Int J Mol Epidemiol Genet 2013;4(3):140-149
www.ijmeg.org /ISSN:1948-1756/IJMEG1306005

Original Article

Phospholipase A2G1B polymorphisms and risk of colorectal neoplasia

Clare Abbenhardt[1], Elizabeth M Poole[2,3], Richard J Kulmacz[4], Liren Xiao[2], Karen Curtin[5], Rachel L Galbraith[2], David Duggan[6], Li Hsu[2], Karen W Makar[2], Bette J Caan[7], Lisel Koepl[2], Robert W Owen[1], Dominique Scherer[1], Christopher S Carlson[2], Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) and CCFR, John D Potter[2,8,9], Martha L Slattery[4], Cornelia M Ulrich[1,2,9]

[1]National Center for Tumor Diseases/German Cancer Research Center, Im Neuenheimer Feld 460, 69120 Heidelberg, Germany; [2]Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, PO Box 19024, Seattle, WA, USA; [3]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston MA 02115, USA; [4]University of Texas Health Science Center at Houston, 6431 Fannin Street, Houston, TX 77030, USA; [5]Department of Medicine, School of Medicine, University of Utah, 30 N. 1900 E., Salt Lake City, UT 84132, USA; [6]Translational Genomics Research Institute, 445 N Fifth St, Phoenix, AZ 85004, USA; [7]Department of Research, Kaiser Permanente Medical Research Program, 2000 Broadway, Oakland, CA 94612, USA; [8]Centre for Public Health Research, Massey University, PO Box 756, Wellington, New Zealand; [9]Department of Epidemiology, University of Washington, PO Box 357236, Seattle, WA 98195, USA

Received June 23, 2013; Accepted July 28, 2013; Epub September 12, 2013; Published September 15, 2013

Abstract: Pancreatic phospholipase A_2, product of *PLA2G1B*, catalyzes the release of fatty acids from dietary phospholipids. Diet is the ultimate source of arachidonic acid in cellular phospholipids, precursor of eicosanoid signaling molecules, linked to inflammation, cell proliferation and colorectal carcinogenesis. We evaluated the association of *PLA2G1B* tagging single-nucleotide polymorphisms with colorectal neoplasia risk. A linkage-disequilibrium-based tagSNP algorithm (r^2=0.90, MAF≥4%) identified three tagSNPs. The SNPs were genotyped on the Illumina platform in three population-based, case-control studies: colon cancer (1424 cases/1780 controls); rectal cancer (583/775); colorectal adenomas (485/578). Evaluating gene-wide associations, principal-component and haplotype analysis were conducted, individual SNPs were evaluated by logistic regression. Two *PLA2G1B* variants were statistically significantly associated with reduced risk of rectal cancer (rs5637, 3702 G>A Ser98Ser, p-trend=0.03; rs9657930, 1593 C>T, p-trend=0.01); principal component analysis showed that genetic variation in the gene overall was statistically significantly associated with rectal cancer (p=0.02). NSAID users with the rs2070873 variant had a reduced rectal cancer risk (P-inter=0.02). Specific associations were observed with tumor subtypes (TP53/KRAS). The results suggest that genetic polymorphisms in *PLA2G1B* affect susceptibility to rectal cancer.

Keywords: Phospholipase A2G1B, polymorphism, colorectal neoplasia, case-control study

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide [1]. Substantial advances have been made in understanding the molecular mechanisms of colorectal carcinogenesis, including the role of inflammation in cancer development and progression [2, 3].

Eicosanoids derived from arachidonic acid (AA) have important roles in inflammation and a wide range of other pathophysiologic processes [4, 5]. Laboratory and epidemiologic studies link aberrant arachidonic-acid metabolism, via the production of prostanoids (COX pathway) or leukotrienes (by lipoxygeneases), to the promotion of carcinogenesis [6-9]. The use of non-steroidal-anti-inflammatory drugs (NSAIDs), which inhibit PTGS (COX)-mediated conversion of AA to prostanoids is associated with decreased risk of CRC [10, 11] and *PTGS2* overexpression is directly correlated with the degree of dysplasia [12]. NSAID effects on survival may be mediated by *PIK3CA* mutation status [13].

Eicosanoid synthesis utilizes AA released from membrane phospholipids by specialized PLA2s

[4] but the ultimate source of AA is diet. Mammals cannot synthesize AA *de novo* and must obtain the fatty acid or a precursor from dietary lipids [14]. During digestion, pancreatic phospholipase A_2 (PLA2G1B), secreted into the intestinal lumen and proteolytically activated, releases fatty acids from the sn-2 position of dietary phospholipids [15-17]. This fosters incorporation of AA into membrane phospholipids of cells throughout the body, available for subsequent release and use in eicosanoid signaling.

Given the role of PLA2G1B in supplying fatty acids as precursors for eicosanoid synthesis and in releasing lysophospholipid signaling molecules [18], we hypothesized that genetic variation in *PLA2G1B* may affect colorectal neoplasia. We evaluated the association of *PLA2G1B* tagSNPs with colorectal adenoma, colon cancer, and rectal cancer. We further examined whether the reduction of CRC risk by use of NSAIDs differs by specific haplotypes and genotypes [18]. The availability of tumor samples allowed us to analyze for differences in mutations of TP53 and K-ras [19].

Material and methods

The analyses are based on three US population-based case-control studies of colorectal adenomas [20], colon cancer [21], and rectal cancer [22] using subjects with available DNA from blood and tissue samples. Methods have been described in detail elsewhere [20-22]. Participants consented and the Institutional Review Board at FHCRC approved the study.

