Published in final edited form as:

Mutat Res. 2009 January 15; 660(1-2): 12–21. doi:10.1016/j.mrfmmm.2008.10.001.

Colon tumor mutations and epigenetic changes associated with genetic polymorphism: Insight into disease pathways

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Abstract

Variation in genes associated with serum levels of proteins may be useful for examining specific disease pathways. Using data from a large study of colon cancer, we examine genetic variants in insulin, inflammation, estrogen, metabolizing enzymes, and energy homeostasis genes to explore associations with microsatellite instability (MSI), CpG Island methylator phenotype (CIMP), mutations of *p53* in exons 5 through 8, and mutations in codons 12 and 13 of *Ki-ras*. Insulin-related genes were associated with CIMP positive and MSI tumors, with the strongest associations among aspirin users. The Fok1 Vitamin D Receptor (*VDR*) polymorphism was associated with CIMP positive/*Ki-ras* mutated tumors; the Poly A and *CDX2 VDR* polymorphisms were associated only with *Ki-ras* mutated tumors. *NAT2* was associated with CIMP positive/*Ki-ras* mutated tumors but not with MSI tumors. The *TCF7L2* rs7903146 polymorphism was associated with *p53* mutated tumors. Most associations varied by recent aspirin/NSAID use: *IL6* rs1800796 and rs1800795 polymorphisms were associated inversely with tumor mutations in the presence of aspirin/NSAIDs; *POMC* significantly reduced risk of *Ki-ras*- mutated tumors when aspirin/NSAIDs were not used; the *TCF7L2* rs7903146 was associated with reduced risk of *Ki-ras*-mutated tumors in the presence of aspirin and increased risk in the absence of aspirin. These data, although exploratory, identify specific tumor subsets that may be associated with specific exposures/polymorphism combinations. The important modifying effects of aspirin/NSAIDs on associations with genetic polymorphisms reinforce the underlying role of inflammation in the etiology of colon cancer.

Keywords

Insulin; polymorphisms; colon cancer; aspirin; non-steroidal anti-inflammatory; CIMP; *p53*; MSI; *Ki-ras*

In 1988 it was proposed that colorectal cancers (CRC) followed a series of mutational events, with the majority of tumors initially having an *APC* mutation that was followed by mutations in the *p53* and *Ki-ras* genes [1]. Existing evidence suggests that CRC pathways that once were believed to be overlapping, are often unique, and that many tumors do not have multiple mutations [2]. It is possible that insight into unique pathways resulting in epigenetic changes and mutations in colorectal tumors can be obtained when evaluated in conjunction with inherited susceptibility, diet, lifestyle, and environmental exposure data.

Although studies are limited, there is evidence that examination of epigenetic and genetic mutations in colon tumors and adenomas are associated with certain exposures that alter the likelihood of developing those mutations. For instance, cigarette smoking has been associated with greater risk of developing tumors that have microsatellite instability (MSI) [3] and CpG Island methylator phenotype (CIMP) [4] and specifically with hyperplastic rather than adenomatous polyps [5]. Dietary folate has been associated with specific *Ki-ras* mutations in adenomas and cancers [6,7]. We also have reported that polymorphisms in certain insulin-related genes influence *p53* mutations [8]. While several pathways, including insulin, estrogen, and inflammation have been proposed as being important in colon cancer etiology [9–12], we have not systematically examined how these pathways influence the broad spectrum of acquired genetic and epigenetic changes in tumors.

Genetic variation in genes associated with serum levels of various proteins may be a useful for examining specific pathways. For instance the –202 *IGFBP3* genetic variant has been associated with serum levels of IGFBP-3 [13,14], variants of the *IGF1* gene has been associated with serum IGF-1 levels [15]; and variants of the *IL6* gene has been associated with IL-6 levels [16]. Thus, assessment of genetic variants with tumor markers may further our understanding of how major pathways lead to specific types of mutations in tumors.

In this study, we examine genetic variants that are linked to the pathways hypothesized to contribute to colon cancer. Because little is known about factors that contribute to specific tumor mutations, we explore variation in genes in pathways including insulin, estrogen, carcinogen metabolism, inflammation, and energy homeostasis to describe exposure-specific pathways that can be linked to specific tumor markers. Although the analyses are exploratory in nature, we believe that the results add to our previous work by simultaneously examining a wide spectrum of epigenetic and genetic changes in tumors, including MSI, CIMP, *p53*, and *Ki-ras*, and will help us to gain insight into the carcinogenic process and generate hypotheses that can be tested in other studies.

Methods

The Institutional Review Board from all centers approved all aspects of the study. A case-control study of 1839 colon cancer cases and 2014 controls was conducted among black, white, or Hispanic people from either the Kaiser Permanente Medical Care Program (KPMCP) of Northern California, an eight county area in Utah (Davis, Salt Lake, Utah, Weber, Wasatch, Tooele, Morgan, and Summit counties), or the Twin Cities Metropolitan area in Minnesota. Eligibility criteria for cases included diagnosis with first-primary incident colon cancer (ICD-O 2nd edition codes 18.0, 18.2 to 18.9) between October 1, 1991 and September 30, 1994, between 30 and 79 years of age at time of diagnosis, and mentally competent to complete the interview. Cases with adenocarcinoma or carcinoma of the rectosigmoid junction or rectum (defined as the first 15 cm from the anal opening), with known familial adenomatous polyposis, ulcerative colitis, or Crohn's disease were not eligible. Of all cases asked to participate, 75.6% cooperated. We obtained tumor blocks from 96% of cases identified in Utah, from 81% of cases at KPMCP, and from 38% of cases in Minnesota.

Controls, in addition to the eligibility criteria for cases, could not at the time of recruitment have had a previous colorectal tumor. Controls were selected from eligibility lists for KPMCP, driver's license lists for Minnesota, and random-digit-dialing, driver's license lists, or Health Care Finance Administration (HCFA) lists for Utah. These methods have been described in detail [17]. Of all controls selected, 63.7% participated.

