon cancer, and risk of colorectal adenomas observed a significant interaction between *COX-2* 765 G>C genotype, NSAID use, and risk of colorectal adenomas (41).

To our knowledge, no one has reported on the association between variants in cytokine genes and risk of colorectal adenoma recurrence, as well as the possible modification of the association between cytokine gene SNPs and susceptibility to adenoma recurrence by NSAIDs. We therefore investigated the association between proinflammatory cytokine SNPs in the interleukin genes *IL-1 β* (-511 C>T), *IL-6* (-174 G>C), and *IL-8* (-251 T>A) and two anti-inflammatory cytokine SNPs in *IL-10* (-819 C>T and -1082 G>A) and risk of adenoma recurrence in the Polyp Prevention Trial. In addition, we investigated interactions between the inflammatory cytokine polymorphisms and use of NSAIDs.

Subjects and Methods

Study Population. Participants in this study were from the Polyp Prevention Trial, a multicenter randomized clinical trial to evaluate the effects of a high-fiber, high fruit and vegetable, low-fat diet on the recurrence of colorectal polyps. Men and women, ages ≥ 35 years and with at least one histologically confirmed adenoma removed in the prior 6 months, were randomized to the dietary intervention group or control group for 4 years. Eligible participants had no history of colorectal cancer, surgical resection of adenomas, or inflammatory bowel disease; weighed no more than 150% of the recommended level; were not taking lipid-lowering drugs; and had no medical conditions or dietary restrictions that would limit their compliance with the protocol. A total of 2,079 participants were enrolled in the trial, with 1,037 randomized to the intervention diet and 1,042 assigned to

their usual diet. The study was completed by 1,905 participants (91.6%), 958 in the intervention group and 947 in the control group.

All participants received a colonoscopy 1 year (T1) and 4 years (T4) after randomization. The 1-year colonoscopy served to detect and remove any lesions missed at the baseline colonoscopy (T0). The participants were then followed for ~ 4 years after randomization, at which time the subjects returned to their usual endoscopist for colonoscopy. A detailed description of the study design, dietary intervention, study population, and end-point assessment is reported elsewhere (42-44).

For the purposes of this analysis, the outcome of "any recurrence" was defined as those Polyp Prevention Trial participants who had any recurrence by any endoscopic procedure during the 3 years following the 1-year colonoscopy. We examined a subset of cases with "multiple adenoma recurrence" who were individuals with >1 adenoma identified at defined intestinal sites during their follow-up endoscopic procedure ($n = 381$). We did not have enough power to investigate the association between genotype and risk of advanced adenoma recurrence ($n = 125$), defined by any adenoma >1 cm, had evidence of high-grade dysplasia, or $>25\%$ villous elements. Controls are those participants who did not have a polyp recurrence at the end of the 4 years of follow-up.

Among the 1,905 participants who completed the Polyp Prevention Trial, 1,723 (90.4%) of the participants, 673 (89.3%) cases and 1,050 (91.2%) controls, had available DNA for genotyping. The analysis is limited to those participants identifying themselves as African American or Caucasian, as those participants endorsing "other" race were excluded due to small numbers ($n = 43$). The study was approved by the institutional review boards of the National Cancer Institute and those of the collaborating centers. All subjects provided written informed consent.

Data Collection and Variable Coding. Demographic characteristics, dietary intake, medical history, and health-related behavior information, including NSAID use, were collected in-person by a trained interviewer at the baseline visit and at each of four annual visits. The questionnaires collected information such as age, sex, education, race, income, and first-degree family history of colon cancer. In addition, data were collected on lifestyle factors such as physical activity, tobacco use, medication use, and medical history. Also at these visits, participants completed a Four Day Food Record and Food Frequency Questionnaire, as well as the Block Health Habits and History Questionnaire (45, 46), which was modified to account for the intake of high-fiber, low-fat foods.

Participants were also questioned about their use of various medications, including the use of NSAIDs. Information on prescription and nonprescription NSAID use was ascertained at each visit by asking participants if they were currently taking any medication, including NSAIDs, on a regular basis (defined as once per month or more frequently). In addition, participants were asked to bring any prescription or nonprescription medication with them to each visit for the interviewers to verify the medication name and dose. For the purposes of this analysis, regular NSAID use includes both aspirin and nonaspirin NSAIDs.

For continuous covariates, median cut points were determined on the basis of distributions among the entire cohort. These covariates included total energy intake, percent of calories from fat, total fiber intake, servings of fruits and vegetables per day, and physical activity. Type of recurrence, any adenoma and multiple adenomas, was determined from hospital pathology reports and confirmed by the study pathologists.

Additional covariates evaluated for confounding included regular vitamin/mineral supplement use (>1 week over the last year, <1 week over the last year); cigarette smoking (never, former, or current); education level (<high school graduate, >high school graduate); first-degree relative with colon cancer (yes, no); age (continuous); multiple polyps diagnosed at baseline, body mass index, and physical activity. Body mass index was computed based on measured weight and height at the baseline interview and categorized as normal (<24.9), overweight (25.0-29.9), and obese (>30.0); (ref. 47). Physical activity was measured by asking participants how much time during the past year they typically spent on weekends and on weekdays in moderate or vigorous activity for combined occupational, nonoccupational, and nonwork/weekend activities. Data are expressed in terms of average hours per week spent in either moderate or vigorous activities of all types.

SNP Selection

We identified both proinflammatory and anti-inflammatory genes that have a role in inflammation and choose variants in these genes that were either reported to result in a functional change of the SNP or associated with inflammatory disease or cancer. We limited our selection to SNPs with a reported frequency of >10% to adequately examine the main effect of the SNP given our sample size. We assessed three SNPs in three different proinflammatory genes, *IL-1B* -511 C>T (rs16944), *IL-6* -174 G>C (rs1800795), and *IL-8* -251 T>A (rs4073), and two SNPs in the anti-inflammatory gene *IL-10*, -819 C>T (rs1800871) and -1082 G>A (rs1800896).