Colorectal adenoma cases (n=485) and polyp-free controls (n=578) were recruited through a large, multiclinic, gastroenterology practice in the Twin Cities area of Minnesota. Eligible participants: were aged 30-74 years; first diagnosed with a colorectal adenoma between 1991-1994; had no known genetic CRC syndrome; had and no history of cancer (except non-melanoma skin cancer), prior colorectal polyps, or inflammatory bowel-disease. All participants underwent colonoscopy; participation was 68%.

Colon cancer cases (n=1424) and controls (n=1780) and rectal cancer cases (n=583) and controls (n=775) were recruited from Utah, the Northern California Kaiser Permanente Medical Care Program (KPMCP), and the Twin Cities Metropolitan area of Minnesota (colon only). Participants aged between 30-79 years with no previous diagnosis of CRC, familial adenomatous polyposis, Crohn's disease or ulcerative colitis were eligible. Colon cancer cases were first diagnosed 1991-1994 [21] whereas rectal cancer cases – including cancer of the recto-sigmoid junction, or rectum – were first diagnosed 1997-2001 [22]. Participation among contacted colon cancer cases was 76% (controls: 69%), among contacted rectal cancer cases 73% (controls: 69%).

Information on health behaviors, anthropometry, medical history, family history of cancer, medication, and demographics were obtained by questionnaire as described previously (referent year 2 years prior to diagnosis/selection) [20-22]. A history of regular use of NSAIDs was defined as using any NSAID at least twice/week for ≥1 month.

Tumor DNA was obtained from paraffin-embedded tissue, categorized by *TP53* or *KRAS* mutations, microsatellite instability (MSI) or the CpG-island methylator phenotype (CIMP) as previously described [23-26]. The proportion of MSI+ tumors in rectal cases was <3% and thus not investigated further. To compare cancer patients with specific molecular types of tumors controls, a generalized-estimating equation with a multinomial outcome was used.

We applied a linkage-disequilibrium (LD)-based, tagging-single-nucleotide-polymorphism (tagSNP) selection algorithm ($r^2 \geq 0.90$, MAF≥4%) to identify 3 tagSNPs in *PLA2G1B* (rs5637 3702 G>A Ser98Ser, rs9657930 1593 C>T and rs2070873 3027 G>T). Germline DNA was extracted from buffy coats for genotyping.

We used the same genotyping platform (Illumina™ GoldenGate) for all three studies. Intraplate and interplate replicates and blinded duplicates were included (5%) for quality control, as were data from 30 CEPH trios (Coriell Cell Repository) genotyped by HapMap. Genotypes were excluded if any of the following was 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; <85% concordance with blinded or non-blinded duplicates; or Hardy-Weinberg *p*-value >0.05.

Unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence

Table 1. Characteristics of the three study populations[a]

	Adenoma Study			Colon Cancer Study			Rectal Cancer Study		
	Cases (N=485)	Controls (N=578)	*p*-value	Cases (N=1424)	Controls (N=1780)	*p*-value	Cases (N=583)	Controls (N=775)	*p*-value
Age	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
	58 (9.6)	52.9 (11.0)	<0.01	65.2 (9.7)	65.1 (10.3)	NA[b]	62.3 (10.8)	62.6 (10.5)	NA[b]
Location	N (%)	N (%)		N (%)	N (%)		N (%)	N (%)	
Proximal	104 (22)	NA	NA	688 (50)	NA	NA	NA	NA	NA
Distal	300 (62)	NA		700 (50)	NA		NA	NA	
Rectal	77 (16)	NA		NA	NA		583	NA	
Sex									
Male	304 (63)	227 (39)	<0.01	797 (56)	946 (53)	NA[b]	346 (59)	428 (55)	NA[b]
Female	181 (37)	351 (61)		627 (44)	834 (47)		237 (41)	347 (45)	
Study Center									
Kaiser Northern California	NA	NA	NA	617 (43)	647 (36)	<0.01	349 (60)	449 (58)	0.48
Minnesota	485 (100)	578 (100)		565 (40)	791 (44)		NA	NA	
Utah	NA	NA		242 (17)	342 (19)		234 (40)	326 (40)	
Regular use of aspirin or NSAIDs									
Yes	180 (37.1)	257 (44.5)	0.02	562 (39.5)	865 (48.6)	<0.01	263 (45.1)	417 (53.8)	<0.01
No	305 (62.9)	321 (55.6)		862 (60.5)	915 (51.4)		320 (54.9)	358 (46.2)	
Body mass index									
Normal/Underweight	159 (33.5)	225 (39.8)	0.10	475 (33.5)	708 (39.8)	<0.01	184 (31.7)	258 (33.5)	0.31
Overweight (25-29.9)	204 (43.0)	213 (37.7)		578 (40.7)	726 (40.9)		242 (41.7)	325 (42.2)	
Obese (30+)	111 (23.4)	127 (22.5)		366 (25.8)	343 (19.3)		155 (26.7)	187 (24.3)	

[a]Percentages may not total to 100% due to rounding and missing values. [b]NA – these were matching factors.

intervals (CIs) for associations between genotypes and outcomes. Genotypes were modeled using indicator variables for the heterozygous and the homozygous variant genotypes (unrestricted/co-dominant model); the dominant model (combining heterozygous and homozygous variants) was used if <10 cases or controls were involved. Models were adjusted for age, sex, and study center. For trend tests, genotypes were treated as a continuous variable. Analyses were restricted to non-Hispanic Caucasians (97% in the adenoma study, 91% and 82% in the colon and rectal cancer studies). A two-sided *p*-value <0.05 was considered statistically significant.

