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Association of common polymorphisms in *IL10*, and in other genes related to inflammatory response and obesity with colorectal cancer

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Abstract

Objective and methods—The association of 17 candidate single nucleotide polymorphisms (SNPs) in *IL10* and other immune response genes (*CRP*, *TLR4*, *IL6*, *IL1B*, *IL8*, *TNF*, *RNASEL*) and genes related to obesity (*PPARG*, *TCF7L2*, *ADIPOQ*, *LEP*) with colorectal cancer was investigated. Haplotype tagging SNPs were chosen for *IL10*, *CRP*, and *TLR4*. Incident colorectal cancer cases ($n = 208$) and matched controls ($n = 381$) were identified between baseline in 1989 and 2003 among participants in the CLUE II cohort. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated using conditional logistic regression.

Results—Compared with the AA genotype at the candidate *IL10*-1082 locus (rs1800896), carrying one (OR, 0.79; 95% CI, 0.53–1.18) or two (OR, 0.58; 95% CI, 0.35–0.95) G alleles, a known higher producer of the anti-inflammatory cytokine IL-10, was associated with lower risk of colorectal cancer ($p_{\text{trend}} = 0.03$). Statistically significant associations with colorectal cancer were observed for three tagSNPs in *IL10* (rs1800890, rs3024496, rs3024498) and one common haplotype, but these associations were due to high linkage disequilibrium with *IL10*-1082. Two *CRP* haplotypes (global $p = 0.04$) and *TLR4* tagSNPs (rs7873784, rs11536891), but not *TLR4* haplotypes, were associated with colorectal cancer.

Conclusions—Our study suggests that polymorphisms in *IL10*, and also possibly in *CRP* and other genes related to immune response or obesity may be associated with colorectal cancer.

Keywords

Inflammation; Obesity; Colorectal cancer; Genetic epidemiology; Prospective study

Introduction

Diet and lifestyle contribute to colorectal cancer risk [1], possibly via chronic inflammation and the sequelae of obesity, including energy/insulin dysregulation and diabetes [2]. Markers of systemic inflammation, obesity, and diabetes have been associated with colorectal cancer risk in prospective epidemiologic studies [3, 4], including studies in the CLUE II cohort [5, 6]. The propensity, nature, and extent of an immune response and of the sequelae of adiposity are influenced by genetic variation. Candidate genes involved in these pathways have been associated with colorectal neoplasia in epidemiologic studies [7–13], and common allelic variants have been shown to have biological effects (Table 1).

Chronic inflammation is, in part, characterized by modifications in cytokine profiles and concentrations. Of note, interleukin-10 (IL-10) has a pivotal role in the immunity of the gastrointestinal tract [14]. Cytokines secreted by immune cells signal other such cells to produce reactive oxygen and nitrogen species, which may promote cancer development by cell membrane and DNA damage. Cytokine action can be crudely described as proinflammatory (e.g., IL-1 β , IL-6, IL-8, tumor necrosis factor- α [TNF- α] and C-reactive protein [CRP]) or anti-inflammatory (e.g., IL-10). Toll-like receptors (TLRs), involved in innate immune recognition of invading bacteria and viruses, activate signaling cascades that lead to the induction of proinflammatory cytokines [15]. Endoribonuclease L (RNASEL) is implicated in mediating apoptosis in response to viral infections [16].

Obesity results from excess dietary energy intake, and is associated with an increased risk of insulin resistance and diabetes as well as is correlated with modifications in adipokine profiles and concentrations. Adipokines (e.g., leptin and adiponectin) have important functions in energy regulation and insulin resistance [17], and act as growth factors for colon cancer cells [18]. The peroxisome proliferator-activated receptors (PPARs) are ligand-dependent transcription factors that have key roles in the regulation of lipid and glucose metabolism and produce selected insulin-sensitizing and anti-inflammatory effects [19]. Single nucleotide polymorphisms (SNPs) in the transcription factor-7-like 2 (*TCF7L2*) gene are reported to be strongly associated with impaired insulin secretion and type 2 diabetes [20].

Based on an abundance of evidence that chronic inflammation, obesity and its sequelae influence colorectal neoplasia, and that the expression of cytokines, adipokines and related enzymes and receptors is under genetic control, it was hypothesized that a genetic profile that is proinflammatory and/or favors metabolic perturbations related to obesity would be associated with increased susceptibility to colorectal cancer. Thus, the association of 17 candidate SNPs in *IL10*, *CRP*, *TLR4*, *IL6*, *IL1B*, *IL8*, *TNF*, *RNASEL*, *PPARG*, *TCF7L2*, *ADIPOQ*, and *LEP* and 19 haplotype tagging SNPs (tagSNPs) in *IL10*, *CRP*, and *TLR4* with colorectal cancer was evaluated in a case-control study nested in the CLUE II cohort of Washington County, Maryland.

Materials and methods

Study population

Colorectal cancer cases and controls were identified among members of the prospective CLUE II cohort, established in 1989 to investigate potential serologic risk factors for cancer and heart disease. The cohort consists of 32,894 residents of Washington County, MD and neighboring areas. For this analysis, the study population was restricted to 22,887 Washington County residents aged ≥ 18 year old. Participants provided a blood sample and completed a brief medical and lifestyle exposure history questionnaire at baseline. Seventy-five percent of the participants returned a food frequency questionnaire at baseline. In 1996, 1998, 2000, and 2003, questionnaires were mailed to participants to update lifestyle, medical, and family histories. The blood samples were drawn into 20 ml heparinized Vacutainer tubes (Becton-Dickinson, Rutherford, NJ), kept at 4°C until the plasma was separated, and was divided into aliquots of plasma, buffy coat, and red blood cells within 24

h. The Institutional Review Board at the Johns Hopkins Bloomberg School of Public Health approved the study.