SNP Genotyping. Genotyping was done by BioServe Biotechnologies, Ltd. (Laurel, MD) via a two-step PCR process and mass spectrometry (Masscode, Qiagen Genomics, Bothel, WA) as described by Kokoris et al. (48). A 2- μ L PCR master mix containing 1.73 μ L of water, 0.2 μ L of 10 \times buffer (Qiagen), 0.04 μ L of 10 mmol/L deoxynucleotide triphosphates (Roche Applied Science, Indianapolis, IN), 0.01 μ L of 100% formamide, and 0.02 μ L of 5 units/ μ L HotStarTaq (Qiagen) was added to 3.5 ng of genomic DNA and external primers. Touchdown PCR protocol was used, with an additional 20 cycles with annealing at 50°C instead of 25 cycles. The second PCR used two allele-specific primers, differing at their 5' ends by a tag-specific sequence and at their 3' ends with the complementary base of the two possible alleles, and two universally tagged primers with a photolytically cleavable mass spectrometry tags with 5' end sequences identical to the allele-specific primers. A primer mix of 0.25 μ L (0.01 μ L of a 50 μ mol/L stock of each allele specific primer, and 0.23 μ L of a 44 \times stock of universally tagged primer mix) and 7.75 μ L of master mix [6.478 μ L of water, 1.0 μ L of 10 \times mix (300 mmol/L Tris-HCl pH 8.7, 17 mmol/L MgCl₂, 360 mmol/L KCl, 40 mmol/L (NH₄)₂SO₄), 0.16 μ L of 10 mmol/L each deoxynucleotide triphosphates (Roche), 0.04 μ L of 100% formamide, and 0.072 μ L of 5 units/ μ L HotFireTaq (Solis Biodyne, Tartu, Estonia)] were added and a touchdown PCR identical to the locus-specific PCR was done. The allele-specific PCR products were pooled from 384-well plates and purified using a 96-well QIAquick (Qiagen) plate according to the instructions of the manufacturer.

An auto-injector HTS PAL (CTC Analytics, Zwingen, Switzerland) was used to load the samples into the mass spectrometer. Before the samples reached the mass spectrometer, they were passed through a photolysis unit containing a mercury lamp emitting UV light at 254 nm. An Agilent 1100 series LC/MSD with an APCI ionization chamber was used to

analyze the samples. The single quadrupole filters and channels the ions according to their mass/charge ratios. Datagen software was used to make automatic or manual calls of the SNP alleles.

The quality control used for this high-throughput genotyping consists of repeated assays on ~10% of randomly selected samples from each experiment as well as the inclusion of blinded controls. The genotyping results of the DNA as a "sample" and as a "quality control duplicated sample" were compared. The quality control concordance rate between duplicate samples for this analysis was $\geq 98\%$.

Data Analysis. We estimated allele frequencies (number of alleles / number of chromosomes) and genotype frequencies (number of participants with genotype / total number of participants) among individuals without an adenoma recurrence and those with any adenoma recurrence or with multiple adenoma recurrence. Each SNP was tested in the entire cohort to ensure that observed genotype frequencies exhibited Hardy-Weinberg equilibrium.

Unconditional logistic regression was used to determine odds ratios (OR) and 95% confidence intervals (95% CI) for the association between genotype and risk of any adenoma recurrence after 4 years in the trial, as well as risk of multiple adenoma recurrence, using the PROC LOGISTIC function of the software package SAS (version 8.1, SAS Institute, Cary, NC), adjusting for age, race, sex, and body mass index. For the association of polymorphisms, homozygosity for the most frequent allele was set as the reference category, and ORs were calculated comparing the heterozygote to the reference category (homozygote for the common allele) and the homozygote for the rarer allele to the reference category using dummy variables. For the stratified analyses by regular NSAID use, we combined the heterozygote and the homozygote for the rare allele into a dominant model to increase statistical power. A two-sided significance level of 5% was used for these analyses.

As previously mentioned, we were missing available DNA on 168 participants and thus were not able to include them in our genotyping analysis. χ^2 statistics was used to determine whether significant differences in categorical variables were present between participants with genotype data and those without genotype data (data not shown). In our study, individuals with a family history of colon cancer were more likely to have genotype data compared with those without a family history ($P = 0.03$) and individuals who were current smokers were less likely to have genotype data ($P = 0.02$). However, these differences in distribution of covariates were nondifferential by disease status.

Several potential confounders were identified from a review of the literature and from previous publications using this data and were retained in models based on a $\geq 10\%$ change in the β coefficients for genotype (homozygous wild-type set as the reference genotype) between the crude and the adjusted models. The multivariate adjusted models for genotype and risk of all outcome categories of polyp recurrence were adjusted for age, race, sex, and body mass index.

To assess heterogeneity in main effect of the genotype, we conducted stratified analyses by sex and by NSAID use. For our stratification by NSAID use, we defined regular NSAID users as individuals who reported current, regular NSAID use at three or more study year visits compared with individuals who reported no use over the entire study period (at baseline and at all four study year visits). We evaluated departures from expectations for multiplicative joint effects using the log-likelihood ratio test comparing the change in deviance ($-2 \log$ likelihood) between the model that included the interaction term to the model with only the main effects ($\alpha = 0.05$, likelihood ratio test).

Table 1. Demographic and other characteristics of participants in the Polyp Prevention Trial with genotype data by adenoma recurrence