All data were collected in-person by trained and certified interviewers [17] using a laptop-administered computerized questionnaire. The referent period for the study was the calendar year approximately two years prior to date of diagnosis for cases and a comparable time period from selection for controls. Information was collected on demographic factors such as age, sex, and center, diet, physical activity [17,18], adult height and weight two and five years prior to diagnosis, regular use of aspirin and/or non-steroidal anti-inflammatory drugs, cigarette smoking history, and medical history.

Genetic Analysis

Tumor DNA was obtained from paraffin-embedded tissue as described previously. Tumors were previously characterized by their genetic profile that include sequence data for exons 5 through 8, or the mutation hotspots of the *p53* gene; sequence data for *Ki-ras* codons 12 and 13; five CpG Island markers MINT1, MINT2, MINT31, *p16*, and *hMLH1*; and microsatellite instability (MSI). This study uses MSI markers *Bat-26* (a mononucleotide repeats which is a very good measure of generalized instability), *TGF β RII* (a coding mononucleotide repeat which is unstable in most colorectal cancers with MSI) and a panel of 10 tetranucleotides repeats [19–22] since it precedes the establishment of the Bethesda Panel for microsatellite instability [23]. The CIMP markers used were those used in early studies and have been able to discriminate associations with lifestyle exposures in this study population [4]. Tumor marker data were available for 1212 MSI stable and 212 MSI unstable, 920 CIMP negative and 334 CIMP positive, 732 *p53* non-mutated and 642 *p53* mutated, and 911 *Ki-ras* non-mutated and 433 *Ki-ras* mutated cases that also had germline data for polymorphism testing.

Germline DNA obtained from blood samples provided by study participants or from normal tissue in the paraffin blocks was used to examine variation in genes along important colon cancer disease pathways. The polymorphisms examined are shown in Table 2 and represent major pathways hypothesized to influence colon cancer risk: inflammation; energy homeostasis; hormones including both insulin and sex steroid hormones; and metabolizing genes. Several genes, such as *apoE*, *PPAR γ* , the vitamin D receptor (*VDR*), and the adenomatous polyposis coli (*APC*) gene may influence multiple pathways. The polymorphisms assessed were initially selected as candidate polymorphisms hypothesized to be associated with colon cancer; the main effects of most of these polymorphisms have been reported previously [12,24–34]. All genes examined were in Hardy-Weinberg equilibrium in non-Hispanic white controls except for *CYP2C9* I359L and *IGF1R*.

Statistical Methods

Using the maximum efficiency robust test (MERT), we compared the trend tests for each mode of inheritance to determine the most appropriate model for each gene, as being either recessive, dominant, or additive [35]. Briefly, MERT is a linear combination of the test statistics for the recessive and dominant models that tests genotype trend in cases where the mode of inheritance is unknown. Using this technique, we determined if the best fitting model for each gene was recessive, dominant or additive. However, in some instances the homozygote variant was sufficiently rare that it was only possible to evaluate the dominant model.

The polymorphism associations were run using polytomous logistic regression models that included the outcomes of control, MSI, CIMP, *p53*, *Ki-ras*. Odds ratios (OR) and 95% Confidence Intervals (CI) are reported along with the p value for a linear trend. Although we report the exact p value, we refer to the 0.05 level as being statistically significant in the text and consider p values of <0.10 as borderline significant. Some cases were in multiple categories if they had a mutation in more than one of the tumor markers. To account for the fact that some cases were in multiple categories, we treated each subject as a cluster or primary sampling unit rather than each mutation in order to estimate the variance among subjects. In these models, adjustments were made for factors associated with colon cancer, including age, gender, body mass index (kg/m²), long-term vigorous physical activity, use of aspirin/NSAIDs, usual number of cigarettes smoked per day, and dietary calcium and energy. Additionally, we stratified data by use of aspirin and non-steroidal anti-inflammatory drugs within the past two years (labeled as NSAIDs) because previous analysis suggests that NSAIDs are major effect modifiers for many risk factors [36]. The data were analyzed using the procedure SURVEYLOGISTIC in SAS version 9.1 (Cary, NC). We examined all genes described in Table 2 for overall associations and for associations that varied by recent use of aspirin/NSAIDs. We present data for genes that appear to have unique associations with specific tumor markers as determined by either a significant linear trend across genotypes or because of significant association for the homozygote variant genotype.

Results

Characteristics of the population are described in Table 1. The majority of the population was over 60 years of age. Approximately half of tumors were proximal and half were distal. Controls were more likely to report having recently used aspirin/NSAIDs within the past two years than cases. The most common tumor variant was a *p53* mutation (46.7% of tumors) and the least common was MSI (14.9%).

Genes and specific polymorphisms examined are shown in Table 2 along with their minor allele frequency (MAF) among non-Hispanic white controls. Because many genes can function in several pathways, we have noted the pathways that we believe are most relevant to the specific gene examined.

The polymorphism in *POMC* was associated with *Ki-ras* mutations and of borderline significance with *p53* mutations (Table 3). *LEPR* was associated with colon cancer overall and had similar levels of association with *p53* mutations although the confidence intervals ranged from 0.52 to 1.05. Genes associated with insulin and/or inflammation related pathways were associated with several tumor markers. The CG/GG genotypes of *PPAR γ* were significantly associated with reduced likelihood of having an MSI or *p53* tumor and of borderline significance with CIMP-positive tumors. *VDR* polymorphisms were associated with a variety of tumor markers, depending upon the polymorphism evaluated. For instance, the polyA and Bsm1 (data not shown in table for Bsm1) and *CDX2* polymorphisms were associated with *Ki-ras* mutations. On the other hand, the *VDR* Fok1 polymorphism was directly associated with *Ki-ras* and CIMP-positive tumors and inversely associated with MSI tumors. *NAT2* intermediate/rapid acetylators have the strongest associations with CIMP-positive and *Ki-ras*-mutated tumors and no association with MSI tumors. Statistically significant associations were noted for the CA repeat in the *IGF1* gene with *Ki-ras*-mutated tumors; *IGF1R*, *IRS1*, and *IRS2* were associated mainly with CIMP-positive and MSI tumors. *TCF7L2* was directly associated with *p53*-mutated tumors.