Selection of colorectal cancer cases and controls

As described previously [5], participants were eligible to be selected as a case or control if they never had a diagnosis of cancer (except possibly for non-melanoma skin cancer or cervix in situ) in 1989 or earlier. Cases were identified through linkage with the Washington County Cancer Registry and since 1992 with the Maryland Cancer Registry. Two hundred and eight colorectal cancer cases diagnosed after the date of blood draw in 1989 until February 2003 were ascertained. Ninety-eight percent of cases were confirmed by review of pathology report. Cases were characterized by site (colon, rectum, distal colon, proximal colon), stage (T1 [$n = 42$]: tumor invades the submucosa; T2 [$n = 56$]: tumor invades the muscularis propria; T3 [$n = 47$]: tumor invades through the muscularis propria into the subserosa or into non-peritonealized pericolic or perirectal tissues; T4 [$n = 23$]: tumor directly invades other organs or structures and/or perforates the visceral peritoneum), and histologic grade (well [$n = 5$], moderate [$n = 118$], poor differentiation [$n = 58$]). One hundred sixty cases had colon cancer (74 distal, and 86 proximal) and 48 cases had rectal cancer. The staging and grading were done following the American Joint Committee on Cancer (AJCC) Cancer Staging Manual 4th edition. For 40 cases with unknown AJCC stage, staging was classified into broad categories of local (tumor confined), regional (spread to lymph nodes), and distant (metastatic to other organs/areas) based on information from each patient's medical record.

For each case, up to two controls (173 cases had two controls and 35 cases had one control) matched on age (± 1 year), sex, race, and date of blood draw (± 1 month) were selected. To be eligible to be sampled, a possible control must have been alive at the time the case was diagnosed and not have a diagnosis of cancer through February 2003. Three hundred eighty-one controls were selected.

Assessment of gene polymorphisms

DNA was extracted from the buffy coat fraction of the blood specimens using an AutoPure DNA analyzer machine from Qiagen (Valencia, CA). SNPs were genotyped using Applied Biosystems' Taqman 5' exonuclease assays, Taqman Universal PCR Master Mix, and 2.5 nanograms of genomic DNA. Laboratory personnel were blinded to case-control status.

Single nucleotide polymorphism selection was performed in two stages. First, 17 candidate SNPs in 12 genes (*IL10*, *CRP*, *TLR4*, *IL6*, *IL1B*, *IL8*, *TNF*, *RNASEL*, *PPARG*, *TCF7L2*, *ADIPOQ*, and *LEP*) involved in the inflammatory response or related to obesity were selected (Table 1). These candidate SNPs were selected based on allele frequency ($< 5\%$ minor allele frequency in Whites), functional data related to the production or activity of each gene product (Table 1), and/or evidence of association with risk of colorectal or other cancers [7–11, 13]. Three SNPs (rs4986790, rs486907, and rs1801282 in *TLR4*, *RNASEL*, and *PPARG*, respectively) were non-synonymous. Genotype data for each candidate SNP were successfully obtained on 94.1–98.5% of study subjects.

After conducting preliminary analysis, associations for SNPs in *IL10*, *CRP*, and *TLR4* were observed; therefore, in the second stage tagSNPs in these genes were selected (Table 1). TagSNPs were chosen using Tagger (www.broad.mit.edu/mpg/tagger). The targeted genomic regions for *IL10*, *CRP*, and *TLR4* included 10 kb before the transcription start site and 5 kb after the transcription end site. The selection criteria were a pair-wise r^2 of 0.8 and a minor allele frequency 5%. Based on the resulting tagSNP list, a more concise set of SNPs was selected after taking into account proximity to the candidate gene region. Seven tagSNPs were chosen for *IL10*, four for *CRP*, and eight for *TLR4*. Genotype data for each tagSNP were successfully obtained on more than 90–96.9% of study subjects with the exception of four *TLR4* tagSNPs (rs2737190, 89.3%; rs10116253, 85.7%; rs11536898, 87.6%; and rs2149356, 75%).

Assessment of other variables

Self-reported current height, weight, and smoking history were collected at baseline in 1989. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Participants were asked at baseline whether they had used any medications in the past 48 h of blood draw. Any prescription or over-the-counter medications that contained aspirin or non-aspirin nonsteroidal anti-inflammatory drugs were coded as nonsteroidal anti-inflammatory drugs (NSAIDs). Participants who reported use of medications to treat diabetes were considered to be diabetics. Women were asked at baseline whether they currently or in the past took oral contraceptives or hormone replacement therapy. Daily intake of alcohol (g/day), folate ($\mu\text{g/day}$), calcium (mg/day), fiber (g/day), energy (kcal/day), saturated fat (g/day), and red meat (g/day) were estimated from a food frequency questionnaire at baseline. The follow-up questionnaire in 1996 ascertained whether participants had a family history of colorectal cancer.

Statistical analysis

Matched sets containing the case and at least one control with information for any given SNP were included in the analysis. The distributions of baseline characteristics were compared between cases and controls by regression models. To account for the matched design and the sampling of two controls per case for some sets, generalized estimating equations with an exchangeable correlation structure among each matched set and robust estimation of standard errors were used. Right skewed baseline characteristics were natural logarithm transformed. Hardy–Weinberg equilibrium was tested among controls using χ^2 tests. For SNPs in the same gene, pair-wise linkage disequilibrium measures (D' statistic and r^2) were estimated among the controls.

Matched odds ratios (ORs) of colorectal cancer and corresponding 95% confidence intervals (CIs) were estimated using conditional logistic regression. ORs were estimated assuming either a codominant or a dominant model of inheritance. Tests for trend were evaluated by entering into the model an ordinal variable for each SNP with values corresponding to the number of variant alleles. Multivariable adjustment for diet and lifestyle risk factors (those in Table 2) provided identical results with the matched analysis; therefore, only the results from the matched model are presented in text and tables. Analyses separately by cancer location, stage, and grade were also performed.