Characteristics	All subjects (n = 1,723)	No recurrence (n = 1,050)	Any adenoma recurrence (n = 673)	Multiple adenoma recurrence (n = 286)
Age (y)	61.0 ± 10.0	59.8 ± 10.2	62.9 ± 9.3*	64.8 ± 9.3*
Body mass index (kg/m ²)	27.6 ± 3.9	27.5 ± 3.9	27.7 ± 3.9	27.9 ± 3.8 [†]
Sex (%)				
Male	1,103 (64.2)	624 (59.6)	479 (71.4)*	215 (75.4)*
Female	615 (35.8)	423 (40.4)	192 (28.6)	70 (24.6)
Race (%)				
White	1,540 (89.6)	938 (89.6)	602 (89.7)	264 (92.6)
African American	135 (7.9)	84 (8.0)	51 (7.6)	16 (5.6)
Other	43 (2.5)	25 (2.4)	18 (2.7)	5 (1.8)
Education (%)				
<High school	425 (24.7)	252 (24.1)	173 (25.8)	79 (27.7)
>High school	1,293 (75.3)	795 (75.9)	498 (74.2)	206 (72.3)
Smoking history (%)				
Never	683 (39.8)	428 (40.9)	255 (38.0)	111 (38.9)
Former	814 (47.4)	489 (46.7)	325 (48.4)	134 (47.0) [†]
Current	221 (12.9)	130 (12.4)	91 (13.6)	40 (14.0) [†]
Family history of colorectal cancer (%)				
No	472 (27.5)	283 (27.0)	189 (28.2)	87 (30.5) [†]
Yes	1,246 (72.5)	764 (73.0)	482 (71.8)	198 (69.5) [†]
Drinks alcohol (servings/wk)	3.7 ± 6.2	3.4 ± 5.7	4.2 ± 6.9	3.5 ± 6.1
Vigorous or moderate activity (h/wk)	12.0 ± 12.6	11.7 ± 11.6	12.3 ± 14.0	11.8 ± 13.8
Total energy intake (kcal/d)	1,912.9 ± 575.7	1,896.5 ± 555.6	1,938.5 ± 605.3	1,913.6 ± 560.2
Fat (% kcal/d)	35.5 ± 7.4	35.8 ± 7.4	35.1 ± 7.3 [†]	35.2 ± 7.4
Fiber intake (g/1,000 cal/d)	9.5 ± 3.9	9.5 ± 3.8	9.6 ± 4.0	9.8 ± 4.0
Fruit and vegetable intake (servings/d)	2.2 ± 1.1	2.2 ± 1.1	2.2 ± 1.1	2.3 ± 1.1
Regular vitamin/mineral use (%)				
No	966 (56.2)	581 (55.5)	385 (57.4)	169 (59.3)
Yes	752 (43.8)	466 (44.5)	286 (42.6)	116 (40.7)
Regular NSAID use [‡] (%)				
No	603 (50.8)	380 (48.7)	253 (54.1)	108 (56.3)
Yes	582 (49.2)	367 (51.3)	215 (45.9)	84 (43.7)

NOTE: Adenomatous polyp recurrence diagnosed after year 1 in the study through postintervention at year 4.

Results are presented as mean ± SD for continuous variables and as percentages for categorical variables. Three participants with no recurrence and two participants with an adenomatous polyp recurrence had missing interview data. *P* values for differences in means were determined by *t* tests and differences in proportions were determined by χ^2 tests. All *P* values are adjusted for the other variables listed in the table.

*Comparison group: no adenoma recurrence; *P* < 0.01.

[†]Comparison group: no adenoma recurrence; *P* < 0.05.

[‡]Regular NSAID use includes current use of NSAIDs on a regular basis (>1 per month) reported at three or more yearly study visits. The reference group of no NSAID use includes individuals who reported no current use of NSAIDs on a regular basis (<1 per month) at all five study visits.

Results

Demographic and lifestyle characteristics of the study participants are presented in Table 1. There were 673 participants (39.1%) who had at least one adenoma recurrence at 4 years and 1,050 (60.9%) participants who did not. The mean age of the study population at baseline was 61 years and 90% of individuals self-identified as White. Participants who had at least one adenoma recurrence were more likely to be older, to be male, and less likely to report current, regular NSAID use at baseline compared with individuals who had no recurrence at 4 years (Table 1).

Genotype and allele frequencies along with associations of the cytokine SNPs are shown in Table 2. Variant allele frequencies in our cohort were similar to distributions among controls in previously reported case-control studies (34, 36). Among all of the SNPs analyzed, the genotype frequencies among the entire cohort were in Hardy-Weinberg equilibrium, except for *IL-1 β* , which exhibited a borderline departure from Hardy-Weinberg equilibrium ($\chi^2 = 4.2$; *P* = 0.04). In our cohort, *n* = 773 (48%) had the heterozygote genotype *IL-1 β* -511 when we expected *n* = 736 (46%) based on allele frequencies. There was a similar shift of observed and expected genotypes for the homozygote wild-type (41% observed, 42% expected) and the homozygote variant genotype (11% observed, 12% expected). We do not attribute this deviation from Hardy-Weinberg equilibrium as due to laboratory or genotyping error because the overall concordance rate for

blinded quality controls was >98% and the overall allelic call rate for this SNP was 94%.

Overall, there were no statistically significant associations between any of the cytokine SNPs investigated in this study and risk of adenoma recurrence (Table 2). Furthermore, investigation of *IL-10* haplotypes constructed from *IL-10* -819 C>T and *IL-10* -1082 G>A SNPs did not add any explanatory power; thus, only the results of the *IL-10* genotype associations are presented. We investigated the association between cytokine genotype and risk of adenoma recurrence, stratified by gender, and did not observe any differences in patterns of the main effect of genotype or statistically significant interactions by gender (data not shown).

Previously, we reported an inverse association between NSAID use and adenoma recurrence in the entire Polyp Prevention Trial cohort of 1995 (11). Similarly, we found current, regular NSAID use for at least 3 years was inversely associated with risk of adenoma recurrence (OR, 0.70; 95% CI, 0.55-0.90) and multiple adenoma recurrence (OR, 0.55; 95% CI, 0.38-0.80) in our cohort of 1,723 Polyp Prevention Trial participants. Therefore, we examined the association of the cytokine polymorphisms and risk of adenoma recurrence separately among non-NSAID users and by regular NSAID use reported for at least 3 years over the study period (Table 3). In our stratified analyses, we observed a borderline significant increased risk of any adenoma recurrence among carriers of the *IL-10* -1082 G>A variant allele among regular NSAID users (OR, 1.55; 95% CI, 1.00-2.43), as well as a suggestion of a

Table 2. Cytokine genotype frequencies by adenomatous polyp recurrence and adjusted ORs and 95% CIs for adenomatous polyp recurrence among participants in the Polyp Prevention Trial