Evaluation of tumor markers among those who recently used aspirin/NSAIDs (Table 4) and those who did not (Table 5) showed several associations that were modified by recent use.

POMC continued to be significantly inversely associated with *Ki-ras* mutations among those not recently using aspirin/NSAIDs (Table 5). *LEP* rs7799039 was inversely associated with MSI tumors among non-aspirin/NSAID users (Table 5) and showed a strong direct association with MSI and CIMP-positive tumors among recent aspirin/NSAIDs users (Table 4). Both *IL6* polymorphisms were associated with a statistically significant reduced risk of having a *p53* mutation among recent aspirin/NSAID users (Table 4). The *VDR* polyA polymorphism was associated with reduced risk of developing a *Ki-ras* mutation only among non-aspirin/NSAID users (Table 5), while the *VDR* Fok1 polymorphism was associated with an increased likelihood of both *Ki-ras* and CIMP-positive tumor for users and non-users of aspirin/NSAIDs alike. Unlike the Fok1 polymorphism, the *CDX2* polymorphism appeared to be associated strongly with *Ki-ras* mutations and have weaker associations with CIMP-positive tumors among those who reported recent use of aspirin/NSAIDs (Table 4). Insulin-related polymorphisms appeared to have stronger associations with CIMP-positive and MSI tumors among people who reported recent use of aspirin/NSAIDs (Table 4). *IGF1R* and *IGFBP3* were strongly associated with increased risk of CIMP-positive and MSI tumors (*IGF1R* only) among those with the variant allele. Having an A allele of the *IRS1* gene was associated with increased colon cancer risk regardless of aspirin/NSAID use. The CC/TT genotype of *TCF7L2* was significantly inversely associated with *Ki-ras*-mutated tumors among recent aspirin/NSAID users (Table 4) and was directly associated with CIMP-positive, *p53*, and *Ki-ras* tumors (Table 5).

Discussion

Results from this study provide some evidence that specific exposure/susceptibility combinations may lead to unique tumor mutations, thus potentially explaining the heterogeneity of colon cancer. Although the analyses presented here were exploratory in nature, they suggest that recent use of aspirin/NSAIDs modify associations with several pathways, most notably those involving insulin and inflammation-related genes. These findings, although descriptive, provide avenues for scientific inquiry into the cause of various mutations that occur in tumors and support the idea of multiple and sometimes interacting pathways that lead to colon cancer development. Many tumors with positive levels of CIMP have been shown to have MSI, whereas another set of CIMP-positive but MSS tumors frequently are accompanied by a *Ki-ras* mutation and are sometimes labeled as CIMP-2 [37]. In our data, approximately 27% of tumors were classified as CIMP-positive, while approximately 15% were MSI. We have reported previously that some risk factors for MSI, such as alcohol, are associated only with those tumors that are CIMP-negative and MSI, while others such as cigarette smoking are associated with both CIMP-positive and MSI tumors [3,4,38,39]. Those data along with the current data suggest that there may be multiple pathways to CIMP-positive and MSI tumors which include both insulin and inflammation. The data presented here lend additional support for a CIMP-positive *Ki-ras* pathway that is independent of MSI. Both CIMP-positive and MSI tumors were associated with *IGF1R*, especially in the presence of aspirin/NSAIDs and *IGFBP3* (−202 C>A variant) was associated with increased risk of CIMP-positive tumors among users of aspirin/NSAIDs, while inversely associated with MSI among non-aspirin/NSAID users. These associations suggest that CIMP appears to be influenced by insulin-related factors that may be, at least in part, regulated by the underlying inflammatory state. Although our study was exploratory in nature, other studies have shown that *IGF1R* influences IGF-1 levels, and that it regulates COX-2 expression via the MAK/Erk pathway [40,41], thus providing some support for our observations. Overall, *IRS1* and *IRS2* appear to have stronger associations with MSI and CIMP-positive tumors than other tumor markers, but these associations appear to be less influenced by aspirin/NSAIDs use.

Genes, such as *POMC*, *LEP*, *LEPR*, and *PPAR γ* have been hypothesized as being involved in multiple pathways including energy homeostasis, inflammation and insulin that could influence colon cancer risk. *POMC* had a greater influence on colon cancer risk in the absence of NSAIDs. Although *POMC* has been associated with energy homeostasis [42], one of the functions of *POMC* is anti-inflammatory. Given the interaction with NSAIDs, *POMC* appears to have a greater effect in the absence of anti-inflammatory drugs, suggesting the anti-inflammatory mechanisms are operational for the *Ki-ras* pathway. While *LEP* and *LEPR* have been hypothesized mainly as involved in regulation of energy homeostasis, involvement in both insulin and inflammation has been suggested [43,44]. Our data suggest possibly greater involvement in the latter two pathways given the effect modification by aspirin/NSAID use. *LEP* (rs7799039) was directly associated with both MSI and CIMP among aspirin/NSAID users and inversely associated with MSI among non-users of aspirin/NSAIDs. *PPAR γ* likewise may be involved in an inflammation-related pathway that appears to alter risk of both MSI and CIMP tumors among non-aspirin/NSAID users. The underlying inflammatory state of the GI tract may be altered by the recent use of aspirin/NSAID thus modifying the ultimate risk associated with inflammation and insulin-related genes.

