Published in final edited form as:
Genes Chromosomes Cancer. 2013 May ; 52(5): 437–449. doi:10.1002/gcc.22042.

Genetic Variation in the Lipoxygenase Pathway and Risk of Colorectal Neoplasia

Sarah E. Kleinstein[1,2], **Laura Heath**[1], **Karen W. Makar**[1], **Elizabeth M. Poole**[1,3], **Brenna L. Seufert**[1], **Martha L. Slattery**[4], **Liren Xiao**[1], **David J. Duggan**[5], **Li Hsu**[1], **Karen Curtin**[4], **Lisel Koepl**[1], **Jill Muehling**[5], **Darin Taverna**[5], **Bette J. Caan**[6], **Christopher S. Carlson**[1], **John D. Potter**[1,2,7], and **Cornelia M. Ulrich**[1,2,8,*]

[1]Cancer Prevention Program, Fred Hutchinson Cancer Research Center, Seattle, Washington, 98109 [2]Department of Epidemiology, University of Washington, Seattle, Washington, 98195 [3]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA [4]Department of Medicine, School of Medicine, University of Utah, Salt Lake City, UT, 84108 [5]The Translational Genomics Research Institute, Phoenix, AZ, 85004 [6]Division of Research, Kaiser Permanente Medical Care Program, Oakland, CA 94612 [7]Centre for Public Health Research, Massey University, Wellington, New Zealand. [8]National Center for Tumor Diseases and German Cancer Research Center, Heidelberg, Germany, D-69120

Abstract

Arachidonate lipoxygenase (ALOX) enzymes metabolize arachidonic acid to generate potent inflammatory mediators and play an important role in inflammation-associated diseases. We investigated associations between colorectal cancer risk and polymorphisms in ALOX5, FLAP, ALOX12, and ALOX15, and their interactions with non-steroidal anti-inflammatory drug (NSAID) use. We genotyped fifty tagSNPs, one candidate SNP, and two functional promoter variable nucleotide tandem repeat (VNTR) polymorphisms in three US population-based case-control studies of colon cancer (1424 cases/1780 controls), rectal cancer (583 cases/775 controls), and colorectal adenomas (485 cases/578 controls). Individuals with variant genotypes of the ALOX5 VNTR had decreased risk of rectal cancer, with the strongest association seen for individuals with one or more alleles of >5 repeats (wildtype=5, OR>5/≥5=0.42, 95% CI 0.20-0.92; p=0.01). Four SNPs in FLAP (rs17239025), ALOX 12 (rs2073438), and ALOX15 (rs4796535 and rs2619112) were associated with rectal cancer risk at p≤0.05. One SNP in FLAP (rs12429692) was associated with adenoma risk. A false discovery rate (FDR) was applied to account for false positives due to multiple testing; the ALOX15 associations were noteworthy at 25% FDR. Colorectal neoplasia risk appeared to be modified by NSAID use in individuals with variant alleles in FLAP and ALOX15. One noteworthy interaction (25% FDR) was observed for rectal cancer. Genetic variability in arachidonate lipoxygenases may affect risk of colorectal neoplasia, particularly for rectal cancer. Additionally, genetic variability in FLAP and ALOX15 may modify the protective effect of NSAID use against colorectal neoplasia.

INTRODUCTION

Inflammation plays a key role in colorectal carcinogenesis. Two essential and competing pathways that are involved in modulating the inflammatory response are the leukotriene and

*Correspondence to: Fred Hutchinson Cancer Research Center 1100 Fairview Ave North PO Box 19024, M4-B402 Seattle, WA 98109-1024 Phone: 206-667-7617 Fax: 206-667-7850 neli.ulrich@nct-heidelberg.de.

prostaglandin pathways, both of which use the same primary precursor, arachidonic acid (AA) (Romano and Claria, 2003). Associations between genetic polymorphisms in prostaglandin synthesis enzymes and colorectal neoplasia risk have been established in multiple studies (Lin et al., 2002; Ulrich et al., 2002, 2004, 2005; Goodman et al., 2004; Koh et al., 2004; Poole et al., 2007, 2010; Cross et al., 2008; Liu et al., 2011), and genetic polymorphisms in a key leukotriene pathway gene family, the arachidonic lipoxygenases (ALOXs), have more recently been implicated in various cancers and inflammatory diseases such as asthma, bone loss, and atherosclerosis (Poole et al., 2005; Ichikawa et al., 2006; Mullin et al., 2007; Paganelli et al., 2007; Tranah et al., 2008; Bhattacharya et al., 2009; Krönke et al., 2009; Feng et al., 2010; Lindley et al., 2010; Liu et al., 2010; Gertow et al., 2011). A primary function of the ALOXs is to convert AA into leukotrienes, a class of paracrine hormones involved in the inflammatory response, as well as other inflammation-mediating eicosanoids which have been implicated in a variety of inflammatory diseases, including colorectal cancer (Funk, 2001; Nie and Honn, 2002).

The bioactive compounds generated by genes in the lipoxygenase pathway result in a variety of biologic activities important for carcinogenesis. Both arachidonate 5-lipoxygenase (ALOX5) and 12-lipoxygenase (ALOX12) have been described as pro-carcinogenic. In particular, ALOX5 and the 5-lipoxygenase-activating protein (FLAP, also known as ALOX5AP), are up-regulated in colon, esophageal, breast, prostate, and pancreatic cancers, and downstream metabolites of the ALOX5 cascade enhance cell proliferation and survival (Anderson et al., 1998; Avis et al., 2001; Shureiqi and Lippman, 2001; Hennig et al., 2002; Tong et al., 2002; Nielsen et al., 2003; Hoque et al., 2005; Jiang et al., 2006). ALOX12 converts AA to 12-hydroperoxyeicosatetraenoic acid (12-HPETE), which increases expression of pro-inflammatory cytokine genes such as tumor necrosis factor-α (Chakrabarti et al., 2009). ALOX15, an oxidizing enzyme that converts AA to 15-hydroperoxyeicosatetraenoic acid (15-HPETE), has anti-carcinogenic properties; it both increases apoptosis and decreases cancer cell proliferation (Feng et al., 2010). The under-expression of ALOX15 has been associated with the development of cancer and osteoporosis, while over-expression has been linked to asthma and atherosclerosis (Tranah et al., 2008; Krönke et al., 2009; Feng et al., 2010; Lindley et al., 2010; Liu et al., 2010; Gertow et al., 2011).

Previous studies point to functional implications of genetic variation in the lipoxygenase pathway. A rare *ALOX15* coding single nucleotide polymorphism (SNP, T560M) that has been implicated in coronary artery disease resulted in reduced enzyme activity in vitro (Schurmann et al., 2011), whereas promoter-region polymorphisms in both *ALOX15* (−292C>T) and *ALOX5* (a variable nucleotide tandem repeat (VNTR) (−176(GGGCGG)2-8)) were associated with transcription modification in vitro (In et al., 1997; Silverman and Drazen, 2000; Wittwer et al., 2006). In an earlier study, we noted evidence for interactions between this *ALOX5* VNTR and a previously identified functional *COX-2* promoter polymorphism (−765G>C) that modulated risk of adenomatous polyps (Poole et al., 2006). In addition, a candidate SNP in the negative regulatory region of the *ALOX5* promoter, rs4986832 (−1700 G>A), has previously been associated with a decreased risk of colon cancer (Goodman et al., 2004).

Long-term NSAID use reduces the risk of colorectal neoplasia and other diseases (Taketo, 1998; Giovannucci, 1999; Baron et al., 2003; Sandler et al., 2003; Ulrich et al., 2006; Chan et al., 2012) by targeting COX-1 and COX-2 to reduce the prostaglandin-induced inflammation response (Vane, 1971; Ulrich et al., 2006). However, prescribing aspirin and other NSAIDs as chemopreventive agents needs to be balanced with risk of adverse effects, such as bleeding (Ulrich et al., 2006). As the leukotriene and prostaglandin pathways are in competition, *ALOX* gene polymorphisms might indirectly interact with NSAID use to alter

the protective effect on colon cancer, which would have pharmacogenetic implications for prescribing NSAIDs long-term for some individuals. Furthermore, in vitro studies show that NSAIDs induce apoptosis in colon cancer cells via up-regulation of ALOX15 (Shureiqi et al., 2000; Shureiqi and Lippman, 2001), suggesting polymorphisms in *ALOX15* may directly interact with NSAID use.

We hypothesized that genetic variability in *ALOX5*, *FLAP*, *ALOX12*, and *ALOX15* influences colorectal carcinogenesis by altering the balance between prostaglandin and leukotriene synthesis and/or by modulating protein expression in the lipoxygenase pathway, with potential implications for effect modification by NSAID use. We present a comprehensive investigation of tagSNPs and functional candidate polymorphisms in the lipoxygenase enzyme pathway across a continuum of colorectal carcinogenesis by including adenoma, rectal cancer, and colon cancer populations. We also identify potential interactions between NSAID use and lipoxygenase polymorphisms that modify colorectal cancer risk.

MATERIALS AND METHODS

Study Populations

Adenoma study—Colorectal adenoma cases (n=485) and polyp-free controls (n=578) were recruited through a large multi-clinic gastroenterological practice in the Twin Cities area of Minnesota (Potter et al., 1996). In brief, patients were identified with a first diagnosis of adenomatous or hyperplastic polyp at the time of colonoscopy; only adenomatous cases were included for further study due to small sample sizes of hyperplastic cases. Eligible participants were aged 30-74 years, with a first colorectal adenoma diagnosis from 1991-1994, no known genetic syndrome associated with increased colon neoplasia risk, and no individual history of cancer (except non-melanoma skin cancer), prior colorectal polyps, or inflammatory bowel disease. All participants underwent colonoscopy. The participation proportion among patients receiving colonoscopy was 68%.