	Total	No recurrence	Adenoma recurrence		Multiple recurrence	
	n (%)	n (%)	n (%)	OR* (95% CI)	n (%)	OR* (95% CI)
<i>IL-1β</i> -511						
C/C	641 (40.8)	385 (40.1)	256 (41.9)	1.0	118 (45.0)	1.0
C/T	751 (47.8)	464 (48.4)	287 (47.0)	0.92 (0.74-1.15)	110 (42.0)	0.75 (0.54-1.06)
T/T	178 (11.3)	110 (11.5)	68 (11.1)	0.91 (0.64-1.29)	34 (13.0)	1.11 (0.68-1.82)
C/T + T/T	929 (59.2)	574 (59.9)	355 (58.1)	0.92 (0.74-1.14)	144 (55.0)	0.82 (0.60-1.12)
<i>IL-6</i> -174						
G/G	578 (37.5)	361 (38.3)	217 (36.1)	1.0	90 (35.6)	1.0
G/C	734 (47.6)	428 (45.4)	306 (50.9)	1.25 (0.99-1.57)	136 (53.8)	1.29 (0.91-1.83)
C/C	231 (15.0)	153 (16.2)	78 (13.0)	0.85 (0.61-1.19)	27 (10.7)	0.57 (0.32-1.01)
G/C + C/C	965 (62.5)	581 (61.7)	384 (63.9)	1.14 (0.91-1.42)	163 (64.4)	1.10 (0.78-1.54)
<i>IL-8</i> -251						
T/T	429 (27.5)	272 (28.4)	157 (26.1)	1.0	69 (26.8)	1.0
A/T	746 (47.9)	447 (46.7)	299 (49.8)	1.18 (0.92-1.52)	125 (48.6)	1.01 (0.69-1.47)
A/A	384 (24.6)	239 (25.0)	145 (24.1)	1.05 (0.77-1.42)	63 (24.5)	1.00 (0.64-1.57)
A/T + T/T	1,130 (72.5)	686 (71.6)	444 (73.9)	1.14 (0.90-1.45)	188 (73.2)	1.00 (0.70-1.43)
<i>IL-10</i> -819						
C/C	921 (57.6)	568 (58.3)	353 (56.7)	1.0	149 (56.2)	1.0
C/T	585 (36.6)	353 (36.2)	232 (37.2)	1.05 (0.85-1.31)	105 (39.6)	1.18 (0.85-1.63)
T/T	92 (5.8)	54 (5.5)	38 (6.1)	1.13 (0.72-1.76)	11 (4.2)	0.96 (0.47-1.97)
C/T + T/T	677 (42.4)	407 (41.7)	270 (43.3)	1.06 (0.86-1.31)	116 (43.8)	1.15 (0.84-1.57)
<i>IL-10</i> -1082						
G/G	333 (20.7)	204 (20.8)	129 (20.6)	1.0	61 (22.8)	1.0
A/G	831 (51.7)	511 (52.1)	320 (51.1)	0.98 (0.75-1.27)	131 (48.9)	0.72 (0.49-1.07)
A/A	442 (27.5)	265 (27.0)	177 (28.3)	1.01 (0.75-1.36)	76 (28.4)	0.83 (0.54-1.28)
A/G + A/A	1,273 (79.3)	776 (79.2)	497 (79.4)	0.99 (0.76-1.27)	207 (77.2)	0.76 (0.53-1.10)

NOTE: Adenomatous polyp recurrence diagnosed after year 1 in the study through postintervention at year 4.

*Multivariate OR and 95% CI adjusted by age, race, sex, and body mass index. Three participants with no recurrence and two participants with an adenomatous polyp recurrence with missing interview data were excluded from models.

40% increased risk of multiple adenoma recurrence. In contrast, among non-NSAID users, we observed a statistically significant decreased risk of multiple adenoma recurrence among individuals who were carriers of the *IL-10* -1082 G>A variant allele (OR, 0.43; 95% CI, 0.24-0.77) and a similar, but nonstatistically significant, 30% decreased risk of any adenoma recurrence. Interestingly, we observed a suggestion of an increased risk of both any adenoma recurrence and multiple

adenoma recurrence among individuals who used NSAIDs regularly for at least 3 years of the study and who were carriers of either of the *IL-10* -1082 G>A or *IL-10* -819 C>T variant alleles.

Finally, we investigated the joint effect of NSAIDs by cytokine gene variants and risk of adenoma recurrence and multiple recurrence (Table 4). We observed a statistically significant interaction between regular NSAID use for >3 years

Table 3. ORs and 95% CIs for interactions between cytokine genotypes and regular NSAID use and adenomatous polyp recurrence among participants of the Polyp Prevention Trial

	Regular NSAID use*					No NSAID use*				
	No recurrence		Any polyp recurrence			No recurrence		Any polyp recurrence		
	n (%)	n (%)	OR [†] (95% CI)	n (%)	OR [†] (95% CI)	n (%)	n (%)	OR [†] (95% CI)	n (%)	OR [†] (95% CI)
Total	361	214		84		338	248		114	
<i>IL-1B</i>										
C/C	143 (41.7)	82 (41.2)	1.0	37 (49.3)	1.0	129 (40.6)	96 (41.2)	1.0	48 (44.9)	1.0
C/T + T/T	200 (58.3)	117 (58.8)	1.02 (0.71-1.47)	38 (41.3)	0.79 (0.45-1.41)	189 (59.4)	137 (58.8)	1.00 (0.70-1.43)	59 (55.1)	0.79 (0.47-1.32)
<i>IL-6</i> -174										
G/G	126 (37.6)	72 (37.7)	1.0	25 (34.3)	1.0	130 (41.3)	82 (36.1)	1.0	39 (38.2)	1.0
G/C + C/C	209 (62.4)	119 (62.3)	1.04 (0.72-1.52)	48 (65.7)	1.19 (0.65-2.18)	185 (58.7)	145 (63.9)	1.18 (0.81-1.73)	63 (61.8)	0.87 (0.51-1.50)
<i>IL-8</i> -251										
T/T	103 (30.2)	48 (25.0)	1.0	21 (29.6)	1.0	87 (27.4)	61 (26.5)	1.0	30 (28.6)	1.0
T/A + A/A	238 (69.79)	144 (75.0)	1.38 (0.91-2.09)	50 (70.4)	0.99 (0.52-1.89)	230 (72.6)	169 (73.5)	1.12 (0.75-1.67)	75 (71.4)	0.88 (0.50-1.55)
<i>IL-10</i> -819										
C/C	215 (62.5)	115 (56.4)	1.0	45 (57.7)	1.0	183 (56.1)	139 (58.4)	1.0	61 (56.5)	1.0
C/T + T/T	129 (37.5)	89 (43.6)	1.30 (0.91-1.88)	33 (42.3)	1.48 (0.84-2.63)	143 (43.9)	99 (41.6)	0.92 (0.65-1.30)	47 (43.5)	0.93 (0.55-1.55)
<i>IL-10</i> -1082										
G/G	85 (24.6)	37 (18.2)	1.0	15 (19.0)	1.0	65 (19.8)	59 (24.8)	1.0	32 (29.6)	1.0
G/A + A/A	260 (75.4)	166 (81.8)	1.55 (1.00-2.43)	64 (81.0)	1.43 (0.72-2.88)	263 (80.2)	179 (75.2)	0.72 (0.48-1.09)	76 (70.4)	0.43 (0.24-0.77)

NOTE: Adenomatous polyp recurrence diagnosed after year 1 in the study through postintervention at year 4.