Analyses were also conducted stratifying by age at diagnosis (cutpoint at the median: 65 vs. >65 years), sex, BMI (cutpoint at the median: 26 vs. >26 kg/m²), and use of NSAIDs (users vs. non-users). Tests for interaction were carried out by using an ordinal variable for each SNP, an indicator variable for the above potential modifiers, and a term for the product of the two variables. For evaluation of modifying factors other than the matching variables, to preserve power the matched sets were broken and logistic regression adjusting for the matching variables performed. Analyses were also conducted among participants who did not use diabetes medications at baseline. All *p* values were two-sided. All analyses were performed using SAS version 9.1 (Cary, NC).

Haplotypes were reconstructed from unphased genotyped data using Haplo Stats in the R statistical package. For the haplotype analysis, participants with missing values on any of the tagSNPs for a gene of interest were excluded. A global score test to assess differences in overall haplotype distribution between cases and controls was used [21]. ORs and 95% CIs for the association between haplotypes and colorectal cancer risk were estimated with generalized linear models using regression substitution as the estimation method and adjusting for the matching variables [22].

Analyses were repeated after multiple imputation of the missing genotypes. Analyses run on the imputed dataset yielded identical inferences; therefore, the results presented in text and tables are from the original non-imputed dataset. To account for the large number of statistical tests made in this study, the false positive report probability was calculated using the method of Wacholder et al. [23].

Results

A greater proportion of cases had a family history of colorectal cancer and used medications to treat diabetes, and a lower proportion of cases used NSAIDs or female hormones (Table 2). Among controls, all genotypes were distributed in accordance with Hardy–Weinberg equilibrium except for the candidate *IL6* SNP rs1800796 (*p* = 0.05), and the *CRP* tagSNPs rs2794521 (*p* = 0.02) and rs2808630 (*p* = 0.02). These SNPs were retained in the analyses because the deviations from the expected genotype frequencies did not appear great.

Candidate SNP analysis

Compared with the wild type AA genotype at *IL10-1082* (rs1800896), carrying one (OR, 0.79; 95% CI, 0.53–1.18) or two (OR, 0.58; 95% CI, 0.35–0.95) variant G alleles, a known higher producer of the anti-inflammatory cytokine IL-10 [24, 25], was associated with a lower risk of colorectal cancer (*p*_{trend} = 0.03; Table 3). Carrying at least one variant A allele of an intronic SNP in *ADIPOQ* (rs1501299) was associated with a borderline significant increased risk of colorectal cancer when compared with the CC genotype (OR, 1.42; 95% CI, 0.99–2.04; *p*_{trend} = 0.09). No other significant associations were observed between the remaining candidate genotypes and colorectal cancer risk (Table 3).

No important differences in the associations were observed by location, stage, and grade (data not shown), aside from possibly the following: The association for the *ADIPOQ* SNP was present for colon cancer (AC/AA [85 cases, 120 controls] vs. CC [70 cases, 153

controls]; OR, 1.68; 95% CI, 1.10–2.57; $p_{\text{trend}} = 0.03$), but not for rectal cancer (AC/AA [20 cases, 41 controls] vs. CC [26 cases, 45 controls]; OR, 0.85; 95% CI, 0.41–1.77; $p_{\text{trend}} = 0.55$). For moderately or well differentiated colorectal cancer, the *TCF7L2* (rs7903146) TT [21 cases, 20 controls] genotype was associated with a 2.11-fold increased risk when compared with the common CC [50 cases, 110 controls] genotype (95% CI, 1.07–4.16; $p_{\text{trend}} = 0.02$); no association was present for poorly differentiated tumors (TT: 4 cases, 6 controls; CC: 33 cases, 56 controls).

Statistically significant interactions by age, sex, BMI, and use of NSAIDs were not observed ($p_{\text{interaction}} > 0.05$), except possibly for the following: among individuals with BMI > 26 kg/m², the AG and AA genotype [52 cases, 106 controls] of the *LEP* SNP (rs2167270) was inversely associated with colorectal cancer (OR, 0.59; 95% CI, 0.35–0.98) compared with the common GG genotype [46 cases, 55 controls]; no association was found in leaner individuals (OR, 1.28; 95% CI, 0.78–2.12; $p_{\text{interaction}} = 0.03$; [AG/AA: 72 cases, 125 controls; GG: 34 cases, 76 controls]). Among NSAID users, the *IL8* SNP (rs4073) was positively associated with colorectal cancer (AT/AA [41 cases, 71 controls] vs. TT [9 cases, 35 controls]; OR, 2.25; 95% CI, 0.98–5.16; $p_{\text{interaction}} = 0.03$); no association was observed in non-users (AT/AA [99 cases, 177 controls] vs. TT [56 cases, 79 controls]; OR, 0.79; 95% CI, 0.52–1.21).

TagSNP and haplotype analysis

Statistically significant associations were also observed for some of the tagSNPs in *IL10*, *CRP*, *TLR4* and colorectal cancer (Table 4). Haplotype analysis was performed for the *IL10*, *CRP*, *TLR4* tagSNPs, and the three *IL6* candidate SNPs. Results were inferentially the same when the combination of the candidate and tagSNPs were used to infer haplotypes for *IL10*, *CRP*, and *TLR4*. Five common haplotypes ($>5\%$) for *IL10*, 4 haplotypes for each *CRP* and *TLR4*, and 3 haplotypes for *IL6* were observed (Table 5). A significant inverse association was seen comparing the *IL10* T-C-A-C-C-T-G with the common A-C-A-C-C-A haplotype (OR, 0.59; 95% CI, 0.40–0.87), although the *IL10* tagSNPs were in high linkage disequilibrium with the candidate *IL10-1082* SNP. The overall haplotype distribution was statistically significantly different between cases and controls only for the *CRP* haplotypes (global $p = 0.04$). The *CRP* T-T-T-C and C-T-C-A haplotypes were statistically significantly positively associated with colorectal cancer compared to the common T-T-T-A haplotype (Table 5).