Colon and rectal cancer studies—Colon cancer cases (n=1424) and controls (n=1780) and rectal cancer cases (n=583) and controls (n=775) were recruited from the northern California Kaiser Permanente Medical Care Program (KPMCP), Utah, and the Twin Cities metropolitan area of Minnesota (colon cancer only) (Slattery et al., 1997; Slattery et al., 2003a). Eligible cases were aged 30-79, with no previous diagnosis of colorectal cancer, familial adenomatous polyposis, ulcerative colitis, or Crohn's disease. Colon cancer cases were first diagnosed from 1991-1994; rectal cancer cases were first diagnosed from 1997-2001. Participation proportions among contacted colon cancer cases were 76% and 64% for controls. Participation proportions among contacted rectal cancer cases were 73% and, for controls, 69%.

Questionnaire Data and Blood Collection

Information on use of NSAIDs, lifestyle factors and diet, anthropometry, demographics, and medical information (including family history of cancer) was obtained by questionnaire, as described previously (Potter et al., 1996; Slattery et al., 1997, 2003a; Ulrich et al., 1999). Buffy coats were obtained within 24 hours of venous blood collection for genomic DNA extraction, quantitation, and genotyping analyses.

SNP Selection and Genotyping

An identical tagSNP selection and genotyping procedure was used in all three studies. The coding regions and 2 kb beyond the 5′ and 3′ ends of *FLAP*, *ALOX12* and *ALOX15* have been resequenced in 23 individuals of European descent by Seattle SNPs (http://pga.gs.washington.edu). TagSNPs were identified in the resequenced individuals using the

Linkage Disequilibrium (LD) Select algorithm (Carlson et al., 2004), with MAF=4% and r2=0.9. Calculations were performed in QUANTO (Gauderman and Morrison, 2006) to determine that for SNPs with MAF>4% and reasonable effect sizes, sample sizes were sufficient and adequately powered in the colon and rectal cancer populations, and more weakly powered in the adenoma population. Thirty-two tagSNPs in *FLAP*, 17 in *ALOX12*, and 21 in *ALOX15* were successfully converted to the Illumina™ GoldenGate genotyping platform as described below. There were no resequencing data available for *ALOX5*, thus only one candidate SNP (rs4986832, −1700G>A) was chosen for inclusion and was genotyped by Taqman assay (Applied Biosystems) at the Fred Hutchinson Cancer Research Center (FHCRC).

Two variable nucleotide tandem repeats (VNTRs) were also sequenced: the *ALOX5* VNTR promoter polymorphism [−176(GGGCGG)2-8] and the *FLAP* poly(A) promoter repeat [−169poly(A)]. The VNTRs were genotyped at FHCRC using GeneScan on an ABI 3130xl, based on previously-published protocols (Sayers et al., 2003; Poole et al., 2006). Briefly, multiplex PCR reactions consisted of 40ng genomic template DNA, 1.5mmol/L MgCl2, 1x PCR Gold Buffer (Applied Biosystems), 0.5 units of Amplitaq Gold Polymerase (Applied Biosystems), 200nmol/L each of oligonucleotide primers ALOX5sp1-F 5′-6FAM-AGGAACAGACACCTCGCTGAGGAGAG-3′, ALOX5sp1-R 5′GAGCAGCGAGCGCCGGGAGCCTCGGC-3′, ALOX5AP-F: 5′ VICCGTGCTCCTCTGCCAAGCCCTGCTTC-3′, and ALOX5AP-R02: 5′-GCTCTGCCTCCAGCTGCACAACCTG-3′, 150μmol/L each of dATP, dCTP and dTTP, 75μmol/L each of dGTP and 7-deaza-2V-dGTP (Roche Diagnostics GmbH, Mannheim, Germany), and 8% (v/v) DMSO (Sigma, St. Louis, MO) in 5 uL. Cycling was as follows: 96°C for 7 minutes, 30 cycles of: 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds, 72°C for 7 minutes. The observed 6FAM-labeled ALOX5 amplicon lengths ranged from 254bp (2 6-bp repeats) to 290bp (8 6-bp repeats) and VIC-labeled FLAP amplicon was either 212bp (19A) or 216bp (23A).

Quality Control

All tagSNPs were genotyped using Illumina™ GoldenGate bead-based genotyping technology at the Translational Genomics Research Institute (TGen, Phoenix, Arizona). Intraplate and interplate replicates (5%) were included for all plates and batches, as well as blinded duplicates. Genotype data from 30 CEPH trios (Coriell Cell Repository, Camden, NJ) that had been genotyped by the HapMap project were used to confirm reliability and reproducibility of the genotyping assays. Quality-control (QC) checks based on Illumina metrics were performed as described previously (Levine et al., 2010). Genotypes were excluded from analyses by TGen if any of the following were true: GenTrain Score <0.4, 10% GC Score <0.25, AB T Dev >0.1239, Call Frequency <0.85, Replicate Errors >2, P-P-C Errors >2. Further exclusions were made for SNPs that had <85% concordance with blinded or non-blinded duplicates and for Hardy-Weinberg Equilibrium (HWE) p-values <0.0001. A total of twenty SNPs failed QC under the guidelines listed previously, thirteen of which were in *ALOX15*. Subsequent mapping of an *ALOX15* pseudogene on chromosome 17 accounted for the *ALOX15* HWE p<0.001 failed SNPs. 28 *FLAP* SNPs, 14 *ALOX12* SNPs, 8 *ALOX15* SNPs, the *ALOX5* SNP, and both VNTRs passed QC measures and were included in the analyses (Supplementary Table 1). When two SNPs were observed to be in high LD (r2≥0.90) in our study population, only one SNP was presented, given that the two SNPs would likely capture the same information.

Statistical Data Analysis

All analyses were conducted using SAS 9.1/9.2 software (SAS Institute, Cary, NC). Logistic regression analysis was used to estimate odds ratios (ORs) and corresponding 95%

confidence intervals (CIs). A co-dominant model was used as long as each cell contained ≥5 individuals; otherwise, a dominant model was analyzed. In a primary analysis examining associations between polymorphisms and colorectal neoplasia risk, trends were also analyzed via a log-additive model. The main effects analyses were adjusted for age, sex, and study site (if applicable) only, as other covariates (such as BMI, calcium intake, smoking, dietary fiber, study site, and activity) did not have significant impacts on the risk ratios. We would not expect, *a priori*, to see associations between health-behavior-related covariates and the candidate genes under study. The analyses were restricted to individuals who self-reported as non-Hispanic white (representing 97%, 91% and 87% in the adenoma, colon and rectal cancer studies, respectively) to limit population stratification.

In the secondary analysis exploring the interaction between the genotyped polymorphisms with NSAID use on colorectal neoplasia risk, multivariate adjustments were used, controlling for age, sex, body mass index (BMI), dietary intake of fiber (gm/day), alcohol (gm/day), energy (calories/day), hours/wk vigorous physical activity, and smoking (cigarettes/day). NSAID use was analyzed comparing regular use to non-regular use (≥1/wk v. ≤1/wk in the adenoma study, ≥3/wk v. ≤3/wk in colon and rectal studies). Regular NSAID use was inversely associated with colon cancer, rectal cancer, and adenoma risk (Bigler et al., 2001; Slattery et al., 2004b) (ORcolon=0.64, 95% CI 0.55-0.74, p<0.0001; ORrectal=0.67, 95% CI 0.54-0.85, p=0.0006; ORadenoma=0.65, 95% CI 0.49-0.85, p=0.0018; adjusted as stated above). Possible effect modification was evaluated using the likelihood ratio test comparing models with and without the multiplicative genotype x NSAID interaction term(s) (with two degrees of freedom (d.f.) for codominant model, 1 d.f. for dominant model).

The *ALOX5* VNTR wildtype genotype (the referent) was defined as five repeats of the six bp motif (-GGGCGG-) in both alleles (noted as 5/5 in Table 2). Individuals with <5 repeats in one or both alleles (<5/≤5) were grouped together, as were individuals with >5 repeats in one or both alleles (>5/≥5). The *FLAP* VNTR is a poly (A) microsatellite containing either 19 (wildtype) or 23 (variant) A's.

All statistical tests were two-sided. The initial statistical significance level was set at a cutoff of α=0.05. Since our study is investigating variation in candidate genes that were hypothesized *a priori* to affect colorectal carcinogenesis, conventional multiple comparisons tests were not applied due to the risk of type II errors (the rejection of true associations) (Rothman, 1990; Streiner and Norman, 2011). Instead, along with presenting uncorrected p-values, the less conservative false discovery rate (FDR) analyses were conducted using the Benjamini and Hochberg (B&H) method (Benjamini and Hochberg, 1995; Benjamini et al., 2001). In brief, rather than protecting against type I errors, the B&H method allows for false positives in the process of discovering true positives at or below a pre-specified FDR, which is the expected proportion of false positives among all the hypotheses determined to be "noteworthy" associations, as described in detail previously (Coghill et al., 2011). For example, 75% of SNPs that are identified as noteworthy by FDR at the 25% level are likely true positives. We set the B&H cutoff for noteworthy SNPs at the 25% level for both main effect and NSAID interaction analyses. FDR analyses were conducted on a gene by gene basis for the tagSNPs and were not conducted for the candidate polymorphism or VNTRs in *ALOX5* and *FLAP*. Tables 2 and 3 include all associations having initial uncorrected p-values≤0.05, and "noteworthy" SNPs below the 25% FDR. Complete results are in Supplementary Tables 2 and 3.