*Regular NSAID use includes current use of NSAIDs on a regular basis (>1 per month) at three or more yearly study visits. The reference group of no NSAID use includes individuals who reported no current use of NSAIDs on a regular basis (<1 per month) at all five study visits.

†Multivariate OR and 95% CI adjusted by age, race, sex, and body mass index. Three participants with no recurrence and two participants with an adenomatous polyp recurrence with missing interview data were excluded from models.

Table 4. Interaction tables for regular NSAID use and adenoma recurrence among participants of the Polyp Prevention Trial

	Any adenoma recurrence				Multiple adenoma recurrence			
	No NSAID use		Regular NSAID use*		No NSAID use		Regular NSAID use*	
	Case/control	OR [†] (95% CI)	Case/control	OR [†] (95% CI)	Case/control	OR [†] (95% CI)	Case/control	OR [†] (95% CI)
<i>IL-1B</i> -511								
C/C	96/129	1.0	82/143	0.69 (0.47-1.02)	39/129	1.00	28/143	0.54 (0.31-0.95)
C/T + T/T	137/192	0.98 (0.69-1.40)	117/200	0.71 (0.49-1.02)	42/192	0.79 (0.47-1.32)	31/200	0.43 (0.25-0.74)
<i>P</i> _{interaction}	0.86				0.98			
<i>IL-6</i> -174								
G/G	82/130	1.0	72/126	0.77 (0.51-1.16)	32/130	1.0	20/126	0.47 (0.25-0.88)
G/C + C/C	145/185	1.19 (0.82-1.73)	119/209	0.80 (0.55-1.16)	44/185	0.89 (0.52-1.52)	36/209	0.55 (0.32-0.96)
<i>P</i> _{interaction}	0.61				0.49			
<i>IL-8</i> -251								
T/T	61/87	1.0	48/103	0.56 (0.34-0.92)	24/87	1.0	17/103	0.46 (0.23-0.94)
T/A + A/A	169/230	1.10 (0.74-1.63)	144/238	0.80 (0.53-1.19)	53/230	0.87 (0.49-1.53)	38/238	0.47 (0.26-0.85)
<i>P</i> _{interaction}	0.38				0.72			
<i>IL-10</i> -819								
C/C	139/183	1.0	115/215	0.61 (0.44-0.85)	46/183	1.0	32/215	0.46 (0.27-0.77)
C/T + T/T	99/143	0.91 (0.65-1.29)	89/129	0.80 (0.55-1.14)	35/143	0.94 (0.56-1.57)	28/129	0.65 (0.38-1.12)
<i>P</i> _{interaction}	0.17				0.29			
<i>IL-10</i> -1082								
G/G	59/65	1.0	37/85	0.39 (0.23-0.67)	26/65	1.0	12/85	0.24 (0.11-0.53)
G/A + A/A	179/263	0.72 (0.48-1.08)	166/260	0.60 (0.40-0.91)	54/263	0.44 (0.25-0.77)	51/260	0.34 (0.19-0.61)
<i>P</i> _{interaction}	0.01				0.01			

NOTE: Adenomatous polyp recurrence diagnosed after year 1 in the study through postintervention at year 4.

*Regular NSAID use includes current use of NSAIDs on a regular basis (>1 per month) at three or more yearly study visits. The reference group of no NSAID use includes individuals who reported no current use of NSAIDs on a regular basis (<1 per month) at all five study visits.

[†] Multivariate OR and 95% CI adjusted by age, race, sex, and body mass index. Three participants with no recurrence and two participants with an adenomatous polyp recurrence with missing interview data were excluded from models.

and carriers of the *IL-10* -1082 G>A variant allele and any adenoma recurrence ($P_{\text{interaction}} = 0.01$), as well as risk of multiple adenoma recurrence ($P_{\text{interaction}} = 0.01$), compared with individuals who were homozygous for the common G allele and who were non-NSAID users. The observed results in Table 4 suggest that, compared with non-regular NSAID users with the *IL-10* -1082 GG common genotype, non-regular NSAID users with any *IL-10* -1082 G>A variant allele are at decreased risk of multiple adenoma recurrence (OR, 0.44; 95% CI, 0.25-0.77), as are those individuals with the *IL-10* -1082 GG common genotype who take NSAIDs regularly (OR, 0.24; 95% CI, 0.11-0.53). However, individuals with the *IL-10* -1082 G>A variant allele do not gain further benefit in their reduction of risk of multiple adenoma recurrence by taking NSAIDs regularly (OR, 0.34; 95% CI, 0.19-0.61). We observed similar patterns and magnitude of the main effect of the *IL-10* -1082 G>A genotype when we stratified by NSAID use only at baseline, as well as significant interactions between NSAID use at baseline, *IL-10* -1082 G>A genotype, and risk of adenoma recurrence (data not shown). We also investigated interactions and associations stratified by NSAID use for the entire study period (baseline and all four study visits) and we also observed similar patterns of association of the main effect if *IL-10* -1082 G>A genotype and risk of adenoma recurrence, but the associations were not statistically significant (data not shown).

