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# Polymorphisms in fatty acid metabolism-related genes are associated with colorectal cancer risk

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**Colorectal cancer (CRC) is the third most common malignant tumor and the fourth leading cause of cancer death worldwide. The crucial role of fatty acids for a number of important biological processes suggests a more in-depth analysis of inter-individual differences in fatty acid metabolizing genes as contributing factor to colon carcinogenesis. We examined the association between genetic variability in 43 fatty acid metabolism-related genes and colorectal risk in 1225 CRC cases and 2032 controls participating in the European Prospective Investigation into Cancer and Nutrition study. Three hundred and ninety two single-nucleotide polymorphisms were selected using pairwise tagging with an $r^2$ cutoff of 0.8 and a minor allele frequency of >5%. Conditional logistic regression models were used to estimate odds ratios and corresponding 95% confidence intervals. Haplotype analysis was performed using a generalized linear model framework. On the genotype level, hydroxyprostaglandin dehydrogenase 15-(NAD) (*HPGD*), phospholipase A2 group VI (*PLA2G6*) and transient receptor potential vanilloid 3 were associated with higher risk for CRC, whereas prostaglandin E receptor 2 (*PTGER2*) was associated with lower CRC risk. A significant inverse association ($P < 0.006$) was found for *PTGER2* GGG haplotype, whereas *HPGD* AGGAG and *PLA2G3* CT haplotypes were significantly ($P < 0.001$ and $P = 0.003$, respectively) associated with higher risk of CRC. Based on these data, we present for the first time the association of *HPGD* variants with CRC risk. Our results support the key role of prostanoid signaling in colon carcinogenesis and suggest a relevance of genetic variation in fatty acid metabolism-related genes and CRC risk.**

## Introduction

Colorectal cancer (CRC) is the third most common malignant tumor and the fourth leading cause of cancer death worldwide with a lifetime risk in Western European and North American populations of around 5% (1). Multiple risk factors, both genetic and environmental, are involved in the etiology and prognosis of CRC. International variations in CRC rates and diet between countries indicate that diet has an important influence on CRC development (2). Current evidence suggests a role for specific dietary fatty acids or their intake profile (3) and a recent meta-analysis indicates a reduced risk of CRC with higher consumption of fish, a rich source of long chain n-3 fatty acids (4). Of particular interest is the arachidonic acid (AA) pathway, an n-6 lipid metabolism pathway, which has been implicated with tumor development (5). AA is released from cell membrane-bound phospholipids by means of phospholipase A2 (PLA2) and converted by prostaglandin (PGs)-endoperoxide synthase isoforms COX-1 (PTGS1) and COX-2 (PTGS2) into prostanoids (thromboxanes, prostacyclin and 2 and 4 series of PGs). PGs are important mediators regulating inflammation and numerous physiological processes with potentially procarcinogenic activity (6,7). PG $E_2$ ($PGE_2$) in particular seems to play an important role in modulating tumor growth (proliferation and apoptosis), cell invasiveness, angiogenesis and inflammatory responses and has a crucial function in CRC development (8,9). Degradation of PGs by oxidation to a keto product is mediated by the $NAD^+$-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH) encoded by hydroxyprostaglandin

**Abbreviations:** AA, arachidonic acid; CI, confidence interval; CRC, colorectal cancer; EPIC, European Prospective Investigation into Cancer and Nutrition; *HPGD*, hydroxyprostaglandin dehydrogenase 15-(NAD); HW, Hardy–Weinberg; LD, linkage disequilibrium; MAF, minor allelic frequency; OR, odds ratios; PG, prostaglandin; 15-PGDH, 15-hydroxyprostaglandin dehydrogenase; $PGE_2$, PG $E_2$; *PTGER2*, prostaglandin E receptor 2; PLA2, phospholipase A2; PLA2G6, phospholipase A2 group VI; SNP, single-nucleotide polymorphism; Tag SNP, tagging SNP; *TRPV3*, transient receptor potential vanilloid.

dehydrogenase 15-(NAD) (*HPGD*). Growing evidence indicates that genetic variants in genes of the AA pathway may modulate the risk for colorectal carcinoma (10–20), but a comprehensive analysis of this pathway has not been published to date. In the context of a case–control study nested within the international prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC) study, we have explored the association of 392 gene variants in 43 selected genes directly and indirectly involved in AA metabolism and CRC risk.