The inflammatory process is thought to be a key underlying component of colon cancer that is initiated by pro-inflammatory cytokines such as IL-6 in response to an inflammatory insult. IL-6 has been shown to stimulate secretion of C-reactive protein (CRP), an important biomarker for pro-inflammatory status in several diseases. Polymorphisms in the *IL6* gene promoter have been reported to be related to levels of circulating C-reactive protein [45], to be associated with different profiles of plasma *IL6* response to immunization [46], and to modify the association between high BMI and incident type-2 diabetes [47]. The rs1800975 (–174) and the rs1800796 polymorphism (–572 G>C) of the *IL6* gene have been associated with IL-6 levels, body size, and colorectal cancer [48–51]. We have previously reported that *IL6* rs1800795 and rs1800796 are associated with reduced risk of colon cancer among people who report recently using aspirin/NSAIDs [29]; the reduced risk for the rs1800795 variant appears to be greatest for *p53* mutations while the rs1800796 appears to influence all tumor types among NSAID users with no influence for any tumor type among non-users of aspirin/NSAIDs.

The transcription factor 7-like 2 (*TCF7L2*) rs7903146 marker was identified in genome-wide association studies as being associated with type-2 diabetes [52]; studies have corroborated these findings with the T allele associated with impaired insulin secretion [53]. In addition to its functional role in insulin regulation, *TCF7L2* is involved in the *Wnt*/ β -catenin signaling pathway that is central to colon cancer [2,54,55]. Although having a T allele was associated with an increased likelihood of having a *p53* mutation, the associations differed by NSAIDs use. We have previously shown that NSAIDs modify the overall colon cancer risk associated with *TCF7L2* [56]. In this study, we show that the inverse association among recent aspirin/NSAID users is confined to a reduced risk of having a *Ki-ras* mutation and to a lesser extent CIMP-positive profile, whereas an increased risk for CIMP-positive, *p53*, and *Ki-ras* mutations is observed among non-aspirin/NSAID users.

Not all polymorphisms in one gene may have the same functional effects or even be involved in the same disease pathway. *VDR* has many functions which may be dependent on the site of the polymorphism examined [57]. Our previous analyses have shown that *VDR* polymorphisms may influence colon and rectal tumor sites differently [30]. In these analyses, we show consistent associations for *Ki-ras* mutations for all *VDR* polymorphisms, however, Fok1 and CDX2 polymorphisms may have additional functions given associations with CIMP-positive tumors. It also is interesting to note that *VDR* polymorphisms can increase risk of CIMP-positive tumors while reducing risk of having a MSI tumor. These

data suggest that a CIMP-positive/*Ki-ras*-mutated tumor may be relevant for some *VDR* markers. The fact that these associations are at least in part modified of NSAIDs use suggests an underlying inflammation-regulatory component to the observed associations.

The association between *NAT2* and colon cancer is mixed in the literature, with most studies showing no or modest effects [58]. Data evaluating *NAT2* with specific tumor markers has not, to our knowledge, been done previously. In our data, intermediate/rapid acetylators have much lower odds of developing CIMP-positive and *Ki-ras* tumors and to a lesser extent, *p53* tumors; this effect is seen for both users and non-users of NSAIDs, suggesting a pathway that is independent of inflammation. This tumor-specific association could contribute to the inconsistent associations between *NAT2* and colon cancer that exist in the literature.

There are several strengths to the study. We have a large collection of cases with tumor markers that were derived from a population-based sample that included all colon cancer cases identified over a three-year time period from three separate geographic areas. Thus, we believe our tumor data are representative of the most common tumor mutations that exist in colon cancers. However there are also limitations. Errors in genotyping could contribute to misclassification of exposure or outcome. Polymorphism exposures had elements of quality control to minimize, if not prevent genotyping errors. As part of the quality control, each genotype was included in every plate analyzed; all genotypes were in HWE. There is a greater likelihood of errors in outcomes since as we have described we did not assess the entire gene for *p53* and *Ki-ras*, but looked at the common hotspots. Likewise, while our markers of CIMP have been shown to have validity in terms of associations, it is possible that other CIMP markers may also have been informative. We do not have tumor-mutation data on the *APC* gene, which given the size of the gene and the cost of sequencing is beyond the scope of this study. Additionally, while we have polymorphism data from many candidate genes that are based on existing data that suggest that these polymorphisms have functional components, other polymorphisms of these genes may have different associations. Thus, while using our existing data, we have only been able to look at a limited set of polymorphisms within the candidate pathways explored. Additional genes within these pathways need to be considered and may be important in defining associations with specific tumor markers. We used MERT to help define the best statistical model -- dominant, recessive, or additive -- to evaluate each polymorphism. Also we have made many comparisons, however, since this is a study exploring pathways we report all associations that were detected using confidence intervals along with exact p values. We were able to define recent aspirin/NSAID use, which appears to be an important component in defining risk for many factors rather than long-term use [59]. However, our recent aspirin/NSAID use variable does not take into account dosage.

Although these analyses should be viewed as exploratory, we believe that they are valuable given that little is known about various exposures and pathways and how they influence specific tumor subsets. We believe that we have added to our understanding of exposure-specific pathways to colon cancer. It is however vital that others replicate these results to help determine factors that lead to specific disease pathways as determined by genetic and epigenetic alterations in tumors.

Acknowledgments

Grant support: This study was funded by CA48998, CA85846, and CA61757 to Dr. Slattery. This research was supported by the Utah Cancer Registry, which is funded by Contract #N01-PC-67000 from the National Cancer Institute, with additional support from the State of Utah Department of Health and the University of Utah, the Northern California Cancer Registry, and the Sacramento Tumor Registry. The contents of this manuscript are

solely the responsibility of the authors and do not necessarily represent the official view of the National Cancer Institute.