Discussion

Candidate SNPs in *IL10* and in seven other genes known to be involved in the inflammatory response and in four genes related to obesity and its metabolic sequelae were evaluated. Statistically significant associations with colorectal cancer risk were observed for the candidate *IL10-1082* SNP, three tagSNPs in *IL10* (rs1800890, rs3024496, rs3024498) and one common *IL10* haplotype. The *IL10* tagSNPs were in high linkage disequilibrium with the *IL10-1082* SNP. Statistically significant associations with colorectal cancer were also observed for an ADIPOQ SNP (rs1501299), for *CRP* haplotypes, for two tagSNPs in *TLR4* (rs7873784, and rs11536891), but not for the *TLR4* haplotypes.

IL-10 is an anti-inflammatory cytokine that regulates the growth and differentiation of B cells, natural killer cells, cytotoxic, helper and regulatory T cells [14]. IL-10 deficient mice have been shown to develop colitis and colorectal cancer [26, 27]. A recent genome-wide association study implicated a downstream *IL10* SNP (rs3024505) to ulcerative colitis susceptibility [28]. The two candidate *IL10* SNPs (rs1800872 and rs1800896) that were investigated are located in the promoter region and have been consistently associated with changes in transcriptional activity and expression [24, 25]. Our finding of an inverse association between the G allele at the *-1082* locus and colorectal cancer risk is consistent with the prior observation that the G allele increases IL-10 expression [24, 25]. Two case-control studies did not report statistically significant associations for the *IL10-1082* SNP [7, 9]; however, the association in both of the studies was in the same direction but much weaker compared to our finding. One of the studies was conducted in the United States, the other in Scotland; they were slightly bigger than our study but the minor allele frequency in the controls for the *IL10-1082* SNP was very similar to the frequency in our population.

Adiponectin is a protein produced by fat cells that increases insulin sensitivity by increasing tissue fat oxidation, resulting in reduced circulating fatty acid levels and, reduced intracellular triglyceride contents in liver and muscle [29]. Adiponectin knockout mice exhibit moderate to severe diet-induced insulin resistance [30]. Circulating adiponectin concentrations have been inversely associated with colorectal neoplasia in prospective studies [31, 32]. Adiponectin may attenuate carcinogenesis by promoting apoptosis and inhibiting angiogenesis [33]. The *ADIPOQ* (rs1501299) A allele has been shown to increase expression of blood adiponectin [34, 35]. Our observed association is in the opposite direction to what was expected based on the expression studies. A recent publication of two case-control studies showed inconsistent results for the rs1501299 SNP, but a consistent statistically significant association for another SNP in the 5' flanking region of *ADIPOQ*, which however belonged in a different haplotype block than the rs1501299 SNP [36].

C-reactive protein is an inflammatory mediator produced by the liver in response to cytokines released by phagocytes. C-reactive protein mimics antibodies by acting as an opsonin and activates the complement system [37]. It is positively associated with incidence of cardiovascular disease, diabetes, total mortality, and poorer colorectal cancer prognosis [38, 39]. Epidemiologic findings for colorectal cancer incidence have been inconsistent [3]; however, a prior study in the CLUE II cohort with the same case population observed a strong positive association between plasma concentration of C-reactive protein and colorectal cancer [5], consistent with the observed haplotype association reported in the current study. *CRP* gene polymorphisms have been associated with altered expression [40–42], including a recent study in the CLUE II cohort [43]. One prospective epidemiologic study reported no association between *CRP* SNPs (rs1205, rs1130864, and rs3093068) and colorectal cancer [44]; our study is in agreement for rs1205, but we did not study the other two SNPs. However, in our study several tagSNPs were statistically significantly associated with colorectal cancer risk leading to a significantly different distribution of haplotypes between cases and controls.

Statistically significant findings were observed for two *TLR4* tagSNPs (rs7873784 and rs11536891) and colorectal cancer risk but the haplotype analysis did not reveal any

statistically significant patterns. The candidate *TLR4* SNPs were not associated with colorectal cancer. No association was observed for rs4986790 and colorectal cancer in one epidemiologic study [45]. Finally, the *LEP-19G>A* polymorphism has been previously observed to be inversely associated with colorectal cancer risk in a case–control study, and significant interactions were observed by BMI and NSAID use [10]. Although, we observed no overall association for *LEP-19G>A*, we also found an interaction with BMI of the same direction, but not with NSAID use. Overweight or obese carriers of the A allele, which is correlated with higher plasma leptin, were at decreased risk for colorectal cancer compared to the GG genotype. This result may suggest that certain overweight/obese individuals who possess *LEP* mutations and as a result produce lower blood leptin levels are at increased risk for colorectal cancer not via the growth promoting effect of leptin but via excess adiposity pathways (e.g., insulin resistance).

Overall the association between *TCF7L2* rs7903146 was not statistically significantly associated with colorectal cancer risk except in the subgroup of moderately or well-differentiated colorectal cancer, where the TT genotype was associated with a significant 2.11-fold increased risk. The T allele has been consistently associated with type 2 diabetes [46, 47] and diabetes is a risk factor for colorectal cancer [48]. *TCF7L2* encodes HMG box transcription factor 4 involved in Wnt/ β -catenin signaling [49]. Association of nuclear β -catenin with this transcription factor promotes the expression of several compounds that have important roles in the development and progression of colorectal cancer (e.g., c-myc, cyclin D1) [50]. The T allele of this SNP was statistically significantly positively associated with colon cancer risk in a nested case–control study in the Atherosclerosis Risk in Communities study [13], no overall association was observed in a population-based case–control study but a significant interaction by aspirin/NSAID use was observed (positive association among not recent aspirin/NSAID users) [51], and an inverse association was seen in a third study conducted in the Nurses' Health Study and the Health Professionals Follow-up Study that evaluated the association between the rs12255372 SNP (in linkage disequilibrium with the rs7903146 SNP) [52].