## Materials and methods

### *Study settings*

The present study is a nested case–control study within the prospective EPIC cohort. Recruitment in EPIC started in 1992 until 1999 and comprises 519 978 participants (70% women and 30% men) aged mainly 35–69 years. Study details have been reported previously in detail (21). In brief, the EPIC cohort consists of subcohorts of 23 research centers from Denmark, France, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden and the UK allowing comparisons between regions with very different rates of cancer occurrence and distribution of lifestyle factors and dietary habits. Dietary questionnaires, height, weight, lifestyle and personal history data were collected along with biological samples including plasma, serum, leukocytes and erythrocytes. The participants were recruited from the general population of different geographic areas. Exceptions are the French subcohort where participants are women who were members of a health insurance scheme for state school employers, some subcohorts from Italy and Spain where participants were chosen out of individuals who donated blood, part of the Dutch EPIC cohort where women undergoing breast cancer screening were enrolled and the Oxford 'health conscious' subgroup, which includes a high number of vegetarians. All participants signed an informed consent form.

### *Identification of CRC cases and controls*

The follow-up was based on population cancer registries. Exceptions are Germany, France and Greece where a combination of methods, including health insurance records, cancer and pathology registries, and active follow-up of study subjects and their next-of-kin were used. Mortality data were collected from either the cancer or mortality registries at the regional or national level. At the time of establishment of this nested case–control study in June 2005, 1363 eligible CRC cases had occurred. Prevalent cases at baseline were excluded from the study. CRC was defined as a combined group of colon and rectal cancers, excluding anal canal tumors (C18.0–C18.9, C19, C20, 10th Revision of the International Statistical Classification of Diseases, Injury and Causes of Death). Proximal colon cancers were defined as tumors in the cecum, appendix, ascending colon and hepatic flexure, transverse colon and splenic flexure (C18.0–18.5). Distal colon cancers include cancers of the descending and sigmoid colon (C18.6–C18.7). Cancers of the rectum included tumors occurring at the rectosigmoid junction (C19) and rectum (C20).

Two controls were matched to each case following an incidence density matching protocol with the following criteria: gender, age at blood donation (±1 year), study center, fasting status, time of the day of blood collection, menopausal status for women and phase of menstrual cycle for premenopausal women. For some centers, only one control subject was selected. From 3599 available DNA samples, 119 samples were excluded because the case sets were incomplete and 231 samples fulfilled one or more excluding criteria (for example they were derived from prevalent cases or had no valid date of blood donation). Thus, the present study includes a total of 1225 CRC cases and 2032 matched controls with a total of 1554 males and 1703 females.

### *Candidate gene and SNP selection*

Genes were selected according to their role in the fatty acid metabolism and specifically in the AA pathway and their implication in colon carcinogenesis on the basis of previously published experimental and other data. Genetic variants were chosen from the set of common single-nucleotide polymorphisms (SNPs) genotyped in the Caucasian population sample of the HapMap project (Data Release 21a/phaseII Jan07, on National Center for Biotechnology Information B35 assembly, dbSNP build 125). The software Tagger (22), integrated in Haploview (23) was used to select tagging SNPs (Tag SNPs), capturing the variations of all SNP alleles within the gene region with $r^2 > 0.8$ based on the criteria of Gabriel *et al.* (24). The gene region was defined as a stretch of genomic DNA + 5 kb upstream and downstream from the first base of the first known exon to the last base of the last known exon. SNPs were selected with main focus on their tagging characteristics. We restricted the choice to markers, which in HapMap had a Mendelian error rate of 0, a minor allelic frequency (MAF) of at least 5% and a probability of at least 1% that its deviation from Hardy–Weinberg (HW) equilibrium could be explained by chance (HW *P*-value cutoff $\geq$ 0.01). For Illumina-based genotyping, we further selected SNPs with high likelihood of genotyping success (Illumina score $\geq$ 0.6), excluding SNPs with Illumina failure codes. Tag SNPs with an Illumina score <0.6 were replaced by representative variants with higher Illumina score.