RESULTS

Characteristics of the Study Populations

Characteristics of the study populations are shown in Table 1. Briefly, the adenoma study participants were youngest on average, while the colon cancer study participants were oldest on average. Cases of all three study populations were more likely to be male, and controls in all three populations were more likely to be regular users of NSAIDs.

Genetic Variation in *ALOX5*

The *ALOX5* functional VNTR polymorphism was associated with a significantly lower risk of rectal cancer for individuals carrying an allele with any number of repeats other than wildtype (5 repeats) (Table 2) ($OR_{<5/\leq5\ v.\ 5/5}$=0.76; 95% CI 0.60-0.95; $OR_{>5/\geq5\ v.\ 5/5}$=0.42; 95% CI 0.20-0.92; global p=0.01). The same (though statistically non-significant) pattern of lower risk in individuals with at least one non-wildtype allele was seen in both colon cancer and adenoma.

For the *ALOX5* candidate SNP rs4986832 (−1700G>A), individuals who carried a variant allele had a borderline reduced risk of rectal cancer relative to individuals with the wildtype genotype ($OR_{het\ v.\ wt}$=0.82; 95% CI 0.64-1.15, $OR_{hzv\ v.\ wt}$=0.60; 95% CI 0.31-1.18; global p=0.11). However, carriage of the variant allele showed a statistically significant dose-response (p-trend=0.04). Because these analyses focused on pre-hypothesized candidate/functional variants, no FDR calculations were applied. There were no statistically significant associations between the candidate polymorphism and colon cancer or adenoma risk, and there were no statistically significant NSAID interactions.

Genetic Variation in *FLAP*

Two SNPs in *FLAP* were associated with either adenoma or rectal cancer risk. Individuals with the rs12429692 (2439 A>T) homozygous variant genotype had a two-fold higher risk of adenoma ($OR_{hzv\ v.\ wt}$=2.05; 95% CI 1.20-3.53; global p=0.01). Individuals who carried a variant rs17239025 (30185G>C) allele had a borderline significant 43% increased risk of rectal cancer (ORhet/hzv v. wt=1.43; 95% CI 1.01-2.04; global p=0.05). A similar pattern of higher risk in individuals with the variant allele was seen in the colon and adenoma cancer studies, though results were neither statistically significant nor noteworthy (Table 2).

There were four SNP-NSAID interactions at global p≤0.05 in the rectal cancer study (Table 3). Regular NSAID use was associated with a decreased risk of rectal cancer for individuals with the homozygous wildtype alleles of all five SNPs, as would be predicted. For three of these SNPs (rs9508832 (4527 G>A); rs9315053 (29380 T>G); and rs4075692 (113604 G>A)), regular NSAID users with a variant genotype had an additional reduction in rectal cancer risk compared to wildtype individuals (Table 3); this pattern was not seen in non-regular NSAID users.

Additionally, regular NSAID use was associated with a lower risk of colon cancer in wildtype individuals for rs17239025 (30185 G>C), whereas the protective effect conferred by NSAID use was absent among individuals with variant genotypes (p-interaction=0.02) (Table 3). The same loss of protection from NSAIDs in individuals with the variant allele was seen in both rectal cancer and adenoma, though these results were not statistically significant.

Genetic Variation in *ALOX12*

We observed a single significant association among the *ALOX12* tagSNPs investigated. Individuals with the homozygous variant genotype of rs2073438 (639 G>A) had a 34%

lower rectal cancer risk ($OR_{hzv\ v.\ wt}$=0.66; 95% CI 0.42-1.04, global p=0.02) (Table 2). This association was not seen in the colon cancer or adenoma populations and there were no significant NSAID interactions with *ALOX12* polymorphisms.

Genetic Variation in *ALOX15*

We observed evidence for associations between polymorphisms in *ALOX15* and rectal cancer. Individuals with at least one variant allele of rs4796535 (1351G>A) had a 43% higher risk of rectal cancer (ORhet/hzv v. wt=1.43, 95% CI: 1.03-1.97, global p=0.03, noteworthy at 25% FDR). The association between rs2619112 (9562 C>T) and rectal cancer risk was also noteworthy at 25% FDR ($OR_{het\ v.\ wt}$=1.37, 95% CI 1.06-1.77) ($OR_{hzv\ v.\ wt}$=1.13, 95% CI 0.83-1.55, global p=0.05).

We observed a significant and noteworthy NSAID interaction with rs2619112 in rectal cancer that followed the same non-dose-dependent pattern seen in the main effect analysis of this SNP: there was no protective effect for regular NSAID users with heterozygous genotypes, whereas those with homozygous wildtype and homozygous variant genotypes had lower risks (p-interaction=0.01, noteworthy at 25% FDR) (Table 3). Additionally, the lowered risk of adenoma in non-regular NSAID users with the homozygous variant genotype of rs2664593 (−189 G>C) ($OR_{hzv\ v.\ wt}$=0.19, 95% CI 0.06-0.57, global p=0.04) was lost in regular NSAID users with the same homozygous variant genotype.

DISCUSSION

We combined a tagSNP and candidate-polymorphism approach to analyze genetic variation in four leukotriene pathway genes that had previously been implicated in colorectal carcinogenesis. Genetic variability in *ALOX* genes was most prominently associated with rectal cancer risk; although the precise pathogenic mechanisms differentially influencing colon and rectal cancer are still unclear, many studies have proposed the idea that colon and rectal cancer are distinct entities (Frattini et al., 2004; Li and Lai, 2009) in which risk is modulated by different environmental, physiologic, and etiologic mechanisms, including genetics (Caan et al., 1998; Slattery et al., 2003a, 2003b, 2004a, 2011; Haug et al., 2012). In addition, a recent study revealed differential associations between colon and rectal cancer in multiple colorectal cancer-associated loci initially identified by GWAS (Lubbe et al., 2012). Our results support these findings by indicating a differential influence of genetic variability in lipoxygenase enzymes across colorectal neoplasias, with particular importance for rectal cancer. Although these differences could stem from chance findings, the fact that many associations were observed specifically in rectal cancer suggests a true etiologic difference between colon cancer and rectal cancer.

We also observed an increased risk of adenoma associated with a *FLAP* polymorphism, and an NSAID interaction for an *ALOX15* polymorphism in the adenoma study population. However, while adenoma may be a precursor to cancer in some individuals, in many cases adenomatous polyps never progress to cancer. Because of some heterogeneity of adenoma case outcomes, small sample sizes for certain alleles (resulting in less statistical power), and our inability to fine-tune polyp diagnoses any further than hyperplastic vs. adenomatous, this data does not provide support for etiological differences between the two cancers and adenoma.

At the time the data were collected for this study, extensive sequencing of *ALOX5* had not occurred and little information regarding genetic variability was available in existing databases. Now there is evidence for much more variability in *ALOX5*, including another promoter SNP (−1753 G>A) that is in complete LD with the functional candidate SNP in our study rs4986832 (−1700G>A), and which has been associated with a reduced risk of

colon cancer (Goodman et al., 2004). We also observed a trend for lower risk of cancer for individuals with the variant genotype rs4986832, specifically in the rectal cancer population.

The variant genotypes of the *ALOX5* VNTR were also statistically significantly associated with a decreased risk of rectal cancer, with a similar, although statistically non-significant, association in the colon cancer population. Variability in the *ALOX5* VNTR has been associated with transcription modification in vitro (In et al., 1997; Silverman and Drazen, 2000; Wittwer et al., 2006), and downstream eicosanoid production has been shown to be higher in individuals with the wildtype 5/5 genotype than for individuals with at least one allele containing fewer than five repeats (Stephensen et al., 2011). Additionally, this polymorphism has been implicated in inflammation-associated diseases such as asthma (Kalayci et al., 2006) and atherosclerosis (Dwyer et al., 2004), and we previously observed an interaction between this polymorphism and a functional promoter polymorphism in COX-2 in the adenoma case-control study (Poole et al., 2006). Taken together, these findings suggest an important role for the *ALOX5* VNTR in the pathogenesis of inflammatory diseases.

The functional candidate polymorphisms included in our study have not, to our knowledge, been included in a GWAS for colorectal cancer risk. However, even if they had been added to a GWAS SNP panel, pooling of colon and rectal cancer in the study populations may have attenuated the ability to detect associations. In addition, the relative technical difficulty of genotyping VNTRs has limited their inclusion in other studies, though advances in next generation sequencing will allow for easier sequencing of repeat polymorphisms (Schentrup et al., 2009). Further studies are needed to investigate these and more recently discovered polymorphisms in *ALOX5* and their relation to colorectal neoplasia.

FLAP, the 5-lipoxygenase-activing protein, partners with *ALOX5* to regulate leukotriene pathway activity. Four polymorphisms in FLAP showed statistically significant interactions with NSAID use and rectal cancer. Though none of the associations were noteworthy at 25% FDR, the consistent patterns of risk and repeated findings in the rectal population are intriguing. Although *FLAP* polymorphisms have not previously been assessed in the context of colorectal neoplasia, genetic variation in *FLAP* has been associated with increased risk of a variety of inflammatory diseases including stroke, asthma, and cardiovascular disease (Domingues-Montanari et al., 2010; Huang et al., 2010; Via et al., 2010), though some analyses yielded conflicting results (Matarin et al., 2009). It is likely that the result of genetic variation in *FLAP* is too subtle to be detected in most single-gene SNP analyses; rather, there may be gene-gene interactions occurring within the 5-lipoxygenase pathway, such as those detected in asthma between FLAP and downstream leukotriene LTA4H (Holloway et al., 2008; Via et al., 2010). As FLAP is the activating protein for ALOX5, FLAP-ALOX5 interactions seem likely. Though we were not able to properly assess a gene-gene interaction using only candidate polymorphisms in *ALOX5*, we did not detect interactions between individual *ALOX5* and *FLAP* polymorphisms in a preliminary analysis. Potential epistatic effects remain an important avenue of research. Overall, genetic variation in *FLAP* could provide new insights into the lipoxygenase pathway and colorectal carcinogenesis, especially if the focus is on rectal cancer and NSAID use.