We would like to acknowledge the contributions of Dr. Kristen Anderson for her contributions to the data collection and study guidance, Sandra Edwards, Leslie Palmer, and Judy Morse to the data collection and management efforts of this study and to Dr. Hans Albertson, Michael Hoffman, and Erica Wolff for genotyping, and the core facility of the University of Utah Health Sciences Center for sequencing

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Table 1

Description of Study Population

	Cases N	(%)	Controls N	(%)
Center				
Kaiser	913	49.6	822	40.8
Minnesota	580	31.5	809	40.2
Utah	346	18.8	383	19.0
Sex				
Male	1014	55.1	1079	53.6
Female	825	44.9	935	46.4
Age				
<40	30	1.6	41	2.0
40–49	125	6.8	132	6.6
50–59	345	18.8	338	16.8
60–69	641	34.9	697	34.6
70–79	698	38.0	806	40.0
Recent Aspirin/NSAID use				
Yes	562	30.6	827	41.1
No	1265	68.8	1182	58.7
Unknown	12	0.7	5	0.2
Tumor Site				
Proximal	884	48.1		
Distal	909	49.4		
Unknown	46	2.5		
<i>p53</i> Mutated	642	46.7		
<i>Ki-ras</i> Mutated	433	32.2		
CIMP Positive	334	26.6		
MSI Unstable	212	14.9		

Table 2

Candidate polymorphisms examined with colon cancer tumors

Gene	rs Number	MAF	Pathways
Growth hormone secretagogue receptor ligand (GHRL)	rs35682	0.49	EH
Leptin (LEP)	rs7799039	0.45	EH; INF; INS
Leptin Receptor (LEPR)	rs6588147	0.33	EH, INF, INS
Pro-opiomelanocortin (POMC)	rs1866146	0.35	EH, INF, INS
Melanocortin 4 receptor (MC4R)	rs8093815	0.31	EH, INF, INS
Estrogen Receptor 1 (ER α , ESR1)	rs9340799	0.35	EA
Estrogen Receptor 2 (ER β , ESR2)	rs1256049	0.03	EA
Androgen Receptor (AR)	CAG repeat	0.38	EA
Interleukin 6 cytokine (IL6)	rs1800795	0.42	INF, INS
	rs1800796	0.06	
Peroxisome proliferator-activated receptor-gamma (PPAR γ)	rs1801282	0.13	INF, INS
UDP-glycosyltransferase 1 family polypeptide A6 (UGT1A6)	rs2070959	0.31	INF
	rs4365457	0.36	
Vitamin D receptor (VDR)	rs1544410	0.41	INF, INS
	PolyA repeat	0.42	
	rs10735810	0.40	
	rs11568820	0.19	
Adenomatous polyposis coli (APC)	rs459552	0.23	U
Cytochrome p450 1A1 (CPY1A1)	Msp1	0.11	M
	rs1048943	0.03	
Cytochrome p450 subfamily 2C polypeptide 9 (CYP2C9)	rs1799853	0.12	M, INF
	rs1057910	0.06	
Epoxide hydrolase 1 microsomal (MEH))	rs1051740	0.29	M
	rs2234922	0.20	
Insulin-like growth factor 1 (IGF1)	CA repeat	0.35	INS
	rs5742612	0.04	
Insulin-like growth factor 1 receptor (IGF1R)	rs2139924	0.18	INS; INF
Insulin-like growth factor-binding protein 3 (IGFBP3)	rs2854744	0.46	INS
	rs2854746	0.40	
Insulin-receptor substrate 1 (IRS1, G972R)	rs1801278	0.06	INS; EA
Insulin-receptor substrate 2 (IRS2)	rs1805097	0.35	INS; EH
Transcription factor 7-like 2 (TCF7L2)	rs7903146	0.28	INS
	Phenotype/Variant	Frequency	
Apolipoprotein E (apoE)	e3 (C112/R158)	common	INF
	e4 (R112/R158)	0.15	
	e2 (C112/C158)	0.08	
UDP-glycosyltransferase 1 family polypeptide A6 (UGT1A6)	*1 (T181/R184)	common	INF
	*2 (A181/S184)	0.32	
	*3 (T171/S184)	0.04	

Gene	rs Number	MAF	Pathways
Cytochrome p450 subfamily 2C polypeptide 9 (CYP2C9)	*1 (R144/I359)	common	M
	*2 (C144/I359)	0.12	
	*3 (R144/L359)	0.06	
Glutathione-s-transferase, μ -1 (GSTM1)	null/present	0.46	M
N-acetyl transferase 2 (isoniazid inactivation) (NAT2)	slow	common	M
	intermediate	0.35	
	rapid	0.06	

MAF = minor allele frequency based on distribution in non-Hispanic white controls; M = metabolizing, INF = inflammation; INS = Insulin; EH = Energy Homeostasis; EA = Estrogen/Androgen

Table 3

Associations between polymorphisms and specific tumor mutations.