A total of 392 SNPs in 43 genes were selected, ~62% were located in introns, 28% in upstream and downstream areas and 10% in exons. For SNPs located in exons, 45% were in untranslated regions and 55% in coding regions (78% were synonymous and 22% were non-synonymous changes). Information on SNP location was taken from the database SNPselector (National Center for Biotechnology Information assembly 36, dbSNP build 126, http://snpselector.duhs.duke.edu/hqsnp36.html).

### *Laboratory techniques*

Whole genome amplification was performed for all samples using the Phi29 polymerase (Amersham Biosciences, GE Healthcare, Piscataway, NJ) to replicate linear genomic DNA by strand displacement amplification with a minimum of 50 ng high-quality DNA as starting material. To minimize genotyping failure due to insufficient starting amounts of whole genome amplification DNA for the Illumina Golden Gate reaction, the DNA concentration for all samples was evaluated with PicoGreen® (Invitrogen GmbH, Karlsruhe, Germany).

The whole genome amplification DNA was genotyped by two methods, the highly multiplexed Golden Gate® assay (Illumina, San Diego, CA) for 379 SNPs and the polymerase chain reaction-based 5′-nuclease allelic discrimination assay (TaqMan®, Applied Biosystems, Foster City, CA) for 13 SNPs. Quality control was tested for both genotyping methods with 278 blinded duplicated blood DNA pairs with an average of four replicates per plate.

### *Statistical analysis*

Conditional logistic regression models were used to estimate odds ratios (OR) and corresponding 95% confidence intervals (95% CI) for the matched case–control sets using SAS version 9.1 (SAS Institute, Cary, NC). Genotype data for the investigated 392 SNPs in 43 candidate genes were categorized by genotype (homozygote minor allele, heterozygote and homozygote major allele) and checked for dominant, recessive model or test for trend. Tests for trend were performed by modeling the number of rare alleles (0, 1 or 2) as a continuous variable. SNPs with a MAF ranging from 0.07 to 0.41 have under a dominant model an expected percentage of carriers of the risk allele between 14 and 65%, which allows the detection of SNPs with low penetrance as well as highly penetrant SNPs. All *P*-values are two tailed. OR and 95% CI were adjusted for gender, age at blood donation, body mass index (<18.5, 18.5 to <30 and ≥30), physical activity at work and leisure time (minimum, moderate, intense and unknown), energy intake, smoking (never, former, smoker and unknown) and alcohol consumption (never, former drinker, drinkers only at recruitment, lifetime drinkers and unknown). Stratified analysis was performed for cancer site [colon (colon distal and colon proximal) and rectum] and gender. We used the Bayesian false discovery probability, an approach first proposed by Sholom Wacholder and colleagues as the false-positive reporting probability (25), and further developed by Jon Wakefield in a Bayesian decision theory (26,27) to determine the noteworthiness of the SNP main associations with CRC risk that achieved *P*-values <0.01. The Bayesian false discovery probability calculates the probability of no association given the data and a specified prior on the presence of an association, and has a noteworthy threshold defined in terms of the costs of false discovery and non-discovery. Our choices of these values to adjust for multiple testing follow the proposals given by Wakefield. We used the $\chi^2$ test statistic to test for heterogeneity between countries, gender and/or subtype-specific SNP effects, comparing the deviations of logistic b-coefficients observed in each subgroup relative to the overall b-coefficient. Each polymorphism was tested for HW equilibrium in the control population.

Haplotype analysis was performed at the level of haplotype blocks within a gene. Haplotype blocks were reconstructed using Haploview (24). The method haplo.glm, which is included in the haplo.stats R-library (28), jointly models observed multipoint SNP genotype and phenotype using a generalized linear model framework. Haplo.glm was used to estimate haplotype associations. The most common haplotype was chosen as reference. To account for the uncertainty of the haplotype estimation, each haplotype pair, consistent with the genotype of an individual and weighted by its estimated probability, was used to model the individual's phenotype. Haplotypes with a frequency of <5% were rated as rare and were subsequently pooled. Suggestive haplotype findings were controlled for multiple testing with simulation *P*-values (29) for confirmation.

## Results

The nested case–control study characteristics including cancer site and the main CRC risk factors are shown in Table I.