For principal component analysis (PCA) [27], we determined the number of principal components that explained >80% of the variance in the gene and performed logistic regression using these components. Gene-level significance was determined using a likelihood-ratio test. Each PCA was adjusted for age, sex, and study center.

Effect modification of the genetic association by NSAID use was evaluated by testing for a difference in trends within strata (never vs. ever) of NSAID use. Because use of NSAIDs may be confounded by other risk factors for colorectal neoplasia, we adjusted these analyses for age, sex, study center, smoking status, body mass index, physical activity, and intakes of calcium, total energy, and dietary fiber.

Results

Characteristics of the three study populations are presented in **Table 1**. Adenoma patients were younger than colon and rectal cancer patients. Overall tumors in the CRC cases (n=2007) were distributed approximately equally in the rectum, distal colon, and proximal colon. The tagSNPs in *PLA2G1B* were in modest LD (r^2<0.6).

Polymorphisms in *PLA2G1B* were statistically significantly associated with rectal cancer risk. PCA showed statistically significant results for the gene-level association with rectal cancer (p=0.02) (**Table 2**). For two *PLA2G1B* variants, rs5637 (3702 G>A Ser98Ser) and rs9657930 (1593 C>T), we observed statistically significant trends (p-trend=0.03 and 0.01) towards reduced risk of rectal cancer, with a greater number of variant alleles (**Table 2**). Carrying

Table 2. TagSNPs in *PLA2G1B* and risk of colorectal neoplasia, adjusted for age, sex and study center

		Adenoma[a]					Colon Cancer[b]					Rectal Cancer[b]				
SNP	Genotype	Cases	Controls	OR	95% CI	p-trend[d]	Cases	Controls	OR	95% CI	p-trend[d]	Cases	Controls	OR	95% CI	p-trend[d]
rs5637	GG	335	414	1.00	.		1022	1284	1.00	.	.	428	536	1.00		.
3702 G>A	GA	134	158	1.05	0.79-1.40		362	461	0.99	0.84-1.16	.	147	215	0.85	0.66-1.08	.
Ser98Ser	AA	14	10	1.67	0.69-4.04	0.40	36	27	1.70	1.02-2.82	0.39	8	23	0.43	0.19-0.98	0.03
rs2070873	GG	366	447	1.00	.	.	1082	1329	1.00	.	.	436	574	1.00	.	.
3027 G>T	GT	109	129	1.09	0.80-1.48	.	307	405	0.93	0.79-1.10	.	139	181	1.00	0.78-1.29	.
	TT	7	6	1.34	0.43-4.20	0.49	24	34	0.85	0.50-1.44	0.32	6	19	0.42	0.17-1.06	0.36
rs9657930	CC	388	476	1.00	.	.	1161	1446	1.00	.	.	488	614	1.00	.	.
1593 C>T	CT	87	103	1.08	0.77-1.50	.	234	311	0.94	0.78-1.13	.	89	148	0.75	0.56-1.00	.
	TT	7	2	3.47	0.70-17.29	0.30	14	13	1.34	1.63-2.87	0.76	3	10	0.39	0.11-1.43	0.01
		Principal component analysis[c] p=0.49					Principal component analysis[c] p=0.60					Principal component analysis[c] p=0.02				

[a]Adjusted for age and sex. [b]Adjusted for age, sex, and study center. [c]Principal component analysis (PCA) tests for statistical significance at the gene level overall. [d]Tests for significant trends with an increasing number of alleles.

Table 3. *PLA2G1B* haplotypes (≥5% frequency in controls) and risk of rectal cancer

Haplotype[a]	Case (%)	Control (%)	OR (95% CI)
GGA	73.0%	69.0%	1.00 (ref.)
GTA	13.0%	14.1%	0.86 (0.68-1.08)
AGC	8.2%	10.8%	0.70 (0.54-0.92)
ATA	5.8%	6.0%	0.90 (0.65-1.24)
global p=0.05			

[a]Order of SNPs in haplotype: rs5637 (G>A), rs2070873 (G>T), rs9657930 (C>T).

two variant alleles of rs5637 or rs9657930 in *PLA2G1B* was associated with >50% reduction in the risk of rectal cancer for the homozygous variant compared to the wild-type genotype in a log additive model (rs5637, p-trend=0.03; rs9657930, p-trend=0.01). The haplotype ACC was statistically significantly associated with lower rectal cancer risk (OR: 0.70; 95% CI (0.54-0.92), (**Table 3**). We did not observe any statistically significant associations for colorectal adenomas or colon cancers.

Associations stratified by molecular subtypes of colon cancer are shown in Supplementary Table 1. Homozygous variant genotypes were significantly associated with colon tumors characterized by *TP53* or *KRAS* mutations (rs5637 and rs9657930) even though this analysis should be considered exploratory, because the number of cases was small. No association was observed for CIMP+ or MSI+ colon cancers, Supplementary Table 2.

For rectal cancer, we observed a statistically significant interaction between NSAID use and rs2070873, Supplementary Table 3. Among those with the wild-type GG genotype, NSAID use was associated with a ~40% reduction in risk. The variant genotype was associated with a ~30% reduction in risk, but no additional reduction in risk was observed with use of NSAIDs. *PLA2G1B* genotypes did not influence survival (data not shown).