	Control		All Cases*		MSI Unstable		CIMP Positive		p53 Mutation		K12-ras Mutation		
	n	n	OR	(95% CI)	n	OR	(95% CI)	n	OR	(95% CI)	n	OR	(95% CI)
POMC (rs1866146)													
TT	838	730	1.00		88	1.00		130	1.00		243	1.00	
TC	908	683	0.88	(0.76, 1.01)	85	0.87	(0.63, 1.20)	118	0.84	(0.64, 1.10)	234	0.91	(0.74, 1.12)
CC	227	162	0.87	(0.69, 1.09)	16	0.71	(0.41, 1.24)	27	0.81	(0.52, 1.26)	45	0.73	(0.51, 1.04)
P trend			0.07			0.21			0.21			0.06	<.01
LEPR (rs6588147)													
AA	888	766	1.00		99	1.00		133	1.00		256	1.00	
AG	850	655	0.90	(0.78, 1.04)	70	0.75	(0.54, 1.04)	114	0.90	(0.69, 1.18)	220	0.89	(0.73, 1.10)
GG	231	155	0.79	(0.63, 1.00)	19	0.77	(0.46, 1.29)	27	0.81	(0.52, 1.27)	48	0.74	(0.52, 1.05)
P trend			0.03			0.09			0.26			0.07	0.62
IL6 (rs1800795)													
GG	728	631	1.00		70	1.00		114	1.00		213	1.00	
GC	897	696	0.89	(0.76, 1.03)	90	1.04	(0.75, 1.44)	114	0.80	(0.60, 1.06)	223	0.85	(0.68, 1.05)
CC	347	246	0.83	(0.68, 1.01)	27	0.81	(0.51, 1.29)	47	0.85	(0.59, 1.23)	87	0.87	(0.65, 1.16)
P trend			0.04			0.56			0.26			0.18	0.37
PPARG (rs1801282)													
CC	1493	1234	1.00		157	1.00		221	1.00		419	1.00	
CG/GG	478	343	0.87	(0.74, 1.03)	31	0.61	(0.41, 0.91)	55	0.78	(0.57, 1.07)	103	0.77	(0.60, 0.98)
P trend			0.14			<.01			0.22			0.05	0.56
VDR Poly A repeat													
>17/>17	690	582	1.00		70	1.00		101	1.00		193	1.00	
□>17/≤17	905	730	0.93	(0.80, 1.08)	83	0.91	(0.65, 1.28)	131	1.00	(0.76, 1.33)	244	0.95	(0.76, 1.18)
≤17/≤17	352	243	0.82	(0.67, 1.00)	35	1.00	(0.65, 1.54)	43	0.85	(0.58, 1.25)	80	0.81	(0.61, 1.09)
P trend			0.05			0.88			0.44			0.17	0.02
VDR Fok1 (rs10735810)													
CC	691	722	1.00		88	1.00		103	1.00		224	1.00	
CT	891	770	0.84	(0.73, 0.97)	85	0.74	(0.54, 1.02)	135	1.01	(0.77, 1.34)	264	0.94	(0.76, 1.15)
TT	279	206	0.73	(0.59, 0.90)	24	0.68	(0.42, 1.11)	74	1.80	(1.28, 2.52)	108	1.25	(0.95, 1.65)

	Control		All Cases*			MSI Unstable			CIMP Positive			p53 Mutation			Ki-ras Mutation		
	n	n	OR	(95% CI)	n	OR	(95% CI)	n	OR	(95% CI)	n	OR	(95% CI)	n	OR	(95% CI)	
P trend			<.01			0.04			<.01			0.27			<.01		
VDR CDX2 (rs11568820)																	
GG	1241	957	1.00		113	1.00		163	1.00		311	1.00		204	1.00		
GA	632	515	1.07	(0.93, 1.24)	63	1.09	(0.79, 1.51)	100	1.23	(0.94, 1.62)	177	1.16	(0.93, 1.43)	123	1.24	(0.97, 1.59)	
AA	99	105	1.29	(0.96, 1.72)	10	1.06	(0.53, 2.10)	13	0.99	(0.54, 1.85)	37	1.40	(0.93, 2.11)	28	1.59	(1.00, 2.52)	
P trend			0.09			0.65			0.32			0.06			0.02		
NAT2																	
Slow	1157	918	1.00		110	1.00		206	1.00		350	1.00		290	1.00		
Intermediate/Rapid	807	690	1.08	(0.94, 1.24)	83	1.11	(0.82, 1.51)	76	0.54	(0.40, 0.71)	185	0.75	(0.61, 0.92)	72	0.35	(0.26, 0.46)	
IGF1 (CA repeats)																	
19/19	804	678	1.00		87	1.00		116	1.00		239	1.00		150	1.00		
19/non19	907	816	1.08	(0.94, 1.24)	88	0.89	(0.65, 1.22)	149	1.14	(0.88, 1.49)	284	1.05	(0.86, 1.28)	186	1.11	(0.87, 1.41)	
non19/non19	247	243	1.14	(0.92, 1.40)	26	0.91	(0.58, 1.45)	49	1.34	(0.93, 1.93)	79	1.02	(0.76, 1.38)	68	1.41	(1.02, 1.96)	
P trend			0.18			0.60			0.11			0.64			0.05		
IGF1R (rs2139924)																	
AA	1311	1078	1.00		121	1.00		181	1.00		362	1.00		245	1.00		
AC	613	456	0.93	(0.80, 1.07)	54	1.00	(0.71, 1.40)	81	1.01	(0.76, 1.34)	146	0.88	(0.71, 1.10)	102	0.91	(0.70, 1.17)	
CC	50	48	1.10	(0.73, 1.66)	12	2.42	(1.23, 4.75)	14	1.86	(0.99, 3.48)	16	1.10	(0.61, 1.97)	8	0.82	(0.39, 1.73)	
P trend			0.57			0.15			0.26			0.44			0.29		
IRS1 (rs1801278)																	
GG	1726	1499	1.00		167	1.00		267	1.00		516	1.00		350	1.00		
GA/AA	231	250	1.29	(1.06, 1.57)	35	1.64	(1.10, 2.45)	50	1.46	(1.04, 2.05)	90	1.36	(1.04, 1.78)	61	1.36	(0.99, 1.85)	
P trend			<.01			0.02			0.03			0.03			0.07		
IRS2 (rs1805097)																	
GG	829	718	1.00		93	1.00		136	1.00		246	1.00		151	1.00		
GA/AA	1135	854	0.89	(0.77, 1.02)	93	0.74	(0.55, 1.01)	137	0.73	(0.57, 0.95)	272	0.82	(0.67, 1.00)	205	1.00	(0.80, 1.27)	
P trend			0.41			0.54			0.18			0.17			0.41		
TCF7L2 (rs7903146)																	
CC	1034	778	1.00		96	1.00		137	1.00		236	1.00		184	1.00		
CT/TT	941	802	1.15	(1.00, 1.31)	92	1.06	(0.78, 1.44)	139	1.13	(0.87, 1.45)	288	1.36	(1.12, 1.66)	171	1.05	(0.84, 1.33)	

	Control	All Cases*		MSI Unstable		CIMP Positive		p53 Mutation		K1-ras Mutation	
	n	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)
P trend			0.09		0.64		0.38		0.02		0.41

Odds Ratios (OR) adjusted for age, sex, BMI, long-term activity level, average number of cigarettes per day, dietary calcium, and energy intake.