*Genes and variants-based analysis*

A list with the selected 43 candidate genes, including their official gene symbols, locus information and rs-number of genotyped Tag SNPs, is available online (Supplementary Table 1 is available at *Carcinogenesis* Online).

The mean genotype call rate was 98.7%. Nineteen SNPs (5%) and 42 DNA samples (1.1%) were excluded from the analysis because of genotyping failure or deficient completion of the setting parameters (Illumina call rate >98%), and 13 SNPs were dropped because of HW *P*-values <0.01. In total, 360 SNPs were included in the statistical analysis. Genotype concordance between repeated samples and completion was >99.9%. ORs and 95% CIs remained essentially the same after adjustment next to gender and age, for body mass index, physical activity, energy intake, smoking and alcohol consumption. Exclusion of the matching variables gender and age at blood donation from the logistic regression model did not have an impact on the results.

Using the Bayesian false discovery probability, variants in *HPGD* (rs2612656 and rs8752), PLA2, group VI (*PLA2G6*) (rs4821737), prostaglandin E receptor 2 (*PTGER2*) (rs17831718) and transient receptor potential vanilloid (*TRPV3*) (rs11078458) (Table II) remained significantly associated with CRC risk with a prior of 1% or greater. Compared with non-carriers, carriers of the minor allele of two *HPGD* polymorphisms (rs8752 and rs2612656) were at 22 and 24% higher risk of CRC, respectively [unadjusted $P_{dominant} \leq 0.009$; 95% CI = (1.05–1.43) and (1.07–1.44), respectively]. The MAFs of these two SNPs were 0.26 and 0.44 in CRC cases compared with 0.22 and 0.40, respectively, in the EPIC controls. The homozygote minor *PLA2G6* rs4821737C allele was associated with higher CRC risk (OR = 1.26, 95% CI = 1.06–1.50, unadjusted $P_{recessive} = 0.009$), whereas a Tag SNP located downstream of *PTGER2* (rs17831718) was inversely associated with CRC risk (OR = 0.73, 95% CI = 0.58–0.91, unadjusted $P_{dominant} = 0.006$). A notable positive disease association was observed for a synonymous-coding *TRPV3* SNP (rs11078458) (OR = 1.32, 95% CI = 1.10–1.59, unadjusted $P_{recessive} = 0.003$).

*PLA2G6* (rs4821767 and rs4821737), stearoyl-CoA desaturase (*SCD* rs11190483), arachidonate 5-lipoxygenase (*ALOX5* rs2291427), thromboxane A2 receptor (*TBXA2R* rs10411250) and *PPARGC1A* (rs3774921) remained significant with a prior of 1% or greater when analysis was stratified by cancer site (Table II). The higher risk among female carriers of at least one copy of the minor *TRPV3* rs6502729 G allele stayed significant with a prior of 0.1%

**Table I.** Characteristics and risk factors for CRC in cases and matched controls

| Characteristics | Cases ($N = 1225$) | | Controls ($N = 2032$) | | $P^a$ |
|---|---|---|---|---|---|
| | *N* | % | *N* | % | |
| Body mass index | | | | | 0.01 |
| <18.5 | 11 | 1 | 9 | 0 | |
| 18.5 to <30 | 1632 | 80 | 1680 | 83 | |
| ≥30 | 389 | 19 | 343 | 17 | |
| Alcohol consumption (g/day) | | | | | <0.01 |
| 0 | 108 | 6 | 138 | 8 | |
| 1–18 | 1187 | 67 | 1193 | 68 | |
| ≥19 | 470 | 27 | 434 | 24 | |
| Gender | | | | | 0.54 |
| Male | 593 | 48 | 961 | 47 | |
| Female | 632 | 52 | 1071 | 53 | |
| Smoking | | | | | 0.15 |
| Never | 518 | 42 | 931 | 46 | |
| Former | 404 | 33 | 658 | 32 | |
| Smoker | 292 | 24 | 424 | 21 | |
| Unknown | 11 | 1 | 19 | 1 | |
| Education | | | | | 0.70 |
| None | 54 | 4 | 105 | 5 | |
| Primary school completed | 393 | 33 | 685 | 34 | |
| Technical/ professional school | 302 | 25 | 499 | 25 | |
| Secondary school | 212 | 18 | 348 | 17 | |
| Longer education/ University degree | 220 | 18 | 333 | 17 | |
| Not specified | 30 | 2 | 43 | 2 | |
| Physical activity | | | | | 0.01 |
| Inactive | 208 | 18 | 256 | 13 | |
| Moderately inactive | 341 | 29 | 541 | 27 | |
| Moderately active | 478 | 40 | 863 | 42 | |
| Active | 106 | 9 | 202 | 10 | |
| Missing | 92 | 8 | 170 | 8 | |
| Cancer site[b] | Men | Women | Men | Women | |
| Colon (C18.0–18.9) | 351 (59%) | 419 (66%) | 577 (60%) | 728 (68%) | |
| Colon distal (C18.6–18.7)[a] | 170 | 201 | 274 | 346 | |
| Colon proximal (C18.0–18.5)[a] | 142 | 184 | 230 | 322 | |
| Rectum (C19–C20) | 242 (41%) | 213 (34%) | 384 (40%) | 343 (32%) | |
| Unspecified (C18.8–C18.9) | 39 | 34 | 73 | 60 | |