Discussion

Our results provide the first evidence that genetic polymorphisms in the *PLA2G1B* gene can modify susceptibility to rectal cancer. Individuals with variant alleles for rs5637 and rs9657930 had a >50% reduced risk. It was proposed, almost 30 years ago, that CRCs distal to the splenic flexure involve different etiologic factors from proximal cancers [28, 29]. The data presented here support this notion. Most recently it has been argued that a continuum of molecular pathologic patterns, rather than a dichotomy, exists across lower gastrointestinal cancers [30, 31].

We attempted replication of our main effects in the GECCO and CCFR consortia (for consortia description see [32, 33]). Our strongest hit, SNPs rs9657930, was associated with lower rectal cancer risk in the larger US study populations (i.e. CCFR and WHI); however, discordant results were observed in the GECCO-consortium's European-based studies DACHS and ASTERISK, as well as some other US-based studies with small number of rectal cases, resulting in inconsistent replication (Figure S1). Underlying differences in lifestyle, BMI, and NSAID use could be causes for this inconsistency. No molecular tumor subtypes are yet available from this consortium.

Although we observed an association between SNPs in *PLA2G1B* with rectal cancer risk, we found no association with risk of the precursor lesions, i.e., rectal adenomas; this may have been due to the limited statistical power. Nevertheless, it remains possible that genetic variability in these *PLA2G1B* SNPs affects the progression from adenoma to cancer, rather than the early stages of neoplasia.

Our results indicate that general NSAID use is associated with a reduced risk of rectal cancer, as shown previously [34]. An important new finding here, however, is that the risk reduction may be limited to individuals with the *PLA2G1B* rs2070873 wild-type genotype, further supporting the concept of NSAID pharmacogenetics [34, 35].

The SNPs identified as risk modifiers of rectal cancer most probably differ in their impact on the function of the PLA2G1B protein. TagSNP rs5637 is in exon 1, but is a synonymous mutation. Therefore, rs5637 could plausibly impact mRNA stability or protein expression levels and lead to altered PLA2G1B activity. PLA2G1B activity is a major determinant of digestive intake of fatty acids from dietary phospholipid. Thus, *PLA2G1B* SNPs could affect the supply of arachidonic acid available for loading into cellular phospholipids thereby modulating eicosanoid signaling in all tissues [36, 37]. As noted,

arachidonic acid is metabolized by the PTGS (COX)/LOX pathways to prostaglandins and leukotrienes, which have been shown to influence carcinogenesis and specifically colon cancer risk [6-9]. It may be relevant that *PLA2G1B* rs5637 was previously found to be associated with an increased risk of obesity in women [38]: obesity is a well-defined risk factor for CRC [39]. The *PLA2G1B* tagSNP rs9657930 and SNP rs2070873 are located in introns of the gene and could have indirect effects on PLA2G1B activity via altered mRNA processing or splicing.

Eicosanoids may modulate tumor progression through several mechanisms: e.g., by activating receptors on tumor epithelial cells to regulate cell proliferation, apoptosis, and migration/invasion or by inducing epithelial cells to secrete growth factors, pro-inflammatory mediators, and angiogenic factors that switch the microenvironment to one that supports tumor growth [40]. The eicosanoid prostaglandin E_2 mediates pro-inflammatory and tumor-promoting effects of PTGS2, and is strongly connected to CRC development and progression [41].

We observed statistically significant results with colon, but not rectal, tumors after stratification on *TP53* and *KRAS*. Because of the limited sample size for these subset analyses, results should be considered suggestive. Combining research efforts on interactions of molecular changes and i.e. clinical outcomes could possibly lead to a better understanding, described recently as "molecular pathologic epidemiology" by Ogino et al [42-45].

The study has several strengths. The design, including adenoma patients, as well as those with both colon and rectal cancer, covers the range of the colorectal carcinogenesis paradigm. Comparable results are ensured by the use of standardized methods. Molecular subtyping in the colon and rectal cancer studies allows exploration of subtype-specific associations. One limitation of the study is the lack of information on functional impact of the identified SNPs. In this regard, we have used *in silico* approaches as first steps. Further observational and functional follow-up studies are needed for rs5637 (associated with obesity) and rs9657930.

In conclusion, the results provide evidence that polymorphisms in the *PLA2G1B* gene contribute to the risk of rectal cancer and may be associated with colon tumor subtypes. Protection by NSAID use against rectal cancer was confirmed, but may be limited to individuals with specific *PLA2G1B* genotypes.

Acknowledgements

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, Donna Morse, Dave Taverna and Jill Muehling for their contributions to the colon and rectal cancer studies. Asian Consortium: The authors wish to thank the study participants and research staff for their contributions and commitment to this project, Regina Courtney for DNA preparation, and Jing He for data processing and analyses. The french Association STudy Evaluating RISK for sporadic colorectal cancer: The authors are very grateful to Dr Bruno Buecher without whom this project would not have existed. The authors also thank all those who agreed to participate in this study, including the patients and the healthy control persons, as well as all the physicians, technicians, and students. Darmkrebs: Chancen der Verhütung durch Screening: The authors thank all participants and cooperating clinicians, and Ute Handte-Daub, Renate Hettler-Jensen, Utz Benscheid, Muhabbet Celik, and Ursula Eilber for excellent technical assistance. GECCO: The authors would like to thank all those at the GECCO Coordinating Center for helping to bring together the data and people who made this project possible. Health Professionals Follow-up Study, Nurses' Health Study, and Physicians' Health Study: The authors would like to acknowledge Patrice Soule and Hardeep Ranu of the Dana Farber Harvard Cancer Center High-Throughput Polymorphism Core who assisted in the genotyping for Nurses' Health Study, Health Professionals Follow-up Study, and Physician's Health Study under the supervision of Dr Immaculata Devivo and Dr David Hunter, Qin (Carolyn) Guo, and Lixue Zhu who assisted in programming for Nurses' Health Study and Health Professionals Follow-up Study, and Haiyan Zhang who assisted in programming for the Physicians' Health Study. The authors would like to thank the participants and staff of the Nurses' Health Study and the Health Professionals Follow-up Study for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR,