Table 4
Associations between polymorphisms and tumor markers among those who have recently used aspirin/NSAIDs

	Control		All Cases*		MSI Unstable		CIMP Positive		p53 Mutation		Ki-ras Mutation		
	n	n	OR	(95% CI)	n	OR	(95% CI)	n	OR	(95% CI)	n	OR	(95% CI)
LEP (rs7799039)													
GG	280	150	1.00		13	1.00		27	1.00		51	1.00	
GA	391	249	1.24	(0.95, 1.61)	32	1.82	(0.92, 3.58)	44	1.20	(0.71, 2.02)	87	1.28	(0.86, 1.89)
AA	148	95	1.27	(0.91, 1.78)	12	2.06	(0.91, 4.70)	24	1.84	(1.00, 3.39)	36	1.36	(0.82, 2.25)
P trend			0.11			0.06			0.05			0.16	
IL6 (rs1800795)													
GG	286	214	1.00		18	1.00		35	1.00		82	1.00	
GC	382	201	0.70	(0.54, 0.90)	29	1.18	(0.63, 2.18)	42	0.90	(0.56, 1.46)	68	0.63	(0.43, 0.90)
CC	147	77	0.70	(0.50, 0.99)	9	1.03	(0.44, 2.41)	16	0.90	(0.47, 1.73)	24	0.58	(0.35, 0.99)
P trend			0.01			0.81			0.68			<.01	
IL6 (rs1800796)													
GG	713	447	1.00		52	1.00		88	1.00		159	1.00	
GC/CC	104	45	0.69	(0.47, 1.01)	4	0.54	(0.18, 1.58)	6	0.47	(0.19, 1.13)	14	0.61	(0.33, 1.12)
P trend			0.07			0.19			0.05			0.06	
VDR Fok1 (rs10735810)													
CC	273	213	1.00		28	1.00		35	1.00		68	1.00	
CT	370	242	0.83	(0.65, 1.06)	25	0.61	(0.34, 1.10)	41	0.82	(0.50, 1.35)	91	1.00	(0.70, 1.45)
TT	128	60	0.62	(0.43, 0.90)	6	0.43	(0.17, 1.10)	26	1.54	(0.87, 2.73)	32	1.11	(0.68, 1.81)
P trend			<.01			0.03			0.22			0.58	
VDR CDX2 (rs11568820)													
GG	510	290	1.00		30	1.00		48	1.00		102	1.00	
GA	261	170	1.19	(0.93, 1.53)	22	1.48	(0.83, 2.64)	39	1.63	(1.02, 2.59)	63	1.26	(0.88, 1.82)
AA	39	29	1.22	(0.73, 2.05)	3	1.48	(0.42, 5.28)	5	1.39	(0.51, 3.83)	9	1.06	(0.48, 2.36)
P trend			0.16			0.19			0.06			0.33	
NAT2													
Slow	457	287	1.00		28	1.00		72	1.00		120	1.00	
Intermediate/Rapid	347	213	0.94	(0.74, 1.19)	30	1.48	(0.85, 2.60)	24	0.43	(0.26, 0.70)	59	0.62	(0.43, 0.88)
												0.29	(0.17, 0.49)

Control		All Cases*			MSI Unstable			CIMP Positive			p53 Mutation			Ki-ras Mutation		
n	n	OR	(95% CI)	n	OR	(95% CI)	n	OR	(95% CI)	n	OR	(95% CI)	n	OR	(95% CI)	
IGF1R (rs2139924)																
AA	550	329	1.00	37	1.00		64	1.00		114	1.00		76	1.00		
AC	246	149	0.98 (0.76, 1.26)	14	0.85 (0.44, 1.64)		24	0.83 (0.49, 1.39)		55	1.06 (0.74, 1.53)		28	0.80 (0.50, 1.29)		
CC	17	15	1.36 (0.66, 2.82)	5	4.56 (1.51, 13.83)		6	3.22 (1.15, 8.99)		5	1.37 (0.46, 4.11)		0	0.00 (0.00, 0.00)		
P trend			0.78		0.24			0.49			0.59			0.13		
IGFBP3 (~202 C>A)																
CC	264	143	1.00	13	1.00		19	1.00		63	1.00		26	1.00		
CA	367	270	1.36 (1.04, 1.77)	33	1.86 (0.96, 3.62)		56	2.17 (1.25, 3.74)		92	1.08 (0.75, 1.55)		72	2.05 (1.26, 3.32)		
AA	178	120	1.20 (0.87, 1.65)	12	1.39 (0.60, 3.18)		25	1.91 (1.00, 3.64)		40	0.92 (0.58, 1.47)		24	1.34 (0.73, 2.47)		
P trend			0.19		0.36			0.04			0.58			0.33		
IRS1 (rs1801278)																
GG	714	458	1.00	48	1.00		84	1.00		162	1.00		107	1.00		
GA/AA	94	77	1.29 (0.93, 1.80)	11	1.71 (0.84, 3.48)		18	1.60 (0.91, 2.83)		32	1.58 (1.00, 2.49)		18	1.37 (0.77, 2.43)		
P trend			0.12		0.12			0.08			0.07			0.23		
TCF7L2 (rs7903146)																
CC	397	269	1.00	30	1.00		52	1.00		87	1.00		66	1.00		
CT/TT	414	222	0.79 (0.63, 1.00)	25	0.80 (0.46, 1.39)		41	0.76 (0.49, 1.18)		87	0.99 (0.70, 1.40)		38	0.55 (0.35, 0.87)		
P trend			0.04		0.27			0.08			0.46			<.01		

* Includes cases without tumor marker data.

Odds Ratios adjusted for age, sex, BMI, long-term activity level, average number of cigarettes per day, dietary calcium, and energy intake.