The major strength of our study is the prospective design, where controls are selected from the same source population that gave rise to the cases. In addition, our study had a low genotyping failure proportion, colorectal cancer cases were confirmed with pathology records, and detailed information was available on cancer location, stage, and grade as well as several potential modifying factors. The limitations of our study include a small sample size, and a large number of statistical tests, which together increase the probability of false positive findings. To investigate this limitation, a method proposed by Wacholder et al. that incorporates Bayesian principles was used [23]. Accepting a false positive report probability threshold for noteworthy findings of 0.5, only the *IL10-1082* and possibly the *CRP* haplotype findings may be considered noteworthy. All remaining findings, especially those in subgroups, may be considered only for descriptive purposes. Null findings may very well be due to inadequate power instead of a true lack of an association. The subgroup analysis by NSAID use may be additionally problematic due to the lack of detailed information of NSAID use at baseline. Three SNPs (rs1800796, rs2794521, rs2808630) did not conform to Hardy–Weinberg equilibrium. To examine whether this might have affected our inferences,

odds ratios were calculated by comparing the observed genotype distributions in the cases with the expected distributions, based on the observed allele frequencies, in the controls. These estimates were inferentially the same as the findings in Tables 3 and 4.

In conclusion, our prospective study suggests that polymorphisms in *IL10*, and also possibly in *CRP* and other genes related to immune response or obesity and its metabolic sequelae may be associated with risk of colorectal cancer. More and larger studies are needed to verify our findings and to study untested SNPs in immune response and obesity related genes.

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Genes and single nucleotide polymorphisms related to inflammatory response and obesity evaluated in association with colorectal cancer in the CLUE II cohort of Washington county, Maryland, 1989

Table 1

Gene	Function	Polymorphism	Location	dbSNP no.	Variant phenotype
Candidate SNPs					
<i>IL10</i>	Anti-inflammatory, regulates T cell and macrophage function	-1082A>G	Promoter	rs1800896	G allele: increased expression [24, 25]
<i>CRP</i>	Proinflammatory, activates the complement system	-592C>A	Promoter	rs1800872	G allele: increased expression [24, 25]
		1059G>C	Exon 2	rs1800947	G allele: increased expression [40-42]
		1082C>T	3' UTR	rs1205	T allele: decreased expression [40, 41]
<i>TLR4</i>	Proinflammatory, recognizes invading microorganisms	896A>G (Asp299Gly)	Exon 4	rs4986790	A allele: increased expression [53]
<i>IL6</i>	Proinflammatory, affects proliferative & anti-apoptotic pathways	11381G>C	3' UTR	rs11536889	C allele: increased risk for prostate cancer [54]
		-597G>A	Promoter	rs1800797	G allele: increased expression [55]
		-572G>C	Promoter	rs1800796	C allele: increased expression [56]
<i>IL1B</i>	Proinflammatory, key immunomediator	-174G>C	Promoter	rs1800795	C allele: decreased expression [55-57]
		-31T>C	Promoter	rs1143627	Inconsistent evidence [58-60]
		-251T>A	Promoter	rs4073	A allele: increased expression [61]
<i>TNF</i>	Proinflammatory, key immunomediator	-308G>A	Promoter	rs1800629	A allele: increased expression [62]
<i>RNASEL</i>	Mediates antiviral and proapoptotic activities	-1385G>A (Arg462Gln)	Exon 1	rs486907	A allele: decreased expression [63]
<i>PPARG</i>	Insulin-sensitizing and anti-inflammatory effects	-49C>G (Pro12Ala)	Exon 4	rs1801282	C allele: increased risk for diabetes [64]
<i>TCF7L2</i>	Part of the WNT signaling pathway; critical for cell proliferation and adipogenesis	47833C>T	Intron	rs7903146	T allele: increased glucose, decreased insulin secretion [46, 47]
<i>ADIPOQ</i>	Insulin-sensitizing and anti-inflammatory effects	276C>A	Intron 2	rs1501299	A allele: increased expression [34, 35]
<i>LEP</i>	Inhibits food intake and stimulates energy expenditure	-19G>A	Exon 1	rs2167270	G allele: decreased expression [65]
tagSNPs					
<i>IL10</i>		-459A>T	Promoter	rs1800890	
		-885C>T	Promoter	rs1800894	
		-192A>C	Intron 1	rs3021094	
		19C>T	Intron 3	rs1554286	
		6543C>T	Intron 3	rs3024509	
		7951C>T	3' UTR	rs3024496	
<i>IL10</i>		8286A>G	3' UTR	rs3024498	

Gene	Function	Polymorphism	Location	dbSNP no.	Variant phenotype
<i>CRP</i>		-717T>C	Promoter	rs2794521	No association with expression [41, 66]
		29T>A	Intron	rs1417938	Inconsistent evidence [40-42, 67, 68]
		3407T>C	3' UTR	rs2808630	No association with expression [40, 67]
		4639A>C	3' UTR	rs3093077	Alters expression [68]
<i>TLR4</i>		-2570A>G	Promoter	rs2737190	
		-2431T>C	Promoter	rs10116253	
		-2025A>G	Promoter	rs1927914	
		-423G>A	Intron 1	rs1927911	
		-468G>T	Intron 3	rs2149356	
		12185G>C	3' UTR	rs7873784	
		12586T>C	3' UTR	rs11536891	
		13459C>A	Downstream	rs11536898	

SNP single nucleotide polymorphism; UTR untranslated region; tagSNP haplotype tagging SNP