The downstream product of ALOX12, 12-HETE, can enhance carcinogenesis by stimulating cell proliferation, angiogenesis, and tumor spread, and inhibiting apoptosis (Chopra et al., 1991; Tang et al., 1996). These findings have generated research interest in *ALOX12* polymorphisms in a variety of diseases, since altered enzyme expression caused by genetic variation could have a direct influence on cancer risk. R261Q, the single most extensively studied *ALOX12* polymorphism in relation to colorectal neoplasia risk, was previously observed to be associated with a 40% lower colorectal adenoma risk and had a significant

NSAID interaction in an adenomatous polyp study (Gong et al., 2007). Conversely, the same polymorphism was associated with higher risk of both rectal and colon cancer in another study (Tan et al., 2007); and yet another study reported no associations between this SNP and risk of colon cancer (Goodman et al., 2004). We did not observe a statistically significant association with cancer risk in the included tagSNP that is in perfect LD with (and has a similar MAF to) R261Q (rs434473 N322G, r^2=1). However, the Gong, et al. and the Goodman, et al. studies had substantially smaller sample sizes than our study (N=162 cases and 178 cases respectively), which can lead to false positives and inconsistencies; additionally, the Tan, et al. study, which had more robust sample sizes (N=403 colon cancer and 597 rectal cancer cases) was focused on a Chinese Han population. It is possible that there are different modifying alleles or health behaviors in these different ethnic populations. The one *ALOX12* SNP in our study that was statistically significantly associated with a 44% lower rectal cancer risk (rs2073438) is in moderate LD (r2 ~ 0.5) with these two functional *ALOX12* polymorphisms, though this probably represents an independent effect. Overall, in light of the inconsistency in results among the previous studies in different populations, studies of interactions with modifying alleles or health behaviors in specific populations are warranted.

The *ALOX15* SNP rs2619112 (9562G>A) emerged as an interesting variant in our study, with a borderline significant increased risk of rectal cancer in individuals carrying the heterozygous AG genotype, and a statistically significant and noteworthy NSAID interaction. Similar patterns of elevated risk for disease in individuals with the heterozygous genotype of this polymorphism have been observed in Chinese Han populations with coronary artery disease (Zhang et al., 2010). Furthermore, interactions between rs2619112 and menopausal status modified risk of low bone mineral density in women (Cheung et al., 2008). One additional *ALOX15* SNP (rs4796535, 1351G>A) was associated with an increased risk of rectal cancer, also at 25% FDR. Previous studies have shown that down-regulation of *ALOX15* can lead to colorectal carcinogenesis, warranting further study of this pathway as a potential therapeutic target (Bhattacharya et al., 2009).

Our study design has several strengths and limitations. We were able to investigate more rare and functional polymorphisms not covered in current GWAS. Use of identical genotyping platforms across three independent case-control studies enabled us to investigate the full continuum of genetic variation across colorectal carcinogenesis. Sample sizes were relatively large, though stratification by NSAID use was sometimes underpowered, and this study was not powered to capture the impact of rarer polymorphisms or associations in multiple ethnicities. Few associations were noteworthy at 25% FDR, and none would have been significant after very stringent methods of correction for multiple comparisons. However, if we view these results as avenues toward potentially fruitful areas of future research rather than definitive or clinically actionable findings, discussion of uncorrected results with $p \leq 0.05$ can be considered valid, though we acknowledge that we are more open to type I errors when we minimize type II errors (Streiner and Norman, 2011). Also, assessing the genetic variation in *ALOX5* and *ALOX15* was limited due to the lack of resequencing of the *ALOX5* gene at the time of the study, and general difficulties in genotyping this gene due to the presence of an *ALOX15* pseudogene.

Our results suggest that genetic variability in the lipoxygenase pathway impacts the risk of colorectal neoplasia, and that variability in *FLAP* and *ALOX15*, in particular, may modify the protective association of NSAID use against colorectal neoplasia, especially rectal cancer. NSAIDs are emerging as potent cancer-preventive agents, yet have a risk of adverse effects (Ulrich et al., 2006; Cross et al., 2008; Chan et al., 2012); tailoring NSAIDs to individuals rather than prescribing them indiscriminately is a key goal of pharmacogenetics. These and other studies examining genetic variability, risk of colorectal cancer, and NSAID

use interactions provide valuable insight into disease etiology, future therapeutic targets, and the potential for individualized treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank Dr. Roberd Bostick and Lisa Fosdick for their contributions to the initial establishment of the adenoma study, Dr. Kristin Anderson, Sandie Edwards, and Donna Morse for their contributions to the colon and rectal cancer studies.

Supported by: National Cancer Institute grants R01 CA114467, R03 CA123577, R25 CA094880, CA48998, and T32 CA009001

REFERENCES

Anderson KM, Seed T, Vos M, Mulshine J, Meng J, Alrefai W, Ou D, Harris JE. 5-Lipoxygenase inhibitors reduce PC-3 cell proliferation and initiate nonnecrotic cell death. Prostate. 1998; 37:161–173. [PubMed: 9792133]

Avis I, Hong SH, Martinez A, Moody T, Choi YH, Trepel J, Das R, Jett M, Mulshine JL. Five-lipoxygenase inhibitors can mediate apoptosis in human breast cancer cell lines through complex eicosanoid interactions. Faseb J. 2001; 15:2007–2009. [PubMed: 11511519]

Baron JA, Cole BF, Sandler RS, Haile RW, Ahnen D, Bresalier R, McKeown-Eyssen G, Summers RW, Rothstein R, Burke CA, Snover DC, Church TR, Allen JI, Beach M, Beck GJ, Bond JH, Byers T, Greenberg ER, Mandel JS, Marcon N, Mott LA, Pearson L, Saibil F, van Stolk RU. A randomized trial of aspirin to prevent colorectal adenomas. New Engl J Med. 2003; 348:891–899. [PubMed: 12621133]

Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. Behav Brain Res. 2001; 125:279–284. [PubMed: 11682119]

Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing Journal of the Royal Statistical Society. Series B (Methodological). 1995; 57:289–300.

Bhattacharya S, Mathew G, Jayne DG, Pelengaris S, Khan M. 15-lipoxygenase-1 in colorectal cancer: a review. Tumour Biol. 2009; 30:185–199. [PubMed: 19752603]

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; 61:3566–3569. [PubMed: 11325819]

Caan BJ, Coates AO, Slattery ML, Potter JD, Quesenberry CP Jr. Edwards SM. Body size and the risk of colon cancer in a large case-control study. Int J Obes Relat Metab Disord. 1998; 22:178–184. [PubMed: 9504326]

Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet. 2004; 74:106–120. [PubMed: 14681826]

Chakrabarti SK, Cole BK, Wen Y, Keller SR, Nadler JL. 12/15-Lipoxygenase Products Induce Inflammation and Impair Insulin Signaling in 3T3-L1 Adipocytes. Obesity (Silver Spring). 2009; 17:1657–1663. [PubMed: 19521344]

Chan AT, Arber N, Burn J, Chia WK, Elwood P, Hull MA, Logan RF, Rothwell PM, Schror K, Baron JA. Aspirin in the chemoprevention of colorectal neoplasia: an overview. Cancer Prev Res (Phila). 2012; 5:164–178. [PubMed: 22084361]

Cheung CL, Chan V, Kung AW. A differential association of *ALOX15* polymorphisms with bone mineral density in pre- and post-menopausal women. Hum Hered. 2008; 65:1–8. [PubMed: 17652958]

Chopra H, Timar J, Chen YQ, Rong XH, Grossi IM, Fitzgerald LA, Taylor JD, Honn KV. The lipoxygenase metabolite 12(S)-HETE induces a cytoskeleton-dependent increase in surface expression of integrin alpha IIb beta 3 on melanoma cells. Int J Cancer. 1991; 49:774–786. [PubMed: 1937964]

Coghill AE, Newcomb PA, Poole EM, Hutter CM, Makar KW, Duggan D, Potter JD, Ulrich CM. Genetic variation in inflammatory pathways is related to colorectal cancer survival. Clin Cancer Res. 2011; 17:7139–7147. [PubMed: 21976545]

Cross JT, Poole EM, Ulrich CM. A review of gene-drug interactions for nonsteroidal anti-inflammatory drug use in preventing colorectal neoplasia. Pharmacogenomics J. 2008; 8:237–247. [PubMed: 18195728]

Domingues-Montanari S, Fernandez-Cadenas I, del Rio-Espinola A, Corbeto N, Krug T, Manso H, Gouveia L, Sobral J, Mendioroz M, Fernandez-Morales J, Alvarez-Sabin J, Ribo M, Rubiera M, Obach V, Marti-Fabregas J, Freijo M, Serena J, Ferro JM, Vicente AM, Oliveira SA, Montaner J. Association of a genetic variant in the ALOX5AP with higher risk of ischemic stroke: a case-control, meta-analysis and functional study. Cerebrovasc Dis. 2010; 29:528–537. [PubMed: 20357438]

Dwyer JH, Allayee H, Dwyer KM, Fan J, Wu H, Mar R, Lusis AJ, Mehrabian M. Arachidonate 5-lipoxygenase promoter genotype, dietary arachidonic acid, and atherosclerosis. N Engl J Med. 2004; 350:29–37. [PubMed: 14702425]