Discussion

We investigated the associations between several cytokine gene polymorphisms and risk of recurrent adenomatous polyps. Although we failed to observe main effects of a series of SNPs in pro- and anti-inflammatory cytokines, we observed a statistically significant interaction between the *IL-10* -1082 G>A genotype, regular NSAID use, and risk of adenoma recurrence ($P = 0.01$) and multiple adenoma recurrence ($P = 0.01$). Specifically, we observed an increase in risk for any adenoma and multiple adenoma recurrence among regular

NSAID users who were carriers of the *IL-10* -1082 G>A variant allele; among non-NSAID users, there was a nonsignificant decrease in risk for any adenoma recurrence and a significant decreased risk for multiple adenoma recurrence in those who were carriers of the *IL-10* -1082 G>A variant allele. Although a few reports have investigated the association between cytokine polymorphisms and risk of colon cancer with mixed results (34, 35), our study is among the first to investigate the association between the cytokine gene polymorphisms *IL-1B* -511 C>T, *IL-6* -174 G>C, *IL-8* -251 T>A, *IL-10* -818 C>T, and -1082 G>A and risk of colorectal adenoma recurrence. These data suggest that the *IL-10* genotype may play a role in the progression of inflammation-associated colon cancer and that this association may be modified by NSAID use.

IL-10, which is produced by a variety of cells, including T lymphocytes, B lymphocytes, and monocytes, has been identified as a cytokine with important anti-inflammatory and immunosuppressive properties, which plays a major role in inhibiting the synthesis of proinflammatory cytokines including *IL-1B*, *IL-6*, *IL-8*, and *IL-12* (49-51). Recent reports observed that carriers of the *IL-10* -1082 A allele produced significantly lower levels of *in vitro* secretions of *IL-10* compared with individuals with the *IL-10* -1082 G>G genotype (51), whereas the *IL-10* A [TCATA] haplotype formed by polymorphisms at positions -3575, -2763, -1082, -819, and -592 in the promoter of the *IL-10* gene has been associated with an increased level of circulating *IL-10* (52). Low *IL-10* levels are associated with risk for prostate, cervical, noncardiac gastric cancers, melanoma, and lymphoma (53). However, other studies show that high levels of *IL-10* may actually be a risk factor for other cancers, hepatocellular, ovarian, melanoma, lymphoma, and myeloma (50). The *IL-10* -592 C>A promoter polymorphism has been associated with a reduced breast cancer risk (37) and the *IL-10* -1082 G>A polymorphism was associated with increased risk of noncardiac gastric cancer (36). Currently, the role of *IL-10* in cancer remains unresolved (53).

Recent evidence suggests that NSAID use may modify the association between polymorphisms in inflammatory genes and risk of colorectal cancer (34, 35) and colorectal adenomas (41). Macarthur et al. (35) investigated the association between the *IL-10* -1082 G>A SNP and risk of colorectal cancer in a small population-based case-control study in Northeast Scotland. In their study, compared with individuals with the *IL-10* -1082 GG genotype, carriers of the variant *IL-10* -1082 A allele who used aspirin had a nonstatistically significant reduced risk of colorectal cancer (35). These differences in results may reflect real differences in the gene-drug association and their effect at different stages of disease (i.e., adenoma recurrence versus invasive colorectal cancer), or may be due to limited sample size, differences in aspirin/NSAID exposure categorization, or due to chance. However, our data mimic *IL-10*-deficient mice that develop spontaneous chronic inflammatory bowel disease, a known risk factor for colorectal cancer (49, 54). *IL-10*-deficient mice have increased production of proinflammatory cytokines and several studies report that *IL-10*-/- mice treated with NSAIDs develop progressive, severe colitis much faster than *IL-10*-/- mice not treated with NSAIDs (49). On the other hand, NSAID-treated wild-type mice did not develop colitis and their colonic epithelium had no evidence of hyperplasia or ulcerations (49). Microscopic examination of NSAID-treated *IL-10*-/- mice revealed severe inflammatory infiltrates in their colonic mucosa and increased mRNA expression of inflammatory cytokines and COX-2 expression compared with NSAID-treated wild-type mice (49). It seems that inhibition of prostaglandin production was central to the development of NSAID-induced colitis. These findings may help to explain our observed findings that individuals who used NSAIDs and were carriers of the *IL-10* -1082 A allele, which is associated with a decreased production of the *IL-10* anti-inflammatory cytokine and proposed, subsequent increased production of proinflammatory cytokines, were at a significant increased risk of multiple adenoma recurrence as well as a suggested increased risk of any adenoma recurrence.

SNPs in the *IL-6* gene proinflammatory genes have been associated with changes in cytokine production and inflammatory diseases (30-33, 55, 56). Landi et al. (34), reported that the *IL-6* -174 C allele was associated with increased risk of colorectal cancer but only in those subjects who did not habitually take NSAIDs. We did not observe any interactions between *IL-6* genotype, NSAID use, and risk of adenoma recurrence in our study. Functional studies investigating the biological role of the substitution *IL-6* -174 G>C have been mixed. Reports indicate that the -174 C allele was associated with lower and higher levels of expression of *IL-6* *in vitro* and *in vivo* (33, 55, 57). Few studies have reported on the functional role of the *IL-8* -251 T/A SNP, but one case-control study, investigating the role of *IL-8* and ulcerative colitis, observed significantly higher *IL-8* concentrations in patients with active ulcerative colitis compared with controls (31), whereas the *IL-8* -251 A allele was associated with a decreased risk of colorectal cancer in one study (34).

There are several strengths to our study. First, we are among the first to report on the association between several cytokine polymorphisms and risk of colorectal adenoma recurrence using a substantial sample adequate to avoid the false positives that plague smaller studies and to investigate effect modification. Data for this analysis came from participants in a large dietary randomized trial in which we were able to assess confounding and joint effects by several dietary and lifestyle factors, including NSAID use. Second, due to the prospective study design, all participants had complete ascertainment of recurrent polyp because all participants received a full colonoscopy at the end of the trial intervention period, which also minimized the chance for misclassification of adenoma status compared with sigmoidoscopy of proximal adenomas.

Some limitations of the study should be noted. First, generalizability of these findings may be limited, as all of the participants had a history of an adenoma, the majority of the participants were male, and >90% self-identified as White. However, it is estimated that close to 40% of adults of ages ≥ 60 years have at least one prevalent polyp; therefore, these findings may be generalizable to a number of individuals at risk for colorectal cancer. Second, we did have limited power to detect joint effects and there were few participants who were regular NSAID users for the entire study period, with only 13% reporting regular NSAID use at baseline and at all four study visits.