Table 2

Baseline characteristics of colorectal cancer cases and matched controls in the CLUE II cohort of Washington County, Maryland, 1989

Characteristic	Cases (n = 208)	Controls (n = 381)	p ^{**}
Age (year), mean (SD)	62.8 (11.4)	62.8 (11.5)	Matched
Female (%)	53.9	54.6	Matched
Family history of CRC (%) ^a	12.2	7.2	0.12
Use of NSAIDs (%)	24.0	29.7	0.16
BMI (kg/m ²), mean (SD)	26.3 (4.3)	26.0 (4.1)	0.31
Use of medication to treat diabetes (%)	7.2	4.2	0.12
Cigarette smoking status			
Never (%)	48.6	52.8	0.22
Former (%)	38.4	34.1	
Current (%)	13.0	13.1	
Ever use of female hormones (%) ^b	23.2	32.7	0.03
Daily intake, mean (SD) ^c			
Alcohol (g)	4.8 (16.6)	6.6 (16.6)	0.30
Folate (μg)	381 (261)	364 (224)	0.70
Red meat (g)	70.1 (66.8)	78.1 (70.0)	0.22
Energy (kcal)	1,678 (572)	1,692 (570)	0.79
Saturated fat (g)	25.2 (12.7)	25.3 (13.1)	0.97
Fiber (g)	12.1 (4.4)	12.5 (4.9)	0.63
Calcium (mg) ^d	857 (509)	867 (420)	0.35

Cases and controls matched on age, sex, race, and date of blood draw

SD standard deviation; CRC colorectal cancer; NSAIDs non-steroidal anti-inflammatory drugs

^{**} Linear or logistic regression with each characteristic as the response variable, accounting for the matched design using generalized estimating equations with an exchangeable correlation structure and robust estimation of standard errors. Highly skewed characteristics were transformed using the natural logarithm

^a Among those who returned questionnaires in 1996 (131 cases and 235 matched controls)

^b Use of oral contraceptives or hormone replacement therapy (112 cases and 208 matched controls)

^c Among those who completed a valid food frequency questionnaire (142 cases and 249 matched controls)

^d Diet and supplement use

Table 3

Odds ratios and 95% confidence intervals of colorectal cancer for 17 candidate single nucleotide polymorphisms in genes related to inflammatory response and obesity in the CLUE II cohort of Washington County, Maryland, 1989

Genotype	Cases, <i>n</i>	Controls, <i>n</i>	OR (95% CI)*
<i>IL10</i>			
rs1800896			
A/A	69	98	1.00 (Ref)
A/G	101	187	0.79 (0.53–1.18)
G/G	35	87	0.58 (0.35–0.95)
$p^{\dagger}_{\text{trend}}$			0.03
G-carrier	136	274	0.72 (0.50–1.04)
rs1800872			
C/C	123	213	1.00 (Ref)
A/C	71	131	0.96 (0.66–1.39)
A/A	9	17	0.98 (0.41–2.32)
$p^{\dagger}_{\text{trend}}$			0.85
A-carrier	80	148	0.96 (0.67–1.38)
<i>CRP</i>			
rs1800947			
G/G	185	331	1.00 (Ref)
C-carrier [‡]	20	42	0.92 (0.52–1.63)
rs1205			
C/C	99	167	1.00 (Ref)
C/T	83	156	0.92 (0.64–1.32)
T/T	24	51	0.81 (0.48–1.37)
$p^{\dagger}_{\text{trend}}$			0.42
T-carrier	107	207	0.89 (0.63–1.25)
<i>TLR4</i>			
rs4986790			
A/A	170	325	1.00 (Ref)
G-carrier [‡]	32	44	1.28 (0.77–2.10)
rs11536889			
G/G	141	270	1.00 (Ref)
C/G	59	89	1.30 (0.88–1.92)
C/C	5	11	0.86 (0.29–2.51)
$p^{\dagger}_{\text{trend}}$			0.38
C-carrier	64	100	1.25 (0.86–1.83)
<i>IL6</i>			
rs1800797			
G/G	70	120	1.00 (Ref)

Genotype	Cases, <i>n</i>	Controls, <i>n</i>	OR (95% CI)*
A/G	96	179	0.94 (0.64–1.38)
A/A	37	68	1.03 (0.62–1.71)
$p^{\dagger}_{\text{trend}}$			0.98
A-carrier	133	247	0.96 (0.67–1.39)
rs1800796			
G/G	180	329	1.00 (Ref)
C/G	19	30	1.20 (0.65–2.20)
C/C	2	3	1.00 (0.16–6.14)
$p^{\dagger}_{\text{trend}}$			0.63
C-carrier	21	33	1.18 (0.66–2.09)
rs1800795			
G/G	68	113	1.00 (Ref)
C/G	93	170	0.87 (0.58–1.30)
C/C	39	71	0.93 (0.57–1.54)
$p^{\dagger}_{\text{trend}}$			0.72
C-carrier	132	241	0.89 (0.61–1.30)
<i>IL1B</i>			
rs1143627			
T/T	92	181	1.00 (Ref)
C/T	91	153	1.13 (0.78–1.65)
C/C	21	37	1.06 (0.58–1.93)
$p^{\dagger}_{\text{trend}}$			0.65
C-carrier	112	190	1.12 (0.78–1.60)
<i>IL8</i>			
rs4073			
T/T	65	114	1.00 (Ref)
A/T	88	162	0.97 (0.64–1.46)
A/A	52	86	1.12 (0.70–1.79)
$p^{\dagger}_{\text{trend}}$			0.66
A-carrier	140	248	1.02 (0.70–1.49)
<i>TNF</i>			
rs1800629			
G/G	146	275	1.00 (Ref)
A/G	55	90	1.15 (0.77–1.71)
A/A	3	7	0.78 (0.20–3.08)
$p^{\dagger}_{\text{trend}}$			0.69
A-carrier	58	97	1.12 (0.76–1.66)
<i>RNASEL</i>			
rs486907			
G/G	84	146	1.00 (Ref)