CA, CO, CT, DE, FL, GA, IA, ID, IL, IN, KY, LA, MA, MD, ME, MI, NC, ND, NE, NH, NJ, NY, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, and WY. Prostate, Lung, Colorectal Cancer, and Ovarian Cancer Screening Trial: The authors thank Drs Christine Berg and Philip Prorok, Division of Cancer Prevention, National Cancer Institute, the Screening Center investigators and staff of the Prostate, Lung, Colorectal Cancer, and Ovarian Cancer Screening Trial, Mr Tom Riley and staff of Information Management Services, Inc, Ms Barbara O'Brien and staff of Westat, Inc, and Drs Bill Kopp, Wen Shao, and staff of SAIC-Frederick. Most importantly, the authors acknowledge the study participants for their contributions to making this study possible. Postmenopausal Hormone study: The authors would like to thank the study participants and staff of the Hormones and Colon Cancer study. Tennessee Colorectal Polyp Study: The authors thank the study participants and the research staff for their contributions and commitment to this project, and Regina Courtney for DNA preparation. Women's Health Initiative: The authors thank the Women's Health Initiative investigators and staff for their dedication, and the study participants for making the program possible. A full listing of Women's Health Initiative investigators can be found at: https://cleo.whi.org/researchers/SitePages/Write%20a%20Paper.aspx. This study was supported by the following grants: Grants R01 CA114467, R03 CA123577, R25 CA094880. The Genetics & Epidemiology of Colorectal Cancer Consortium (GECCO), used for validation of results, is supported by National Institutes of Health (NIH) Grant U01 CA137088, R01 CA059045. Funding for participating studies from GECCO follows. ARCTIC: NIH, U01 CA074783; a GL2 grant from the Canadian Institutes of Health Research; and the Cancer Risk Evaluation (CaRE) Program grant from the Canadian Cancer Society Research Institute. ASTERISK: a Hospital Clinical Research Program (PHRC) and supported by the Regional Council of Pays de la Loire, the Groupement des Entreprises Françaises dans la Lutte contre le Cancer (GEFLUC), the Association Anne de Bretagne Génétique and the Ligue Régionale Contre le Cancer (LRCC). CCFR: NIH, U01 CA122839; NIH, RFA # CA-95-011; and through cooperative agreements with members of the Colon Cancer Family Registry and P.I.s. The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centers in the CFRs, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government or the CFR. Colon CFR centers contributing data: Australasian Colorectal Cancer Family Registry (U01 CA097735), Seattle Colorectal Cancer Family Registry (U01 CA074794) and Ontario Registry for Studies of Familial Colorectal Cancer (U01 CA074783). DACHS: German Research Council (Deutsche Forschungsgemeinschaft, BR 1704/6-1, BR 1704/6-3, BR 1704/6-4 and CH 117/1-1), and the German Federal Ministry of Education and Research (01KH0404 and 01ER0814). HPFS is supported by the NIH (P01 CA 055075, UM1 CA167552, R01 137178, and P50 CA 127003), NHS by the NIH (R01 CA137178, P01 CA 087969 and P50 CA 127003) and PHS by the NIH (CA42182). PLCO: Intramural Research Program of the Division of Cancer Epidemiology and Genetics and supported by contracts from the Division of Cancer Prevention, NCI, NIH, DHHS. Additionally, a subset of control samples were genotyped as part of the Cancer Genetic Markers of Susceptibility (CGEMS) Prostate Cancer GWAS (Yeager, M et al. Nat Genet 2007 May; 39(5): 645-9), Colon CGEMS pancreatic cancer scan (PanScan) (Amundadottir, L et al. Nat Genet. 2009 Sep; 41(9): 986-90 and Petersen, GM et al Nat Genet. 2010 Mar; 42(3): 224-8), and the Lung Cancer and Smoking study. The prostate and PanScan study datasets were accessed with appropriate approval through the dbGaP online resource (http://cgems.cancer.gov/data/) accession numbers phs000207v.1p1 and phs000206.v3.p2, respectively, and the lung datasets were accessed from the dbGaP website (http://www.ncbi.nlm.nih.gov/gap) through accession number phs000093 v2.p2. Funding for the Lung Cancer and Smoking study was provided by NIH, Genes, Environment and Health Initiative (GEI) Z01 CP 010200, NIH U01 HG004446, and NIH GEI U01 HG 004438. For the lung study, the GENEVA Coordinating Center provided assistance with genotype cleaning and general study coordination and the Johns Hopkins University Center for Inherited Disease Research conducted genotyping. PMH: NIH, R01 CA076366. VITAL: NIH, K05 CA154337. WHI: NIH, NHLBI, and HHS through HHSN268-201100046C, HHSN268201100001C, HHSN-268201100002C, HHSN268201100003C, H-

HSN268201100004C, and HHSN271201100-004C.

Disclosure of conflict of interest

None declared.

Address correspondence to: Dr. Cornelia Ulrich, National Center for Tumor Diseases (NCT) and German Cancer Research Center (DKFZ), Im Neuenheimer Feld 460, 69120 Heidelberg, Germany. E-mail: neli.ulrich@nct-heidelberg.de

References

[1] GLOBOCAN. GLOBOCAN 2008 fast stats. IARC 2008.