It is also possible that other functional or regulatory SNPs in linkage disequilibrium with the selected SNPs in this study account for the observed results. However, strong functional data seem to support the role of the *IL-10* -1082 G/A SNP in altering plasma cytokine concentrations and risk of cancer (29, 58). Finally, it is plausible that variants in drug metabolism genes or in the COX-2 gene may modify or inhibit the association between NSAIDs and colon cancer and may explain some of the observed differences in the association between NSAID use and risk of adenoma recurrence (41, 59, 60).

In summary, our results add to recent reports that suggest NSAID use may not be beneficial among individuals with certain inflammatory genotypes and, given our data, may even increase an individual's risk for colorectal adenomas. Specifically, our study provides evidence that carriers of the *IL-10* -1082 A variant allele exhibit decreased risk for recurrent adenomas among non-NSAID users. These results suggest that the *IL-10* -1082 A allele is a potential genotype identifying individuals who may not benefit from the chemoprevention of colorectal cancer by NSAIDs. Verification of this finding in other population-based samples and further investigations of its biological role as an effect modifier of the NSAID-colon cancer association are warranted. Future studies investigating the role of variants in inflammatory genes that modify the chemoprotective effect of NSAIDs in colon carcinogenesis may help to elucidate the biological mechanisms of the disease and identify individuals who may respond best to these chemopreventive agents, as well as aid in the development of public health and clinical intervention programs aimed at preventing colorectal cancer.

References

1. Winawer SJ, Zauber AG, O'Brien MJ, et al. Randomized comparison of surveillance intervals after colonoscopic removal of newly diagnosed adenomatous polyps. The National Polyp Study Workgroup. *N Engl J Med* 1993;328:901-6.
2. Winawer SJ. Screening of colorectal cancer: progress and problems. *Recent Results Cancer Res* 2005;166:231-44.
3. Loeve F, Boer R, Zauber AG, et al. National Polyp Study data: evidence for regression of adenomas. *Int J Cancer* 2004;111:633-9.
4. Betario L, Russo A, Sala P, et al. Risk of colorectal cancer following colonoscopic polypectomy. *Tumori* 1999;85:157-62.
5. Shao J, Sheng H, Inoue H, Morrow JD, DuBois RN. Regulation of constitutive cyclooxygenase-2 expression in colon carcinoma cells. *J Biol Chem* 2000;275:33951-6.
6. Sano H, Kawahito Y, Wilder RL, et al. Expression of cyclooxygenase-1 and -2 in human colorectal cancer. *Cancer Res* 1995;55:3785-9.
7. Soslow RA, Dannenberg AJ, Rush D, et al. COX-2 is expressed in human pulmonary, colon, and mammary tumors. *Cancer* 2000;89:2637-45.
8. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, Dubois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 1994;107:1183-8.
9. Einspahr JG, Krouse RS, Yochim JM, et al. Association between cyclooxygenase expression and colorectal adenoma characteristics. *Cancer Res* 2003;63:3891-3.
10. Williams CS, Tsujii M, Reese J, Dey SK, DuBois RN. Host cyclooxygenase-2 modulates carcinoma growth. *J Clin Invest* 2000;105:1589-94.
11. Tangrea JA, Albert PS, Lanza E, et al. Non-steroidal anti-inflammatory drug use is associated with reduction in the recurrence of advanced and non-advanced colorectal adenomas (United States). *Cancer Causes Control* 2003;14:403-11.