Genotype	Cases, <i>n</i>	Controls, <i>n</i>	OR (95% CI) [*]
A/G	89	165	0.97 (0.66–1.41)
A/A	24	51	0.81 (0.47–1.41)
$p^{\dagger}_{\text{trend}}$			0.52
A-carrier	113	216	0.93(0.65–1.33)
<i>PPARG</i>			
rs1801282			
C/C	165	295	1.00 (Ref)
C/G	37	68	1.03 (0.65–1.61)
G/G	1	6	0.30 (0.04–2.53)
$p^{\dagger}_{\text{trend}}$			0.70
G-carrier	38	74	0.98 (0.63–1.54)
<i>TCF7L2</i>			
rs7903146			
C/C	99	190	1.00 (Ref)
C/T	77	132	1.13 (0.78–1.66)
T/T	26	32	1.44 (0.81–2.57)
$p^{\dagger}_{\text{trend}}$			0.22
T-carrier	103	164	1.20 (0.84–1.71)
<i>ADIPOQ</i>			
rs1501299			
C/C	96	198	1.00 (Ref)
A/C	86	134	1.42 (0.96–2.10)
A/A	19	27	1.41 (0.75–2.63)
$p^{\dagger}_{\text{trend}}$			0.09
A-carrier	105	161	1.42 (0.99–2.04)
<i>LEP</i>			
rs2167270			
G/G	80	131	1.00 (Ref)
A/G	91	170	0.87 (0.59–1.29)
A/A	33	61	0.85 (0.50–1.43)
$p^{\dagger}_{\text{trend}}$			0.47
A-carrier	124	231	0.86 (0.60–1.25)

* From a conditional logistic regression model. Cases and controls were matched on age, sex, race, and date of blood draw

[†] Entered into the model as single ordinal variable with values corresponding to number of variant alleles

[‡] The ORs for variant homozygotes were not estimable because of zero counts in cases or controls

Table 4

Odds ratios and 95% confidence intervals of colorectal cancer for 19 tag single nucleotide polymorphisms in *IL10*, *CRP*, and *TLR4* in the CLUE II cohort of Washington County, Maryland, 1989

Genotype	Cases, <i>n</i>	Controls, <i>n</i>	OR (95% CI)*
<i>IL10</i>			
rs1800890			
A/A	80	124	1.00 (Ref)
A/T	89	174	0.77 (0.53–1.13)
T/T	21	54	0.63 (0.35–1.12)
$p^{\dagger}_{\text{trend}}$			0.08
T-carrier	110	228	0.74 (0.51–1.06)
rs1800894			
C/C	191	333	1.00 (Ref)
C/T	8	21	0.64 (0.27–1.52)
rs3021094			
A/A	173	314	1.00 (Ref)
C-carrier ‡	30	51	1.10 (0.67–1.81)
rs1554286			
C/C	132	245	1.00 (Ref)
C/T	65	108	1.17 (0.80–1.72)
T/T	6	10	1.22 (0.44–3.42)
$p^{\dagger}_{\text{trend}}$			0.41
T-carrier	71	118	1.18 (0.81–1.71)
rs3024509			
C/C	183	320	1.00 (Ref)
T-carrier ‡	19	42	0.79 (0.44–1.43)
rs3024496			
C/C	63	86	1.00 (Ref)
C/T	99	173	0.74 (0.49–1.12)
T/T	36	86	0.51 (0.31–0.86)
$p^{\dagger}_{\text{trend}}$			0.01
T-carrier	135	259	0.66 (0.45–0.97)
rs3024498			
A/A	135	196	1.00 (Ref)
A/G	62	133	0.64 (0.44–0.94)
G/G	6	25	0.33 (0.13–0.88)
$p^{\dagger}_{\text{trend}}$			0.002
G-carrier	68	158	0.60 (0.42–0.85)
<i>CRP</i>			
rs2794521			
T/T	95	206	1.00 (Ref)

Genotype	Cases, <i>n</i>	Controls, <i>n</i>	OR (95% CI)*
C/T	84	122	1.60 (1.10–2.33)
C/C	20	33	1.33 (0.73–2.43)
$p^{\dagger}_{\text{trend}}$			0.05
C-carrier	104	155	1.54 (1.08–2.17)
rs1417938			
T/T	109	177	1.00 (Ref)
A/T	74	140	0.80 (0.55–1.16)
A/A	15	43	0.56 (0.30–1.05)
$p^{\dagger}_{\text{trend}}$			0.06
A-carrier	89	183	0.75 (0.53–1.07)
rs2808630			
T/T	96	204	1.00 (Ref)
C/T	84	124	1.56 (1.07–2.29)
C/C	19	34	1.21 (0.66–2.21)
$p^{\dagger}_{\text{trend}}$			0.10
C-carrier	103	158	1.47 (1.04–2.09)
rs3093077			
A/A	167	335	1.00 (Ref)
C-carrier [‡]	34	35	1.88 (1.11–3.18)
TLR4			
rs2737190			
A/A	88	149	1.00 (Ref)
A/G	77	150	0.86 (0.57–1.28)
G/G	22	40	0.82 (0.45–1.52)
$p^{\dagger}_{\text{trend}}$			0.42
G-carrier	99	190	0.85 (0.58–1.24)
rs10116253			
T/T	116	179	1.00 (Ref)
C/T	63	122	0.72 (0.48–1.09)
C/C	9	16	0.84(0.34–2.11)
$p^{\dagger}_{\text{trend}}$			0.20
C-carrier	72	138	0.74 (0.49–1.10)
rs1927914			
A/A	93	154	1.00 (Ref)
A/G	85	155	0.88 (0.60–1.29)
G/G	23	43	0.81 (0.45–1.47)
$p^{\dagger}_{\text{trend}}$			0.41
G-carrier	108	198	0.87 (0.61–1.24)
rs1927911			
G/G	122	201	1.00 (Ref)