[2] Coussens LM and Werb Z. Inflammation and cancer. Nature 2002; 420: 860-867.

[3] Mantovani A, Marchesi F, Porta C, Sica A and Allavena P. Inflammation and cancer: breast cancer as a prototype. Breast 2007; 16: S27-33. Epub 2007 Aug 2030.

[4] Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science 2001; 294: 1871-1875.

[5] Soberman RJ and Christmas P. The organization and consequences of eicosanoid signaling. J Clin Invest 2003; 111: 1107-1113.

[6] Cuendet M and Pezzuto JM. The role of cyclooxygenase and lipoxygenase in cancer chemoprevention. Drug Metabol Drug Interact 2000; 17: 109-157.

[7] Jones R, Adel-Alvarez LA, Alvarez OR, Broaddus R and Das S. Arachidonic acid and colorectal carcinogenesis. Mol Cell Biochem 2003; 253: 141-149.

[8] Greenhough A, Smartt HJ, Moore AE, Roberts HR, Williams AC, Paraskeva C and Kaidi A. The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. Carcinogenesis 2009; 30: 377-386.

[9] Menna C, Olivieri F, Catalano A and Procopio A. Lipoxygenase inhibitors for cancer prevention: promises and risks. Curr Pharm Des 2010; 16: 725-733.

[10] Asano TK and McLeod RS. Non steroidal anti-inflammatory drugs (NSAID) and Aspirin for preventing colorectal adenomas and carcinomas. Cochrane Database Syst Rev 2004; CD004079.

[11] Ulrich CM, Bigler J and Potter JD. Non-steroidal anti-inflammatory drugs for cancer prevention: promise, perils and pharmacogenetics. Nat Rev Cancer 2006; 6: 130-140.

[12] Cathcart MC, Lysaght J and Pidgeon GP. Eicosanoid signalling pathways in the development and progression of colorectal cancer: novel approaches for prevention/intervention. Cancer Metastasis Rev 2011; 30: 363-385.

[13] Liao X, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, Imamura Y, Qian ZR, Baba Y, Shima K, Sun R, Nosho K, Meyerhardt JA, Giovannucci E, Fuchs CS, Chan AT and Ogino S. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. N Engl J Med 2012; 367: 1596-1606.

[14] Horrobin DF. Nutritional and medical importance of gamma-linolenic acid. Prog Lipid Res 1992; 31: 163-194.

[15] Rimon A and Shapiro B. Properties and specificity of pancreatic phospholipase A. Biochem J 1959; 71: 620-623.

[16] Barros H, Sternby B and Nilsson A. Hydrolysis of phosphatidylinositol by human pancreatic phospholipase A2. Scand J Gastroenterol 1990; 25: 134-140.

[17] Dennis EA. Diversity of group types, regulation, and function of phospholipase A2. J Biol Chem 1994; 269: 13057-13060.

[18] Wang X, Baek SJ and Eling T. COX inhibitors directly alter gene expression: role in cancer prevention? Cancer Metastasis Rev 2011; 30: 641-657.

[19] Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012; 487: 330-337.

[20] Potter JD, Bostick RM, Grandits GA, Fosdick L, Elmer P, Wood J, Grambsch P and 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.

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

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

[23] Slattery ML, Curtin K, Anderson K, Ma KN, Ballard L, Edwards S, Schaffer D, Potter J, Leppert M and Samowitz WS. Associations between cigarette smoking, lifestyle factors, and microsatellite instability in colon tumors. J Natl Cancer Inst 2000; 92: 1831-1836.

[24] Samowitz WS, Curtin K, Schaffer D, Robertson M, Leppert M and Slattery ML. Relationship of Ki-ras mutations in colon cancers to tumor location, stage, and survival: a population-based study. Cancer Epidemiol Biomarkers Prev 2000; 9: 1193-1197.