Table 5

Associations between polymorphisms and tumor markers among those not using aspirin/NSAIDs recently.

	Control		All Cases*		MSI Unstable		CIMP Positive		p53 Mutation		K1-ras Mutation		
	n	n	OR	(95% CI)	n	OR	(95% CI)	n	OR	(95% CI)	n	OR	(95% CI)
POMC (rs1866146)													
TT	493	500	1.00		63	1.00		89	1.00		163	1.00	
TC	532	465	0.87	(0.73, 1.04)	59	0.84	(0.57, 1.23)	73	0.77	(0.55, 1.07)	156	0.90	(0.69, 1.16)
CC	129	107	0.84	(0.63, 1.12)	10	0.64	(0.32, 1.30)	18	0.81	(0.47, 1.39)	28	0.68	(0.43, 1.06)
P trend			0.10			0.19			0.21			0.10	<.01
LEP (rs7799039)													
GG	355	353	1.00		48	1.00		63	1.00		104	1.00	
GA	553	537	1.01	(0.84, 1.23)	64	0.85	(0.57, 1.28)	81	0.82	(0.58, 1.18)	183	1.16	(0.88, 1.54)
AA	250	186	0.78	(0.61, 0.99)	19	0.57	(0.32, 1.01)	36	0.85	(0.54, 1.33)	62	0.88	(0.61, 1.27)
P trend			0.08			0.05			0.38			0.63	0.08
ESR1 Xba (rs9340799)													
xx	433	461	1.00		45	1.00		74	1.00		153	1.00	
xX	497	454	0.85	(0.70, 1.02)	55	1.07	(0.70, 1.62)	81	0.93	(0.65, 1.31)	144	0.79	(0.60, 1.03)
XX	126	99	0.72	(0.54, 0.97)	20	1.52	(0.84, 2.73)	17	0.77	(0.43, 1.37)	35	0.77	(0.50, 1.18)
P trend			0.01			0.20			0.43			0.09	0.05
PPARG (rs1801282)													
CC	863	839	1.00		109	1.00		149	1.00		274	1.00	
CG/GG	288	240	0.87	(0.71, 1.06)	23	0.62	(0.39, 0.99)	34	0.67	(0.45, 1.00)	75	0.83	(0.62, 1.11)
P trend			0.21			0.03			0.07			0.20	0.49
VDR Poly A repeat													
>17/>17	390	387	1.00		50	1.00		65	1.00		128	1.00	
□>17/≤17	529	515	0.98	(0.81, 1.18)	57	0.87	(0.58, 1.31)	85	1.00	(0.71, 1.43)	165	0.96	(0.73, 1.26)
≤17/≤17	218	160	0.75	(0.58, 0.96)	23	0.84	(0.50, 1.43)	31	0.87	(0.55, 1.38)	54	0.78	(0.54, 1.12)
P trend			0.05			0.50			0.57			0.18	0.08
VDR FokI (rs10735810)													
CC	417	505	1.00		59	1.00		67	1.00		156	1.00	
CT	519	522	0.84	(0.70, 1.00)	60	0.81	(0.55, 1.20)	94	1.13	(0.80, 1.60)	172	0.90	(0.70, 1.17)
TT	150	144	0.80	(0.62, 1.05)	18	0.84	(0.47, 1.48)	47	1.98	(1.29, 3.04)	75	1.36	(0.97, 1.91)

	Control <i>n</i>	All Cases*			MSI Unstable			CIMP Positive			p53 Mutation			K _i -ras Mutation		
		<i>n</i>	OR	(95% CI)	<i>n</i>	OR	(95% CI)	<i>n</i>	OR	(95% CI)	<i>n</i>	OR	(95% CI)	<i>n</i>	OR	(95% CI)
<i>P</i> trend			0.04			0.33			<.01			0.25			<.01	
NAT2																
Slow	696	622	1.00		81	1.00		132	1.00		229	1.00		202	1.00	
Intermediate/Rapid	459	475	1.15	(0.97, 1.36)	53	1.01	(0.70, 1.47)	52	0.60	(0.42, 0.85)	125	0.82	(0.64, 1.06)	52	0.38	(0.27, 0.53)
<i>IGFBP3</i> (-202 C>A)																
CC	309	351	1.00		50	1.00		52	1.00		116	1.00		76	1.00	
CA	582	605	0.92	(0.76, 1.12)	67	0.70	(0.47, 1.05)	114	1.17	(0.81, 1.68)	213	1.00	(0.76, 1.30)	142	1.01	(0.74, 1.39)
AA	251	237	0.83	(0.66, 1.05)	25	0.61	(0.36, 1.02)	45	1.04	(0.67, 1.61)	75	0.80	(0.57, 1.13)	63	1.01	(0.69, 1.47)
<i>P</i> trend			0.13			0.03			0.89			0.28			0.93	
<i>IRS1</i> (rs1801278)																
GG	1007	1030	1.00		118	1.00		181	1.00		352	1.00		242	1.00	
GA/AA	137	172	1.29	(1.01, 1.64)	24	1.54	(0.95, 2.52)	32	1.41	(0.93, 2.16)	58	1.27	(0.91, 1.79)	42	1.32	(0.90, 1.94)
<i>P</i> trend			0.03			0.08			0.15			0.17			0.17	
<i>TCF7L2</i> (rs7903146)																
CC	635	502	1.00		66	1.00		84	1.00		147	1.00		118	1.00	
CT/TT	524	576	1.41	(1.19, 1.66)	66	1.21	(0.84, 1.75)	97	1.40	(1.02, 1.92)	201	1.68	(1.31, 2.15)	132	1.40	(1.06, 1.85)
<i>P</i> trend			<.01			0.20			0.02			<.01			<.01	

Odds Ratios (OR) adjusted for age, sex, BMI, long-term activity level, average number of cigarettes per day, dietary calcium, and energy intake.