[a]$\chi^2$ Test for difference in cases and controls; *N*, number of individuals.
[b]C18.0, cecum, ileocecal valve; C18.1, appendix; C18.2, ascending colon; C18.3, hepatic flexure; C18.4, transverse colon; C18.5, splenic flexure; C18.6, descending colon; C18.7, sigmoid colon, sigmoid (flexure), excludes: rectosigmoid junction (C19); C18.8, overlapping lesion of colon; C18.9, colon, unspecified, large intestine not otherwise specified; C19, malignant neoplasm of rectosigmoid junction, colon with rectum, rectosigmoid (colon); C20, malignant neoplasm of rectum, rectal ampulla.

**Table II.** BFDP-derived significant associations for main effects and subgroup analysis under the assumption of a noteworthy threshold of 0.67

| | Model | OR (95% CI) | *P* | BFDP prior | | |
|---|---|---|---|---|---|---|
| | | | | 0.01 | 0.05 | 0.1 |
| CRC | | | | | | |
| ***HPGD* rs2612656** | Dominant | 1.24 (1.07–1.44) | 0.005 | 0.90 | **0.62** | **0.44** |
| ***HPGD* rs8752** | Dominant | 1.22 (1.05–1.43) | 0.009 | 0.95 | 0.79 | **0.65** |
| *PLA2G3* rs2232170 | Trend | 1.50 (1.10–2.05) | 0.008 | 0.97 | 0.85 | 0.73 |
| ***PLA2G6* rs4821737** | Recessive | 1.26 (1.06–1.50) | 0.009 | 0.94 | 0.74 | **0.57** |
| *PLA2G6* rs2076370 | Dominant | 0.83 (0.72–0.97) | 0.002 | 0.96 | 0.83 | 0.70 |
| ***PTGER2* rs17831718** | Dominant | 0.73 (0.58–0.91) | 0.006 | 0.91 | **0.66** | **0.48** |
| ***TRPV3* rs11078458** | Recessive | 1.32 (1.10–1.59) | 0.003 | 0.87 | **0.57** | **0.39** |
| Rectum | | | | | | |
| ***PLA2G6* rs4821767** | Recessive | 0.63 (0.46–0.85) | 0.002 | 0.90 | **0.62** | **0.44** |
| ***SCD* rs11190483** | Dominant | 1.40 (1.09–1.79) | 0.008 | 0.93 | 0.73 | **0.56** |
| Colon | | | | | | |
| *PPARGC1A* rs2970869 | Trend | 1.63 (1.06–2.53) | 0.005 | 0.98 | 0.90 | 0.80 |
| Distal | | | | | | |
| ***ALOX5* rs2291427** | Dominant | 0.64 (0.49–0.84) | 0.001 | 0.83 | **0.49** | **0.31** |
| ***PLA2G6* rs4821737** | Recessive | 1.55 (1.13–2.12) | 0.006 | 0.94 | 0.75 | **0.32** |
| ***TBXA2R* rs10411250** | Recessive | 0.60 (0.43–0.83) | 0.002 | 0.90 | **0.63** | **0.44** |
| Proximal | | | | | | |
| *PPARGC1A* rs2970848 | Dominant | 0.41 (0.22–0.77) | 0.005 | 0.97 | 0.88 | 0.78 |
| ***PPARGC1A* rs3774921** | Recessive | 0.55 (0.38–0.80) | 0.002 | 0.91 | 0.67 | **0.49** |