[25] Samowitz WS, Curtin K, Ma KN, Edwards S, Schaffer D, Leppert MF and Slattery ML. Prognostic significance of p53 mutations in colon cancer at the population level. Int J Cancer 2002; 99: 597-602.
[26] Samowitz WS, Albertsen H, Herrick J, Levin TR, Sweeney C, Murtaugh MA, Wolff RK and Slattery ML. Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. Gastroenterology 2005; 129: 837-845.
[27] Gauderman WJ, Murcray C, Gilliland F and Conti DV. Testing association between disease and multiple SNPs in a candidate gene. Genet Epidemiol 2007; 31: 383-395.
[28] Potter JD and McMichael AJ. Large bowel cancer in women in relation to reproductive and hormonal factors: a case-control study. J Natl Cancer Inst 1983; 71: 703-709.
[29] Potter JD and McMichael AJ. Diet and cancer of the colon and rectum: a case-control study. J Natl Cancer Inst 1986; 76: 557-569.
[30] Yamauchi M, Lochhead P, Morikawa T, Huttenhower C, Chan AT, Giovannucci E, Fuchs C and Ogino S. Colorectal cancer: a tale of two sides or a continuum? Gut 2012; 61: 794-797.
[31] Yamauchi M, Morikawa T, Kuchiba A, Imamura Y, Qian ZR, Nishihara R, Liao X, Waldron L, Hoshida Y, Huttenhower C, Chan AT, Giovannucci E, Fuchs C and Ogino S. Assessment of colorectal cancer molecular features along bowel subsites challenges the conception of distinct dichotomy of proximal versus distal colorectum. Gut 2012; 61: 847-854.
[32] Peters U, Jiao S, Schumacher FR, Hutter CM, Aragaki AK, Baron JA, Berndt SI, Bezieau S, Brenner H, Butterbach K, Caan BJ, Campbell PT, Carlson CS, Casey G, Chan AT, Chang-Claude J, Chanock SJ, Chen LS, Coetzee GA, Coetzee SG, Conti DV, Curtis KR, Duggan D, Edwards T, Fuchs CS, Gallinger S, Giovannucci EL, Gogarten SM, Gruber SB, Haile RW, Harrison TA, Hayes RB, Henderson BE, Hoffmeister M, Hopper JL, Hudson TJ, Hunter DJ, Jackson RD, Jee SH, Jenkins MA, Jia WH, Kolonel LN, Kooperberg C, Kury S, Lacroix AZ, Laurie CC, Laurie CA, Le Marchand L, Lemire M, Levine D, Lindor NM, Liu Y, Ma J, Makar KW, Matsuo K, Newcomb PA, Potter JD, Prentice RL, Qu C, Rohan T, Rosse SA, Schoen RE, Seminara D, Shrubsole M, Shu XO, Slattery ML, Taverna D, Thibodeau SN, Ulrich CM, White E, Xiang Y, Zanke BW, Zeng YX, Zhang B, Zheng W and Hsu L. Identification of Genetic Susceptibility Loci for Colorectal Tumors in a Genome-Wide Meta-analysis. Gastroenterology 2013; 144: 799-807, e724.
[33] Newcomb PA, Baron J, Cotterchio M, Gallinger S, Grove J, Haile R, Hall D, Hopper JL, Jass J, Le Marchand L, Limburg P, Lindor N, Potter JD, Templeton AS, Thibodeau S and Seminara D. Colon Cancer Family Registry: an international resource for studies of the genetic epidemiology of colon cancer. Cancer Epidemiol Biomarkers Prev 2007; 16: 2331-2343.
[34] Ulrich CM, Bigler J and Potter JD. Non-steroidal anti-inflammatory drugs for cancer prevention: promise, perils and pharmacogenetics. Nat Rev Cancer 2006; 6: 130-140.
[35] Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med 1999; 340: 115-126.
[36] Lambeau G and Gelb MH. Biochemistry and physiology of mammalian secreted phospholipases A2. Annu Rev Biochem 2008; 77: 495-520.
[37] Mounier CM, Wendum D, Greenspan E, Flejou JF, Rosenberg DW and Lambeau G. Distinct expression pattern of the full set of secreted phospholipases A2 in human colorectal adenocarcinomas: sPLA2-III as a biomarker candidate. Br J Cancer 2008; 98: 587-595.
[38] Wilson SG, Adam G, Langdown M, Reneland R, Braun A, Andrew T, Surdulescu GL, Norberg M, Dudbridge F, Reed PW, Sambrook PN, Kleyn PW and Spector TD. Linkage and potential association of obesity-related phenotypes with two genes on chromosome 12q24 in a female dizygous twin cohort. Eur J Hum Genet 2006; 14: 340-348.
[39] Kant P and Hull MA. Excess body weight and obesity--the link with gastrointestinal and hepatobiliary cancer. Nat Rev Gastroenterol Hepatol 2011; 8: 224-238.
[40] Wang D and Dubois RN. Eicosanoids and cancer. Nat Rev Cancer 2010; 10: 181-193.
[41] Rigas B, Goldman IS and Levine L. Altered eicosanoid levels in human colon cancer. J Lab Clin Med 1993; 122: 518-523.
[42] Ogino S, Chan AT, Fuchs CS and Giovannucci E. Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. Gut 2011; 60: 397-411.
[43] Ogino S and Stampfer M. Lifestyle factors and microsatellite instability in colorectal cancer: the evolving field of molecular pathological epidemiology. J Natl Cancer Inst 2010; 102: 365-367.
[44] Curtin K, Slattery ML and Samowitz WS. CpG island methylation in colorectal cancer: past, present and future. Patholog Res Int 2011; 2011: 902674.
[45] Spitz MR, Caporaso NE and Sellers TA. Integrative cancer epidemiology--the next generation. Cancer Discov 2012; 2: 1087-1090.

Supplementary Table 1. Associations between selected tagSNPs in *PLA2G1B* and colon cancer subtypes, based on the molecular characteristics *TP53* and *KRAS*

		TP53 mutation										*KRAS* mutation									
SNP	Genotype	Cases			Ctrs.	OR	95% CI	*p*-value	OR	95% CI	*p*-value	Cases			Ctrs.	OR	95% CI	*p*-value	OR	95% CI	*p*-value
		TP53$^+$	*TP53*$^-$	*p*-value[a]		TP53$^+$/Controls			TP53$^-$/Controls			*KRAS*$^+$	*KRAS*$^-$	*p*-value[b]		*KRAS*$^+$/Controls			*KRAS*$^-$/Controls		
Colon tumors																					
rs5637	GG/GA	439	527	0.1	1729	1.00			1.00			286	648	0.001	1729	1.00			1.00		
3702 G>A																					
Ser98Ser	AA	18	11		27	1.85	1.17-2.93	0.001	0.98	0.56-1.73	0.19	18	13		27	3.14	1.98-4.97	<.0001	0.91	0.56-1.46	0.32
rs9657930	AA/AC	444	531	0.04	1741	1.00			1.00			294	649	0.07	1741	1.00			1.00		
1593 C>T	CC	9	3		13	2.35	1.24-4.46	0.02	0.59	0.20-1.73	0.8	7	6		13	2.78	1.34-5.76	0.01	1.01	0.49-2.06	0.59
Rectal tumors																					
rs5637	GG/GA	204	218	0.33	743	1.00			1.00			130	313	0.53	743	1.00			1.00		
3702 G>A																					
Ser98Ser	AA	4	2		22	0.71	.	0.37	0.37	0.11-1.27	0.05	1	5		22	0.28	.	0.09	0.66	0.31-1.37	0.14
rs9657930	AA/AC	204	220	0.04	754	1.00			1.00			129	315	0.82	754	1.00			1.00		
1593 C>T	CC	3	0		9	1.22	.	0.84	<0.01	.	0.03	1	2		9	0.61	.	0.63	0.69	.	0.34