Feng Y, Bai X, Yang Q, Wu H, Wang D. Downregulation of 15-lipoxygenase 2 by glucocorticoid receptor in prostate cancer cells. Int J Oncol. 2010; 36:1541–1549. [PubMed: 20428779]

Frattini M, Balestra D, Suardi S, Oggionni M, Alberici P, Radice P, Costa A, Daidone MG, Leo E, Pilotti S, Bertario L, Pierotti MA. Different genetic features associated with colon and rectal carcinogenesis. Clin Cancer Res. 2004; 10:4015–4021. [PubMed: 15217933]

Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science. 2001; 294:1871–1875. [PubMed: 11729303]

Gauderman, W.; Morrison, J. QUANTO 1.1: A computer program for power and sample size calculations for genetic-epidemiology studies. 2006. http://hydra.usc.edu/gxe

Gertow K, Nobili E, Folkersen L, Newman JW, Pedersen TL, Ekstrand J, Swedenborg J, Kühn H, Wheelock CE, Hansson GK, Hedin U, Haeggström JZ, Gabrielsen A. 12- and 15-lipoxygenases in human carotid atherosclerotic lesions: associations with cerebrovascular symptoms. Atherosclerosis. 2011; 215:411–416. [PubMed: 21316676]

Giovannucci E. The prevention of colorectal cancer by aspirin use. Biomedicine & Pharmacotherapy. 1999; 53:303–308.

Gong Z, Hebert JR, Bostick RM, Deng Z, Hurley TG, Dixon DA, Nitcheva D, Xie D. Common polymorphisms in 5-lipoxygenase and 12-lipoxygenase genes and the risk of incident, sporadic colorectal adenoma. Cancer. 2007; 109:849–857. [PubMed: 17236225]

Goodman JE, Bowman ED, Chanock SJ, Alberg AJ, Harris CC. Arachidonate lipoxygenase (ALOX) and cyclooxygenase (COX) polymorphisms and colon cancer risk. Carcinogenesis. 2004; 25:2467–2472. [PubMed: 15308583]

Haug U, Poole EM, Xiao L, Curtin K, Duggan D, Hsu L, Makar KW, Peters U, Kulmacz RJ, Potter JD, Koepl L, Caan BJ, Slattery ML, Ulrich CM. Glutathione peroxidase tagSNPs: Associations with rectal cancer but not with colon cancer. Genes Chromosomes Cancer. 2012; 51:598–605. [PubMed: 22371331]

Hennig R, Ding XZ, Tong WG, Schneider MB, Standop J, Friess H, Buchler MW, Pour PM, Adrian TE. 5-Lipoxygenase and leukotriene B(4) receptor are expressed in human pancreatic cancers but not in pancreatic ducts in normal tissue. Am J Pathol. 2002; 161:421–428. [PubMed: 12163367]

Holloway JW, Barton SJ, Holgate ST, Rose-Zerilli MJ, Sayers I. The role of LTA4H and ALOX5AP polymorphism in asthma and allergy susceptibility. Allergy. 2008; 63:1046–1053. [PubMed: 18547289]

Hoque A, Lippman SM, Wu TT, Xu Y, Liang ZD, Swisher S, Zhang H, Cao L, Ajani JA, Xu XC. Increased 5-lipoxygenase expression and induction of apoptosis by its inhibitors in esophageal cancer: a potential target for prevention. Carcinogenesis. 2005; 26:785–791. [PubMed: 15661803]

Huang H, Zeng Z, Li J, Zhang L, Chen Y. Variants of arachidonate 5-lipoxygenase-activating protein (ALOX5AP) gene and risk of coronary heart disease: A meta-analysis. Arch Med Res. 2010; 41:634–641. [PubMed: 21199733]

Ichikawa S, Koller DL, Johnson ML, Lai D, Xuei X, Edenberg HJ, Klein RF, Orwoll ES, Hui SL, Foroud TM, Peacock M, Econs MJ. Human ALOX12, but not ALOX15, is associated with BMD in white men and women. J Bone Miner Res. 2006; 21:556–564. [PubMed: 16598376]

In KH, Asano K, Beier D, Grobholz J, Finn PW, Silverman EK, Silverman ES, Collins T, Fischer AR, Keith TP, Serino K, Kim SW, De Sanctis GT, Yandava C, Pillari A, Rubin P, Kemp J, Israel E, Busse W, Ledford D, Murray JJ, Segal A, Tinkleman D, Drazen JM. Naturally occurring mutations in the human 5-lipoxygenase gene promoter that modify transcription factor binding and reporter gene transcription. Journal of Clinical Investigation. 1997; 99:1130–1137. [PubMed: 9062372]

Jiang WG, Douglas-Jones AG, Mansel RE. Aberrant expression of 5-lipoxygenase-activating protein (5-LOXAP) has prognostic and survival significance in patients with breast cancer. Prostaglandins Leukot Essent Fatty Acids. 2006; 74:125–134. [PubMed: 16364620]

Kalayci O, Birben E, Sackesen C, Keskin O, Tahan F, Wechsler ME, Civelek E, Soyer OU, Adalioglu G, Tuncer A, Israel E, Lilly C. ALOX5 promoter genotype, asthma severity and LTC production by eosinophils. Allergy. 2006; 61:97–103. [PubMed: 16364163]

Koh WP, Yuan JM, Van Den Berg D, Lee HP, Yu MC. Interaction between cyclooxygenase-2 gene polymorphism and dietary n-6 polyunsaturated fatty acids on colon cancer risk: The Singapore Chinese Health Study. Br J Cancer. 2004; 90:1760–1764. [PubMed: 15150618]

Krönke G, Uderhardt S, Katzenbeisser J, Schett G. The 12/15-lipoxygenase pathway promotes osteoclast development and differentiation. Autoimmunity. 2009; 42:383–385. [PubMed: 19811308]

Levine AJ, Figueiredo JC, Lee W, Poynter JN, Conti D, Duggan DJ, Campbell PT, Newcomb P, Martinez ME, Hopper JL, Le Marchand L, Baron JA, Limburg PJ, Ulrich CM, Haile RW. Genetic variability in the MTHFR gene and colorectal cancer risk using the colorectal cancer family registry. Cancer Epidemiol Biomarkers Prev. 2010; 19:89–100. [PubMed: 20056627]

Li FY, Lai MD. Colorectal cancer, one entity or three. J Zhejiang Univ Sci B. 2009; 10:219–229. [PubMed: 19283877]

Lin HJ, Lakkides KM, Keku TO, Reddy ST, Louie AD, Kau IH, Zhou H, Gim JS, Ma HL, Matthies CF, Dai A, Huang HF, Materi AM, Lin JH, Frankl HD, Lee ER, Hardy SI, Herschman HR, Henderson BE, Kolonel LN, Le Marchand L, Garavito RM, Sandler RS, Haile RW, Smith WL. Prostaglandin H synthase 2 variant (Val511Ala) in African Americans may reduce the risk for colorectal neoplasia. Cancer Epidemiology, Biomarkers & Prevention. 2002; 11:1305–1315.

Lindley, AR.; Crapster-Pregont, M.; Liu, Y.; Kuperman, DA. 12/15-lipoxygenase is an interleukin-13 and interferon-γ counterregulated-mediator of allergic airway inflammation; Mediators Inflamm. 2010. p. 727305

Liu SH, Shen CC, Yi YC, Tsai JJ, Wang CC, Chueh JT, Lin KL, Lee TC, Pan HC, Sheu ML. Honokiol inhibits gastric tumourigenesis by activation of 15-lipoxygenase-1 and consequent inhibition of peroxisome proliferator-activated receptor-gamma and COX-2-dependent signals. Br J Pharmacol. 2010; 160:1963–1972. [PubMed: 20649594]

Liu W, Poole EM, Ulrich CM, Kulmacz RJ. Polymorphic human prostaglandin H synthase-2 proteins and their interactions with cyclooxygenase substrates and inhibitors. Pharmacogenomics J. 2011; 11:337–347. [PubMed: 20548327]

Lubbe SJ, Whiffin N, Chandler I, Broderick P, Houlston RS. Relationship between 16 susceptibility loci and colorectal cancer phenotype in 3146 patients. Carcinogenesis. 2012; 33:108–112. [PubMed: 22045029]

Matarin M, Brown WM, Dena H, Britton A, De Vrieze FW, Brott TG, Brown RD Jr. Worrall BB, Case LD, Chanock SJ, Metter EJ, Ferruci L, Gamble D, Hardy JA, Rich SS, Singleton A, Meschia JF. Candidate gene polymorphisms for ischemic stroke. Stroke. 2009; 40:3436–3442. [PubMed: 19729601]

Mullin BH, Spector TD, Curtis CC, Ong GN, Hart DJ, Hakim AJ, Worthy T, Wilson SG. Polymorphisms in ALOX12, but not ALOX15, are significantly associated with BMD in postmenopausal women. Calcif Tissue Int. 2007; 81:10–17. [PubMed: 17520163]

Nie D, Honn KV. Cyclooxygenase, lipoxygenase and tumor angiogenesis. Cell Mol Life Sci. 2002; 59:799–807. [PubMed: 12088280]

Nielsen CK, Ohd JF, Wikstrom K, Massoumi R, Paruchuri S, Juhas M, Sjolander A. The leukotriene receptor CysLT1 and 5-lipoxygenase are upregulated in colon cancer. Advances in Experimental Medicine & Biology. 2003; 525:201–204. [PubMed: 12751768]

Paganelli M, Albanese C, Borrolli O, Civitelli F, Canitano N, Viola F, Passariello R, Cucchiara S. Inflammation is the main determinant of low bone mineral density in pediatric inflammatory bowel disease. Inflamm Bowel Dis. 2007; 13:416–423. [PubMed: 17206686]

Poole, EM.; Bigler, J.; Whitton, J.; Potter, J.; Sibert, J.; Ulrich, CM. ALOX5 polymorphism and risk of colorectal polyps. AACR 96th Annual Meeting; 2005.