Genotype	Cases, <i>n</i>	Controls, <i>n</i>	OR (95% CI) [*]
A/G	70	134	0.87 (0.59–1.26)
A/A	12	26	0.80 (0.37–1.73)
$p^{\dagger}_{\text{trend}}$			0.39
A-carrier	82	160	0.86 (0.60–1.23)
rs2149356			
G/G	80	137	1.00 (Ref)
G/T	70	110	1.08 (0.69–1.70)
T/T	18	27	1.01 (0.48–2.14)
$p^{\dagger}_{\text{trend}}$			0.85
T-carrier	88	137	1.06 (0.70–1.63)
rs7873784			
G/G	159	244	1.00 (Ref)
C/G	33	86	0.57 (0.36–0.91)
C/C	4	7	0.71 (0.17–2.86)
$p^{\dagger}_{\text{trend}}$			0.03
C-carrier	37	93	0.58 (0.37–0.91)
rs11536891			
T/T	156	254	1.00 (Ref)
C/T	32	84	0.65 (0.41–1.02)
C/C	5	7	1.07 (0.30–3.83)
$p^{\dagger}_{\text{trend}}$			0.13
C-carrier	37	91	0.68 (0.44–1.04)
rs11536898			
C/C	157	252	1.00 (Ref)
A/C	33	68	0.77 (0.48–1.22)
A/A	2	4	0.79 (0.14–4.46)
$p^{\dagger}_{\text{trend}}$			0.27
A-carrier	35	72	0.77 (0.48–1.21)

* From a conditional logistic regression model. Cases and controls were matched on age, sex, race, and date of blood draw

[†] Entered into the model as single ordinal variable with values corresponding to number of variant alleles

[‡] The ORs for variant homozygotes were not estimable because of zero counts in cases or controls

Table 5

Odds ratios and 95% confidence intervals of colorectal cancer for *IL10*, *CRP*, *TLR4*, and *IL6* haplotypes in the CLUE II cohort of Washington County, Maryland, 1989

Genotype [¶]	Cases (%)	Controls (%)	OR (95% CI) ^{**}
<i>IL10</i> [†]	<i>n</i> = 173	<i>n</i> = 322	
A-C-A-C-C-C-A	0.38	0.32	1.00 (Ref)
T-C-A-C-C-T-G	0.16	0.22	0.59 (0.40–0.87)
T-C-A-C-C-T-A	0.18	0.17	0.90 (0.62–1.32)
A-C-A-T-C-C-A	0.12	0.12	0.88 (0.57–1.36)
A-C-C-T-C-C-A	0.04	0.06	0.71 (0.37–1.35)
<i>p</i> ^{††} _{value}			0.39
<i>CRP</i> [*]	<i>n</i> = 190	<i>n</i> = 349	
T-T-T-A	0.33	0.37	1.00 (Ref)
T-A-T-A	0.26	0.31	0.97 (0.71–1.32)
C-T-C-A	0.31	0.26	1.35 (0.99–1.85)
T-T-T-C	0.05	0.09	2.26 (1.32–3.88)
<i>p</i> ^{††} _{value}			0.04
<i>TLR4</i> [‡]	<i>n</i> = 144	<i>n</i> = 246	
A-T-A-G-G-T-C	0.68	0.68	1.00 (Ref)
G-C-G-A-T-C-C-A	0.11	0.12	0.99 (0.62–1.59)
G-C-G-A-T-G-T-C	0.10	0.09	1.02 (0.63–1.66)
G-T-G-G-T-G-T-C	0.08	0.07	1.31 (0.74–2.31)
<i>p</i> ^{††} _{value}			0.81
<i>IL6</i> [§]	<i>n</i> = 195	<i>n</i> = 343	
G-G-G	0.51	0.51	1.00 (Ref)
A-G-C	0.40	0.43	0.94 (0.73–1.22)
G-C-G	0.06	0.05	1.14 (0.66–1.98)
<i>p</i> ^{††} _{value}			0.68

[†]TagSNPs: rs1800890, rs1800894, rs3021094, rs1554286, rs3024509, rs3024496, and rs3024498. Values of $r^2 = 0.8$ ($D' > 0.98$) were observed for the *IL10* rs1800872-rs1554286, rs1800896-rs1800890, rs1800896-rs3024496, and rs1800890-rs3024496 pairs

^{*}TagSNPs: rs2794521, rs1417938, rs2808630, and rs3093077. Linkage disequilibrium between the *CRP* allelic variants rs2794521 and rs2808630 was complete ($r^2 = 0.99$, $D' = 0.99$). All other pair-wise r^2 values for the *CRP* SNPs were below 0.50

[‡]TagSNPs: rs2737190, rs10116253, rs1927914, rs1927911, rs2149356, rs7873784, rs11536891, and rs11536898. Most *TLR4* tagSNPs were in linkage disequilibrium ($r^2 > 0.8$, $D' > 0.97$) with each other but not with the candidate SNPs ($r^2 < 0.39$)

[§]Candidate SNPs: rs1800797, rs1800796, and rs1800795. Linkage disequilibrium between the *IL6* allelic variants rs1800795 and rs1800797 was complete ($r^2 = 0.96$, $D' = 0.99$). All other pair-wise r^2 values for the *IL6* SNPs were below 0.20

[¶]Haplotypes shown only if present in >5% of individuals

^{**}From a generalized linear model using regression substitution adjusted for age and sex

^{††}From a global score test to assess differences in overall haplotype distribution between cases and controls adjusting for age and sex