BFDP, Bayesian false discovery probability. Bold figures are those that remained noteworthy under the assumption that 95% of the effect size fall between ORs of 0.67 and 1.50 and a noteworthy threshold of 0.67, assuming that the false non-discovery was twice as costly as false discovery.

(data not shown). Complete subtype-specific findings for all SNPs with numbers of cases and controls included in each comparison level are presented in Supplementary Table 2 is available at *Carcinogenesis* Online.

*Haplotype-based analysis*

Given the linkage disequilibrium (LD) patterns of *HPGD* in Caucasian populations, the 10 successfully genotyped *HPGD* Tag SNPs captured 28 of 38 (74%) of the SNPs at the $r^2 = 0.95$ level. We identified four haplotype blocks of closely located SNPs in *HPGD*. Five out of 20 SNPs in block 1 were identified as Tag SNPs sufficient to cover the estimated haplotype distribution without loss of genetic information. Five haplotypes were present on a large proportion of chromosomes in the population (greater 5%) under study (Table III). The most frequent haplotype detected in 30% of the controls was used as the reference haplotype. Consistent with the genotype results for each single SNP, the *HPGD* AGGAG haplotype with a frequency of 15% (containing the risk increasing minor alleles of rs8752 and rs2612656 and the major allele of rs7349744) was statistically significantly associated with a 33% higher CRC risk (95% CI = 1.17–1.49). Individuals carrying the CT haplotype of PLA2, group III (*PLA2G3*) (relative frequency of 22%) containing the rare variants of rs2074739 and rs2232170, were at 17% higher CRC risk (95% CI = 1.05–1.30, *P*-value = 0.003).

Data mining from the HapMap project led to a selection of three SNPs in *PTGER2* that define a single haplotype block covering 100% of all alleles and characterizing the most common haplotypes. In comparison with the AAA reference haplotype (76% frequency), the less common haplotype GGG (6%) was associated with a significantly lower CRC risk (OR = 0.74, 95% CI = 0.53–0.95, *P*-value = 0.006).

## Discussion

In this case–control study nested in the EPIC cohort, 360 polymorphisms in genes directly and indirectly related to the AA metabolism were successfully genotyped in 1225 CRC cases and 2032 matched controls. Four tagging variants (*HPGD* rs2612656 and rs8752, *PLA2G6* rs4821737 and *TRPV3* rs11078458) were positively and one (*PTGER2* rs17831718) was negatively associated with CRC risk. In addition, three haplotypes in *HPGD*, *PTGER2* and *PLA2G3* significantly modified CRC risk (Table III).

In detail, our data support an association of variants in two PLs (*PLA2G3* and *PLA2G6*) with higher CRC risk. PLA2s are involved in the release of AA and other polyunsaturated fatty acids from membrane phospholipids. To date, a number of PLA2s have been identified, all with distinct, tissue-specific roles in various pathophysiological events. *PLA2G3* was shown to be preferentially expressed in microvascular endothelial cells as well as in tumor cells and has the ability to facilitate the growth of human CRC cells both *in vitro* and *in vivo* (30,31). Increased *PLA2G3* gene expression was recently reported to activate Wnt/ß-cat/Tcf-4 pathway genes, representing one of the key pathways in colon carcinogenesis (32). Worth mentioning is the fact that rs4821737 (downstream of *PLA2G6*) is located in intron 4 of BAI1-associated protein 2-like 2 (*BAIAP2L2*)—a gene with little information regarding its function and potential role in colon tumorigenesis.