Poole EM, Bigler J, Whitton J, Sibert JG, Kulmacz RJ, Potter JD, Ulrich CM. Genetic variability in prostaglandin synthesis, fish intake, and risk of colorectal polyps. Carcinogenesis. 2007; 28:1259–63. [PubMed: 17277229]

Poole EM, Bigler J, Whitton J, Sibert JG, Potter JD, Ulrich CM. Prostacyclin synthase and arachidonate 5-lipoxygenase polymorphisms and risk of colorectal polyps. Cancer Epidemiol Biomarkers Prev. 2006; 15:502–508. [PubMed: 16537708]

Poole EM, Hsu L, Xiao L, Kulmacz RJ, Carlson CS, Rabinovitch PS, Makar KW, Potter JD, Ulrich CM. Genetic variation in prostaglandin E2 synthesis and signaling, prostaglandin dehydrogenase, and the risk of colorectal adenoma. Cancer Epidemiol Biomarkers Prev. 2010; 19:547–557. [PubMed: 20086108]

Potter JD, Bostick RM, Grandits GA, Fosdick L, Elmer P, Wood J, Grambsch P, Louis TA. Hormone replacement therapy is associated with lower risk of adenomatous polyps of the large bowel: the Minnesota Cancer Prevention Research Unit Case-Control Study. Cancer Epidemiol Biomarkers Prev. 1996; 5:779–784. [PubMed: 8896888]

Romano M, Claria J. Cyclooxygenase-2 and 5-lipoxygenase converging functions on cell proliferation and tumor angiogenesis: implications for cancer therapy. Faseb J. 2003; 17:1986–1995. [PubMed: 14597668]

Rothman KJ. No adjustments are needed for multiple comparisons. Epidemiology. 1990; 1:43–46. [PubMed: 2081237]

Sandler RS, Halabi S, Baron JA, Budinger S, Paskett E, Keresztes R, Petrelli N, Pipas JM, Karp DD, Loprinzi CL, Steinbach G, Schilsky R. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. New Engl J Med. 2003; 348:883–890. [PubMed: 12621132]

Sayers I, Barton S, Rorke S, Sawyer J, Peng Q, Beghe B, Ye S, Keith T, Clough JB, Holloway JW, Sampson AP, Holgate ST. Promoter polymorphism in the 5-lipoxygenase (ALOX5) and 5-lipoxygenase-activating protein (ALOX5AP) genes and asthma susceptibility in a Caucasian population. Clin Exp Allergy. 2003; 33:1103–1110. [PubMed: 12911785]

Schentrup AM, Allayee H, Lima JJ, Johnson JA, Langaee TY. Genotyping the GGGCGG tandem repeat promoter polymorphism in the 5-lipoxygenase enzyme gene (ALOX5) by pyrosequencing assay. Genet Test Mol Biomarkers. 2009; 13:361–365. [PubMed: 19473080]

Schurmann K, Anton M, Ivanov I, Richter C, Kuhn H, Walther M. Molecular basis for the reduced catalytic activity of the naturally occurring T560M mutant of human 12/15-lipoxygenase that has been implicated in coronary artery disease. J Biol Chem. 2011; 286:23920–23927. [PubMed: 21558275]

Shureiqi I, Chen D, Lee JJ, Yang P, Newman RA, Brenner DE, Lotan R, Fischer SM, Lippman SM. 15-LOX-1: a novel molecular target of nonsteroidal anti-inflammatory drug-induced apoptosis in colorectal cancer cells. J Natl Cancer Inst. 2000; 92:1136–1142. [PubMed: 10904086]

Shureiqi I, Lippman SM. Lipoxygenase modulation to reverse carcinogenesis. Cancer Research. 2001; 61:6307–6312. [PubMed: 11522616]

Silverman ES, Drazen JM. Genetic variations in the 5-lipoxygenase core promoter. Description and functional implications. American Journal of Respiratory & Critical Care Medicine. 2000; 161:S77–80. [PubMed: 10673232]

Slattery ML, Caan BJ, Benson J, Murtaugh M. Energy balance and rectal cancer: an evaluation of energy intake, energy expenditure, and body mass index. Nutr Cancer. 2003a; 46:166–171. [PubMed: 14690792]

Slattery ML, Edwards S, Curtin K, Ma K, Edwards R, Holubkov R, Schaffer D. Physical activity and colorectal cancer. Am J Epidemiol. 2003b; 158:214–224. [PubMed: 12882943]

Slattery ML, Lundgreen A, Bondurant KL, Wolff RK. Interferon-signaling pathway: associations with colon and rectal cancer risk and subsequent survival. Carcinogenesis. 2011; 32:1660–1667. [PubMed: 21859832]

Slattery ML, Neuhausen SL, Hoffman M, Caan B, Curtin K, Ma KN, Samowitz W. Dietary calcium, vitamin D, VDR genotypes and colorectal cancer. Int J Cancer. 2004a; 111:750–756. [PubMed: 15252846]

Slattery ML, Potter J, Caan B, Edwards S, Coates A, Ma KN, Berry TD. Energy balance and colon cancer--beyond physical activity. Cancer Research. 1997; 57:75–80. [PubMed: 8988044]

Slattery ML, Samowitz W, Hoffman M, Ma KN, Levin TR, Neuhausen S. Aspirin, NSAIDs, and Colorectal Cancer: Possible Involvement in an Insulin-Related Pathway. Cancer Epidemiol Biomarkers Prev. 2004b; 13:538–545. [PubMed: 15066917]

Stephensen CB, Armstrong P, Newman JW, Pedersen TL, Legault J, Schuster GU, Kelley D, Vikman S, Hartiala J, Nassir R, Seldin MF, Allayee H. ALOX5 gene variants affect eicosanoid production and response to fish oil supplementation. J Lipid Res. 2011; 52:991–1003. [PubMed: 21296957]

Streiner DL, Norman GR. Correction for multiple testing: is there a resolution? Chest. 2011; 140:16–18. [PubMed: 21729890]

Taketo MM. Cyclooxygenase-2 inhibitors in tumorigenesis (Part II). Journal of the National Cancer Institute. 1998; 90:1609–1620. [PubMed: 9811310]

Tan W, Wu J, Zhang X, Guo Y, Liu J, Sun T, Zhang B, Zhao D, Yang M, Yu D, Lin D. Associations of functional polymorphisms in cyclooxygenase-2 and platelet 12-lipoxygenase with risk of occurrence and advanced disease status of colorectal cancer. Carcinogenesis. 2007; 28:1197–1201. [PubMed: 17151091]

Tang DG, Chen YQ, Honn KV. Arachidonate lipoxygenases as essential regulators of cell survival and apoptosis. Proc Natl Acad Sci U S A. 1996; 93:5241–5246. [PubMed: 8643560]

Tong WG, Ding XZ, Witt RC, Adrian TE. Lipoxygenase inhibitors attenuate growth of human pancreatic cancer xenografts and induce apoptosis through the mitochondrial pathway. Mol Cancer Ther. 2002; 1:929–935. [PubMed: 12481414]

Tranah GJ, Taylor BC, Lui LY, Zmuda JM, Cauley JA, Ensrud KE, Hillier TA, Hochberg MC, Li J, Rhees BK, Erlich HA, Sternlicht MD, Peltz G, Cummings SR, Study of Osteoporotic Fractures (SOF) Research Group. Genetic variation in candidate osteoporosis genes, bone mineral density, and fracture risk: the study of osteoporotic fractures. Calcif Tissue Int. 2008; 83:155–166. [PubMed: 18787887]

Ulrich CM, Bigler J, Bostick R, Fosdick L, Potter JD. Thymidylate synthase promoter polymorphism, interaction with folate intake, and risk of colorectal adenomas. Cancer Research. 2002; 62:3361–3364. [PubMed: 12067974]

Ulrich CM, Bigler J, Potter JD. Non-steroidal anti-inflammatory drugs for cancer prevention: promise, perils, and pharmacogenetics. Nature Reviews Cancer. 2006; 6:130–140.

Ulrich CM, Bigler J, Sparks R, Whitton J, Sibert JG, Goode EL, Yasui Y, Potter JD. Polymorphisms in PTGS1 (=COX-1) and risk of colorectal polyps. Cancer Epidemiol Biomarkers Prev. 2004; 13:889–893. [PubMed: 15159324]

Ulrich CM, Kampman E, Bigler J, Schwartz SM, Chen C, Bostick R, Fosdick L, Beresford SA, Yasui Y, Potter JD. Colorectal adenomas and the C677T MTHFR polymorphism: evidence for gene-environment interaction? Cancer Epidemiol Biomarkers Prev. 1999; 8:659–668. [PubMed: 10744125]

Ulrich CM, Whitton J, Yu JH, Sibert J, Sparks R, Potter JD, Bigler J. PTGS2 (COX-2) −765G > C promoter variant reduces risk of colorectal adenoma among nonusers of nonsteroidal anti-inflammatory drugs. Cancer Epidemiol Biomarkers Prev. 2005; 14:616–619. [PubMed: 15767339]

Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature - New Biology. 1971; 231:232–235.