[a]*p*-value TP53$^+$/TP53$^-$. [b]*p*-value KRAS$^+$/KRAS$^-$. Adjusted for age, sex, study center, smoking status, recent NSAID use, BMI, and family history. Ctrs.=Controls.

Supplementary Table 2. Associations between selected tagSNPs in *PLA2G1B* and colon cancer subtypes, based on the molecular characteristics *CIMP*+ and *MSI*+[a]

		CIMP+ mutation					*MSI*+ mutation				
SNP	Genotype	Cases	Controls	OR	95% CI	*p*-value	Cases	Controls	OR	95% CI	*p*-value
Colon tumors											
rs5637	GG/GA	237	1729	1.00			161	1729	1.00		
3702 G>A											
Ser98Ser	AA	6	27	0.99	0.48-2.07	0.21	6	27	1.53	0.68-3.43	0.06
rs9657930	AA/AC	241	1741	1.00			163	1741	1.00		
1593 C>T	CC	1	13	0.38	0.06-2.64	0.61	2	13	1.11	0.29-4.29	0.43

[a]Adjusted for age, sex, study center, smoking status, recent NSAID use, BMI, and family history.

Supplementary Table 3. *PLA2G1B* variants and interactions with non-steroidal anti-inflammatory drug use in rectal cancer

		NSAID Use[a]								
		Never				Ever				
SNP	Genotype	Cases	Controls	OR	95% CI	Cases	Controls	OR	95% CI	P_{inter}
rs2070873 3027 G>T	GG	253	260	1.00	.	183	314	0.58	0.45-0.75	.
	GT or TT	66	98	0.68	0.47-0.98	79	102	0.72	0.51-1.02	0.02

[a]Adjusted for age, sex, study center, smoking status, BMI, dietary calcium, calories, dietary fiber, physical activity.

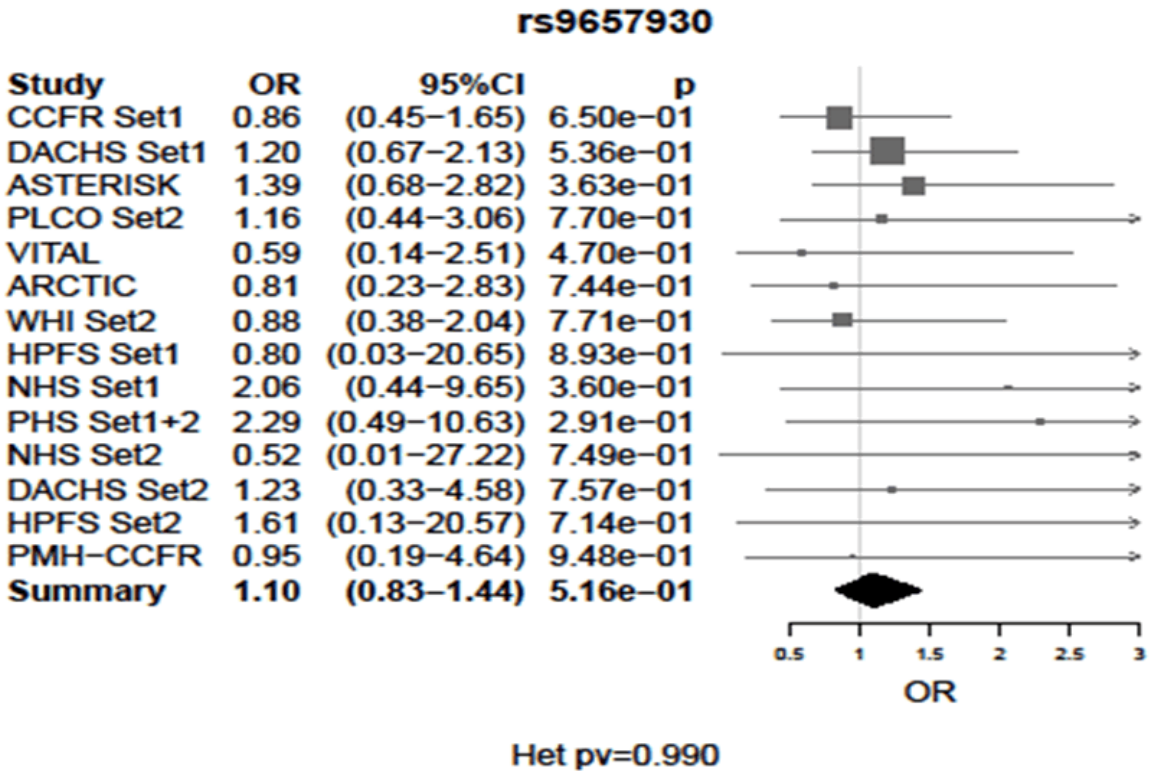

Figure S1. GECCO replication: Forest plot rectal cancer cases only – Including CCFR data. DACHS (Set1 and Set2) and ASTERISK are European studies, the other studies are US based.