12. Sheehan KM, Sheahan K, O'Donoghue DP, et al. The relationship between cyclooxygenase-2 expression and colorectal cancer. *JAMA* 1999; 282:1254-7.
13. Kutcher W, Jones DA, Matsunami N, et al. Prostaglandin H synthase 2 is expressed abnormally in human colon cancer: evidence for a transcriptional effect. *Proc Natl Acad Sci U S A* 1996;93:4816-20.
14. Kargman SL, O'Neill GP, Vickers PJ, Evans JF, Mancini JA, Jothy S. Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. *Cancer Res* 1995;55:2556-9.
15. Langenbach R, Loftin C, Lee C, Tian H. Cyclooxygenase knockout mice. *Biochem Pharmacol* 1999;58:1237-46.
16. Dranoff G. Cytokines in cancer pathogenesis and cancer therapy. *Nat Rev Cancer* 2004;4:11-22.
17. O'Byrne KJ, Dalglish AG. Chronic immune activation and inflammation as the cause of malignancy. *Br J Cancer* 2001;85:473-783.
18. Rhodes JM, Campbell BJ. Inflammation and colorectal cancer: IBD-associated and sporadic cancer compared. *Trends Mol Med* 2002;8:10-5.
19. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001;357:539-45.
20. Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002;420:860-7.
21. Singer II, Kawa DW, Schloemann S, Tessner T, Riehl T, Stenson WF. Cyclooxygenase 2 is induced in colonic epithelial cells in inflammatory bowel disease. *Gastroenterology* 1998;115:297-306.
22. Baron JA, Cole B, Sandler RS, et al. A randomized trial of aspirin to prevent colorectal adenomas. *N Engl J Med* 2003;348:891-9.
23. Sandler RS, Halabi S, Baron JA, et al. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. *N Engl J Med* 2003;348:883-90.
24. Benamouzig R, Deyra J, Martin A, et al. Daily soluble aspirin and prevention of colorectal adenoma recurrence: one-year results of the APACC trial. *Gastroenterology* 2003;125:328-36.
25. Sandler RS, Galanko JC, Murray SC, Helm JF, Woosley JT. Aspirin and nonsteroidal anti-inflammatory agents and risk for colorectal adenomas. *Gastroenterology* 1998;114:441-7.
26. Shiff SJ, Rigas B. Nonsteroidal anti-inflammatory drugs and colorectal cancer: evolving concepts of their chemoprevention actions. *Gastroenterology* 1997;113:1992-8.
27. Asano TK, McLeod RS. Nonsteroidal anti-inflammatory drugs and aspirin for the prevention of colorectal adenomas and cancer: a systematic review. *Dis Colon Rectum* 2004;47:665-73.
28. Baron JA, Sandler RS. Nonsteroidal anti-inflammatory drugs and cancer prevention. *Annu Rev Med* 2000;51:511-23.
29. Bidwell J, Keen L, Gallagher G, et al. Cytokine gene polymorphism in human disease: on-line databases, supplement 1. *Genes Immun* 2001;2:61-70.
30. Arosio B, Trabattini D, Galimberti L, et al. Interleukin-10 and interleukin-6 gene polymorphisms as risk factors for Alzheimer's disease. *Neurobiol Aging* 2004;25:1009-15.
31. Keshavarzian A, Fusunyan RD, Jacyno M, Winship D, MacDermott RP, Sanderson IR. Increased interleukin-8 (IL-8) in rectal dialysate from patients with ulcerative colitis: evidence for a biological role for IL-8 in inflammation of the colon. *Am J Gastroenterol* 1999;94:704-12.
32. Licastro F, Grimaldi LM, Bonafe M, et al. Interleukin-6 gene alleles affect the risk of Alzheimer's disease levels of the cytokine in blood and brain. *Neurobiol Aging* 2003;24:921-6.
33. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest* 1998;102:1369-76.
34. Landi S, Moreno V, Gioia-Patricola L, et al. Associations of common polymorphisms in inflammatory genes interleukin (IL)6, IL8, tumor necrosis factor α , NFKB1, and peroxisome proliferator-activated receptor γ with colorectal cancer. *Cancer Res* 2003;63:3560-6.
35. Macarthur M, Sharp L, Hold GL, Little J, El-Omar EM. The role of cytokine gene polymorphisms in colorectal cancer and their interaction with aspirin use in the northeast of Scotland. *Cancer Epidemiol Biomarkers Prev* 2005;14:1613-8.
36. El-Omar EM, Rabkin CS, Gammon MD, et al. Increased risk of noncardiac gastric cancer associated with proinflammatory cytokine gene polymorphisms. *Gastroenterology* 2003;124:1193-201.
37. Langsenlehner U, Krippel P, Renner W, et al. Interleukin-10 promoter polymorphism is associated with decreased breast cancer risk. *Breast Cancer Res Treat* 2005;90:113-5.
38. Smith KC, Bateman AC, Fussell HM, Howell WM. Cytokine gene polymorphisms and breast cancer susceptibility and prognosis. *Eur J Immunogenet* 2004;31:167-73.
39. Shin HD, Park BL, Kim LH, et al. Interleukin 10 haplotype associated with increased risk of hepatocellular carcinoma. *Hum Mol Genet* 2003; 12:901-6.
40. Howell WM, Turner SJ, Theaker JM, Bateman AC. Cytokine gene single nucleotide polymorphisms and susceptibility to and prognosis in cutaneous malignant melanoma. *Eur J Immunogenet* 2003;30:409-14.
41. Ulrich CM, Whitton J, Yu JH, et al. PTGS2 (COX-2) -756 G>C promoter variant reduces risk of colorectal adenoma among nonusers of nonsteroidal anti-inflammatory drugs. *Cancer Epidemiol Biomarkers Prev* 2005;14: 616-9.
42. Schatzkin A, Lanza E, Corle D, et al. Lack of effect of a low-fat, high-fiber diet on the recurrence of colorectal adenomas. *Polyp Prevention Trial Study Group. N Engl J Med* 2000;342:1149-55.
43. Schatzkin A, Lanza E, Freedman LS, et al. The polyp prevention trial I: rationale, design, recruitment, and baseline participant characteristics. *Cancer Epidemiol Biomarkers Prev* 1996;5:375-83.
44. Lanza E, Schatzkin A, Ballard-Barbash R, et al. The polyp prevention trial II: dietary intervention program and participant baseline dietary characteristics. *Cancer Epidemiol Biomarkers Prev* 1996;5:385-92.
45. Mares-Perlman JA, Klein BE, Klein R, Ritter LL, Fisher MR, Freudenheim JL. A diet history questionnaire ranks nutrient intakes in middle-aged and older men and women similarly to multiple food records. *J Nutr* 1993;123: 489-501.
46. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. A data-based approach to diet questionnaire design and testing. *Am J Epidemiol* 1986;124:453-69.
47. The practical guide: Identification, evolution, and treatment of overweight and obesity in adults. Bethesda, MD: National Institutes of Health, 2000. (http://www.nhlbi.nih.gov/guidelines/obesity/prctgd_c.pdf) (NIH publication no. DO-4084).
48. Kokoris M, Dix K, Moynihan K, et al. High-throughput SNP genotyping with the Masscode system. *Mol Diagn* 2000;5:329-40.
49. Berg DJ, Zhang J, Weinstock JV, et al. Rapid development of colitis in NSAID-treated IL-10-deficient mice. *Gastroenterology* 2002;123:1527-42.
50. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001;19:683-765.
51. Yilmaz V, Yentur SP, Saruhan-Direskeneli G. IL-12 and IL-10 polymorphisms and their effects on cytokine production. *Cytokine* 2005;30: 188-94.
52. Lin MT, Storer B, Martin PJ, et al. Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. *N Engl J Med* 2003;349:2201-10.
53. Brower V. Researchers attempting to define role of cytokines in cancer risk. *J Natl Cancer Inst* 2005;97:1175-7.
54. Sturlan S, Oberhuber G, Beinhauer BG, et al. Interleukin-10-deficient mice and inflammatory bowel disease associated cancer development. *Carcinogenesis* 2001;22:665-71.
55. Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. *J Biol Chem* 2000;275:18138-44.
56. Bennermo M, Herld C, Stemme S, et al. Genetic predisposition of the interleukin-6 response to inflammation: implications for a variety of major diseases? *Clin Chem* 2004;50:2136-40.
57. Vickers MA, Green FR, Terry C, et al. Genotype at a promoter polymorphism of the interleukin-6 gene is associated with baseline levels of plasma C-reactive protein. *Cardiovasc Res* 2002;53:1029-34.
58. Bidwell J, Keen L, Gallagher G, et al. Cytokine gene polymorphism in human disease: on-line databases. *Genes Immun* 1999;1:3-19.
59. Bigler J, Whitton J, Lampe JW, Fosdick L, Bostick RM, Potter JD. CYP2C9 and UGT1A6 genotypes modulate the protective effect of aspirin on colon adenoma risk. *Cancer Res* 2001;61:3566-9.
60. Guengerich FP. Cytochrome P-450 3A4: regulation and role in drug metabolism. *Pharmacol Toxicol* 1999;39:1-17.