Via M, De Giacomo A, Corvol H, Eng C, Seibold MA, Gillett C, Galanter J, Sen S, Tcheurekdjian H, Chapela R, Rodriguez-Santana JR, Rodriguez-Cintron W, Thyne S, Avila PC, Choudhry S, Gonzalez Burchard E. The role of LTA4H and ALOX5AP genes in the risk for asthma in Latinos. Clin Exp Allergy. 2010; 40:582–589. [PubMed: 20067482]

Wittwer J, Marti-Jaun J, Hersberger M. Functional polymorphism in ALOX15 results in increased allele-specific transcription in macrophages through binding of the transcription factor SPI1. Hum Mutat. 2006; 27:78–87. [PubMed: 16320347]

Zhang K, Wang YY, Liu QJ, Wang H, Liu FF, Ma ZY, Gong YQ, Li L. Two single nucleotide polymorphisms in ALOX15 are associated with risk of coronary artery disease in a Chinese Han population. Heart Vessels. 2010; 25:368–373. [PubMed: 20676957]

Table 1

Characteristics of the three study populations

	Colon cancer study			Rectal cancer study			Adenoma study		
	Cases (N=1424)	**Controls (N=1780)**	**p-value**	**Cases (N=583)**	**Controls (N=775)**	**p-value**	**Cases (N=485)**	**Controls (N=578)**	**p-value**
	Mean (SD)	**Mean (SD)**		**Mean (SD)**	**Mean (SD)**		**Mean (SD)**	**Mean (SD)**	
Age (years)	65.2 (9.7)	65.1 (10.3)	NA[a]	62.3 (10.8)	62.6 (10.5)	NA[a]	58 (9.6)	52.9 (11.0)	<0.01
Location [a]	**N** (%)	**N** (%)		**N** (%)	**N** (%)		**N** (%)	**N** (%)	
Proximal	688 (49.6)	NA	NA	NA	NA	NA	104 (21.6)	NA	NA
Distal	700 (50.4)	NA		NA	NA		300 (62.4)	NA	
Rectal	NA	NA		583	775		77 (16.0)	NA	
Sex									
Male	797 (56.0)	946 (53.2)	NA[a]	346 (59.4)	428 (55.2)	NA[a]	304 (62.7)	227 (39.3)	<0.01
Female	627 (44.0)	834 (46.9)		237 (40.7)	347 (44.8)		181 (37.3)	351 (60.7)	
Study Site									
Kaiser Northern California	617 (43.3)	647 (36.4)	<0.01	349 (59.9)	449 (57.9)	0.48	NA	NA	NA
Minnesota	565 (39.7)	791 (44.4)		NA	NA		485 (100)	578 (100)	
Utah	242 (17.0)	342 (19.2)		234 (40.1)	326 (40.1)		NA	NA	
Regular Use of NSAIDs [b]									
Yes	468 (33.2)	748 (42.2)	<0.01	221 (38.1)	359 (46.6)	<0.01	180 (37.1)	257 (44.5)	0.02
No	943 (66.8)	1023 (57.8)		359 (61.9)	412 (53.4)		305 (62.9)	321 (55.6)	

[a] Matching factors

[b] Regular NSAID use defined as at least 1×/week for adenoma stud,y and at least 3×/week for ≥1 month within 2 years of referent date for colon and rectal cancer studies

Genes Chromosomes Cancer. Author manuscript; available in PMC 2014 May 01.

Table 2

Associations between polymorphisms in lipoxygenase pathway and risk of colorectal neoplasia

		Colon cancer					Rectal cancer					Adenomas				
SNPs	**Genotype**	**Cases/ Controls**	**OR**	**95% CI**	**p[a]**	**p_{trend}**	**Cases/ Controls**	**OR**	**95% CI**	**p[a]**	**p_{trend}**	**Cases/ Controls**	**OR**	**95% CI**	**p[a]**	**p_{trend}**
ALOX5																
VNTR	5/5[b] (ref)	979/1188	1.00				430/518	1.00				331/372	1.00			
	<5/≤5	415/530	0.96	(0.82-1.12)			171/274	0.76	(0.60-0.95)			154/200	0.86	(0.65-1.13)		
	>5/≥5	26/50	0.63	(0.39-1.01)	0.14	NA	9/26	0.42	(0.20-0.92)	0.01	NA	18/19	0.92	(0.45-1.87)	0.54	NA
rs4986832	GG	1011/1242	1.00				421/521	1.00				336/389	1.00			
−1700 G>A	AG	372/489	0.94	(0.80-1.10)			149/226	0.82	(0.64-1.05)			135/178	0.87	(0.66-1.16)		
	AA	40/45	1.09	(0.70-1.68)	0.64	0.66	13/27	0.60	(0.31-1.18)	0.11	0.04	12/15	0.94	(0.42-2.14)	0.63	0.40
FLAP																
rs12429692	AA	795/1012	1.00				313/438	1.00				263/318	1.00			
2439 A>T	AT	529/651	1.02	(0.88-1.19)			231/291	1.11	(0.89-1.40)			177/236	0.91	(0.70-1.19)		
	TT	97/113	1.11	(0.84-1.48)	0.75	0.50	39/43	1.26	(0.80-2.00)	0.46	0.21	42/27	2.05	(1.20-3.53)	0.01	0.20
rs17239025	GG	1266/1607	1.00				513/707	1.00				431/528	1.00			
30185 G>C	GC/CC[c]	152/163	1.18	(0.93-1.49)	0.17	NA	70/68	1.43	(1.01-2.04)	0.05	NA	51/54	1.23	(0.80-1.89)	0.34	NA
ALOX12																
rs2073438	GG	704/912	1.00				301/416	1.00				238/303	1.00			
639 G>A	AG	608/712	1.11	(0.96-1.28)			251/292	1.20	(0.96-1.50)			197/231	1.05	(0.80-1.38)		
	AA	104/149	0.91	(0.69-1.19)	0.21	0.74	30/64	0.66	(0.42-1.04)	0.02	0.85	47/47	1.23	(0.77-1.95)	0.68	0.42
ALOX15																
rs4796535	GG	1252/1540	1.00				498/692	1.00				433/546	1.00			
1351 G>A	AG/AA[c]	161/226	0.87	(0.70-1.08)	0.22	NA	85/83	1.43	(1.03-1.97)	*0.03*[d]	NA	50/63	0.96	(0.63-1.45)	0.83	NA
rs2619112	GG	409/513	1.00				143/230	1.00				136/164	1.00			
9562 G>A	AG	721/868	1.03	(0.88-1.22)			316/371	1.37	(1.06-1.77)			244/295	0.98	(0.73-1.32)		
	AA	286/392	0.92	(0.75-1.12)	0.43	0.46	120/172	1.13	(0.83-1.55)	*0.05*[d]	0.34	102/123	1.06	(0.74-1.53)	0.89	0.77

[a] global p-value from co-dominant model; ptrend from log-additive model

[b] Genotype refers to the number of repeats per allele; wildtype = 5 repeats in both alleles (the referent)

[c] the global p-value for the dominant rather than co-dominant model is provided when any cell < 5 (p-trend not applicable)

[d] *Italics underlined* = noteworthy at 25% FDR

Table 3

Interactions between lipoxygenase tagSNPs, NSAID use and risk of colorectal neoplasia

		Colon Cancer NSAID Use					Rectal Cancer NSAID Use					Adenoma NSAID Use				
		Non-Regular		Regular			Non-Regular		Regular			Non-Regular		Regular		
SNP name	**Genotype**	**OR**	**95% CI**	**OR**	**95% CI**	**p_{int}**	**OR**	**95% CI**	**OR**	**95% CI**	**p_{int}**	**OR**	**95% CI**	**OR**	**95% CI**	**p_{int}**
FLAP																
rs17239025	GG	1.00	.	0.60	(0.51-0.71)		1.00	.	0.67	(0.53-0.85)		1.00	.	0.61	(0.50-0.80)	
30185 G>C	GC/CC[a]	0.96	(0.71-1.29)	1.03	(0.70-1.52)	0.02	1.38	(0.88-2.16)	1.06	(0.58-1.93)	0.73	1.23	(0.6-2.4)	0.84	(0.50-1.60)	0.82
rs9508832	GG	1.00		0.58	(0.44-0.76)		1.00		0.64	(0.43-0.95)		1.00		0.55	(0.34-0.89)	
4527 G>A	AG	0.95	(0.77-1.17)	0.62	(0.49-0.79)		0.88	(0.64-1.22)	0.76	(0.54-1.08)		0.68	(0.45-1.02)	0.46	(0.30-0.73)	
	AA	0.92	(0.71-1.18)	0.61	(0.44-0.84)	0.72	1.10	(0.72-1.67)	0.38	(0.23-0.63)	0.02	0.82	(0.49-1.35)	0.45	(0.24-0.84)	0.76
rs9315053	TT	1.00		0.64	(0.51-0.80)		1.00		0.77	(0.55-1.07)		1.00		0.61	(0.41-0.91)	
29380 T>G	GT	1.03	(0.85-1.24)	0.67	(0.53-0.84)		1.00	(0.74-1.36)	0.73	(0.52-1.02)		0.73	(0.50-1.05)	0.45	(0.30-0.69)	
	GG	0.95	(0.71-1.28)	0.49	(0.32-0.76)	0.68	1.58	(0.97-2.59)	0.44	(0.23-0.81)	0.03	0.75	(0.42-1.34)	0.45	(0.20-1.02)	1.00
rs4075692	GG	1.00		0.64	(0.48-0.85)		1.00		0.61	(0.39-0.94)		1.00		0.63	(0.37-1.05)	
13604 G>A	AG	1.00	(0.80-1.24)	0.64	(0.50-0.82)		0.85	(0.60-1.20)	0.75	(0.52-1.08)		0.66	(0.43-1.00)	0.46	(0.29-0.72)	
	AA	0.99	(0.77-1.29)	0.61	(0.44-0.83)	0.97	0.97	(0.64-1.47)	0.40	(0.25-0.64)	0.03	0.82	(0.50-1.33)	0.37	(0.20-0.67)	0.50
rs9551960	AA	1.00		0.57	(0.44-0.74)		1.00		0.53	(0.36-0.79)		1.00		0.75	(0.45-1.23)	
7202 G>A	AG	0.99	(0.81-1.21)	0.71	(0.56-0.89)		0.81	(0.58-1.12)	0.51	(0.36-0.73)		1.20	(0.81-1.78)	0.65	(0.42-1.01)	
	GG	0.95	(0.73-1.24)	0.55	(0.40-0.76)	0.35	0.69	(0.46-1.05)	0.82	(0.51-1.32)	0.05	1.21	(0.72-2.03)	0.73	(0.42-1.29)	0.61
ALOX15																
rs2619112	CC	1.00		0.61	(0.46-0.81)		1.00		0.58	(0.37-0.90)		1.00		0.70	(0.42-1.17)	
9562 C>T	CT	1.01	(0.82-1.25)	0.68	(0.54-0.86)		1.10	(0.79-1.55)		1.00	(0.69-1.43)		0.97	(0.64-1.46)	0.65	(0.42-1.02)
	TT	0.93	(0.71-1.20)	0.53	(0.39-0.73)	0.71	1.30	(0.86-1.97)	0.49	(0.31-0.80)	*0.01* [b]	1.30	(0.78-2.17)	0.55	(0.31-0.98)	0.38
rs2664593	CC	1.00		0.68	(0.56-0.82)		1.00		0.75	(0.57-1.00)		1.00		0.55	(0.38-0.78)	
−189 G>C	CG	1.14	(0.94-1.39)	0.64	(0.50-0.81)		1.07	(0.79-1.45)	0.61	(0.43-0.86)		0.95	(0.66-1.37)	0.61	(0.39-0.96)	
	GG	1.31	(0.86-1.99)	0.95	(0.58-1.56)	0.48	0.93	(0.45-1.92)	0.39	(0.14-1.14)	0.38	0.19	(0.06-0.57)	0.93	(0.26-3.28)	0.04

[a] the p-interaction for the dominant model is provided when any cell < 5

[b] *Italics underlined* = noteworthy at 25% FDR

Table 3A

Interactions between lipoxygenase tagSNPs, NSAID use and risk of colorectal neoplasia

		Colon Cancer NSAID Use					Rectal Cancer NSAID Use					Adenoma NSAID Use				
		Non-Regular		Regular			Non-Regular		Regular			Non-Regular		Regular		
SNP name	**Geno-type**	**Case/ Control**	**OR (95% CI)**	**Case/ Control**	**OR (95% CI)**	**p_{int} *b***	**Case/ Control**	**OR (95% CI)**	**Case/ Control**	**OR (95% CI)**	**p_{int} *b***	**Case/ Control**	**OR (95% CI)**	**Case/ Control**	**OR (95% CI)**	**p_{int} *b***
FLAP																
rs17239025	GG	845/920	1.00	409/684	0.60		311/371	1.00	199/322	0.67		282/299	1.00	149/229	0.61	
30185 G>C			(referent)		(0.51-0.71)			(referent)		(0.53-0.85)			(referent)		(0.50-0.80)	
	GC/CC[a]	94/104	0.96	57/58	1.03	0.02	48/41	1.38	22/27	1.06	0.73	26/25	1.23	25/29	0.84	0.82
			(0.71-1.29)		(0.70-1.52)			(0.88-2.16)		(0.58-1.93)			(0.6-2.4)		(0.50-1.60)	
rs9508832	GG	299/306	1.00	144/238	0.58		121/132	1.00	70/111	0.64		103/87	1.00	65/87	0.55	
4527 G>A			(referent)		(0.44-0.76)			(referent)		(0.43-0.95)			(referent)		(0.34-0.89)	
	AG	453/501	0.95	237/371	0.62		165/209	0.88	121/170	0.76		144/171	0.68	81/126	0.46	
			(0.77-1.17)		(0.49-0.79)			(0.64-1.22)		(0.54-1.08)			(0.45-1.02)		(0.30-0.73)	
	AA	189/213	0.92	86/138	0.61	0.72	73/70	1.10	29/77	0.38	0.02	62/65	0.82	28/45	0.45	0.76
			(0.71-1.18)		(0.44-0.84)			(0.72-1.67)		(0.23-0.63)			(0.49-1.35)		(0.24-0.84)	
rs9315053	TT	412/444	1.00	217/343	0.64		157/191	1.00	107/159	0.77		154/134	1.00	93/117	0.61	
29380 T>G			(referent)		(0.51-0.80)			(referent)		(0.55-1.07)			(referent)		(0.41-0.91)	
	GT	419/449	1.03	215/328	0.67		155/184	1.00	98/157	0.73		124/152	0.73	69/115	0.45	
			(0.85-1.24)		(0.53-0.84)			(0.74-1.36)		(0.52-1.02)			(0.50-1.05)		(0.30-0.69)	
	GG	105/125	0.95	34/73	0.49	0.68	47/36	1.58	16/42	0.44	0.03	31/38	0.75	11/26	0.45	1.00
			(0.71-1.28)		(0.32-0.76)			(0.97-2.59)		(0.23-0.81)			(0.42-1.34)		(0.20-1.02)	
rs4075692	GG	240/256	1.00	129/200	0.64		101/108	1.00	114/214	0.61		95/77	1.00	59/67	0.63	
13604 G>A			(referent)		(0.48-0.85)			(referent)		(0.39-0.94)			(referent)		(0.37-1.05)	
	AG	489/528	1.00	237/377	0.64		177/220	0.85	93/130	0.75		140/168	0.66	88/133	0.46	
			(0.80-1.24)		(0.50-0.82)			(0.60-1.20)		(0.52-1.08)			(0.43-1.00)		(0.29-0.72)	
	AA	212/236	0.99	99/168	0.61)	0.97	81/84	0.97	14/15	0.40	0.03	74/78	0.82	27/58	0.37	0.50
			(0.77-1.29)		(0.44-0.83			(0.64-1.47)		(0.25-0.64)			(0.50-1.33)		(0.20-0.67)	

SNP name	Geno-type	Colon Cancer NSAID Use					Rectal Cancer NSAID Use					Adenoma NSAID Use				
		Non-Regular		Regular			Non-Regular		Regular			Non-Regular		Regular		
		Case/ Control	OR (95% CI)	Case/ Control	OR (95% CI)	p_{int} [b]	Case/ Control	OR (95% CI)	Case/ Control	OR (95% CI)	p_{int} [b]	Case/ Control	OR (95% CI)	Case/ Control	OR (95% CI)	p_{int} [b]
rs9551960	AA	329/352	1.00	149/263	0.57		127/125	1.00	69/117	0.53		98/104	1.00	53/81	0.75	
7202 G>A			(referent)		(0.44-0.74)			(referent)		(0.36-0.79)			(referent)		(0.45-1.23)	
	AG	448/491	0.99	241/341	0.71		167/198	0.81	106/189	0.51		157/166	1.20	84/129	0.65	
			(0.81-1.21)		(0.56-0.89)			(0.58-1.12)		(0.36-0.73)			(0.81-1.78)		(0.42-1.01)	
	GG	161/179	0.95	76/141	0.55	0.35	64/87	0.69	45/53	0.82	0.05	54/52	1.21	36/184	0.73	0.61
			(0.73-1.24)		(0.40-0.76)			(0.46-1.05)		(0.51-1.32)			(0.72-2.03)		(0.42-1.29)	
ALOX15																
rs2619112	CC	278/301	1.00	127/210	0.61		81/123	1.00	48/91	0.58		83/94	1.00	53/70	0.70	
9562 C>T			(referent)		(0.46-0.81)			(referent)		(0.37-0.90)			(referent)		(0.42-1.17)	
	CT	470/501	1.01	245/362	0.68)		174/198	1.10		125/164	1.00	157/166	0.97	87/129	0.65	
			(0.82-1.25)		(0.54-0.86			(0.79-1.55)		(0.69-1.43)			(0.64-1.46)		(0.42-1.02)	
	TT	190/216	0.93	93/174	0.53	0.71	98/88	1.30	46/96	0.49	*0.01*	69/64	1.30	33/59	0.55	0.38
			(0.71-1.20)		(0.39-0.73)			(0.86-1.97)		(0.31-0.80)			(0.78-2.17)		(0.31-0.98)	
rs2664593	CC	561/654	1.00	288/460	0.68		215/255	1.00		0.75		190/185	1.00	110/174	0.55	
−189 G>C			(referent)		(0.56-0.82)			(referent)		(0.57-1.00)			(referent)		(0.38-0.78)	
	CG	320/317	1.14	146/246	0.64		130/137	1.07	70/129	0.61		114/120	0.95	59/78	0.61	
			(0.94-1.39)		(0.50-0.81)			(0.79-1.45)		(0.43-0.86)			(0.66-1.37)		(0.39-0.96)	
	GG	55/46	1.31	33/36	0.95	0.48	14/20	0.93	5/14	0.39	0.38	5/19	0.19	5/6	0.93	0.04
			(0.86-1.99)		(0.58-1.56)			(0.45-1.92)		(0.14-1.14)			(0.06-0.57)		(0.26-3.28)	

[a] the p-interaction for the dominant model is provided when any cell < 5

[b] *Italics underlined* = noteworthy at 25% FDR