The relevance of the AA pathway in the etiology of sporadic CRC was recently supported by data of a large French case–control study that found association of *PLA2G2A* and *COX-1* with CRC risk (33). *PLA2G2A* rs11677, however, was associated with a decreased risk in the French study but was positively associated with CRC risk in our study population ($OR_{AA/GA\ versus\ GG} = 1.16$, 95% CI = 0.98–1.38). Our data did not reveal a significant relevance of the explored variants in *COX-1* and *COX-2* in the etiology of CRC. A recent pooled analysis of *COX* polymorphisms pointed toward an implication of *COX-2* variants in colorectal carcinogenesis mainly for Asian populations (34). To date, most of the published associations of these genes with CRC risk were reported in the context of gene–environment interactions involving non-steroidal anti-inflammatory drug intake or diet (11,17,18,35–37). Joint evaluation of these exposures was not in the center of the manuscript.

Most interestingly, genetic variants in *HPGD* encoding 15-PGDH, an enzyme acting antagonistic to the COX enzymes, appear to modulate CRC risk. 15-PGDH is normally expressed in the gastrointestinal mucosa and catalyses through oxidation the first rate-limiting step in the inactivation of eicosanoids (38). PGDH overexpression does not only increase PG degradation but also promotes the expression of genes involved in PG synthesis. PGDH levels were decreased or absent in CRC and HPGD-deficient mice were more susceptible to develop colon tumors, suggesting its tumor suppressor function. Thus, induction of COX-2 and repression of PGDH appear to be complementary pathways to increase $PGE_2$ levels in tumor tissues. *HPGD* is located on chromosome 4, contains seven exons and several regions within the 5′ flanking region with clustered putative transcription factor-binding sites (39). Two variants in *HPGD* (rs2612656 and rs8752) and one haplotype (AGGAG) were significantly associated with higher CRC risk in this study. Interestingly, mutations in this gene were recently identified as causal for primary hypertrophic osteoarthropathy, a rare familial disorder characterized by several clinical features including digital nail clubbing (40) and a phenotype of isolated congenital nail clubbing (41). To the best of our knowledge, functional data on genetic variants in *HPGD* are lacking and the

**Table III.** Haplotype frequencies and results for their associations with CRC risk in cases and controls of the EPIC study

| Gene/haplotype | OR (95% CI) | $P_{value}$[a] | Relative frequency (%) | | |
|---|---|---|---|---|---|
| | | | Cases | Controls | Pooled |
| *HPGD* rs9312555-rs8752-rs2612656-rs17360144-rs7349744 | | | | | |
| A-A-A-A-A | Reference | | 0.29 | 0.30 | 0.30 |
| A-A-A-A-G | 0.96 (0.84–1.11) | 0.56 | 0.26 | 0.28 | 0.28 |
| G-G-A-A-G | 1.03 (0.89–1.21) | 0.81 | 0.15 | 0.15 | 0.15 |
| **A-G-G-A-G** | **1.33 (1.17–1.49)** | **0.0003** | **0.17** | **0.14** | **0.15** |
| A-G-G-C-G | 1.31 (0.56–3.11) | 0.68 | 0.07 | 0.07 | 0.07 |
| *PLA2G3* rs2074739-rs2232170 | | | | | |
| T-C | Reference | | 0.57 | 0.58 | 0.58 |
| C-C | 0.92 (0.81–1.06) | 0.28 | 0.18 | 0.20 | 0.20 |
| **C-T** | **1.17 (1.05–1.30)** | **0.003** | **0.24** | **0.21** | **0.22** |
| PTGER2 rs17831718-rs17125362-rs1254580 | | | | | |
| A-A-A | Reference | | 0.77 | 0.76 | 0.76 |
| A-G-G | 1.08 (0.94–1.25) | 0.29 | 0.14 | 0.13 | 0.14 |
| **G-G-G** | **0.74 (0.53–0.95)** | **0.006** | **0.05** | **0.07** | **0.06** |

*P*-values < 0.05 were presented in bold.
[a]Simulated *P*-values, adjusted for multiple comparisons.

present study appears to be the first to evaluate possible associations between genetic heterogeneity in *HPGD* and CRC risk.

Once produced, prostanoids are released from the cell into the extracellular compartment where they induce their biological functions. Each prostanoid activates distinct receptors. $PGE_2$ actually may activate four receptor isoforms, i.e. EP1–EP4 (PTGER 1–4). $PGE_2$ signaling via PTGERs is associated with several pathologic conditions, impaired host immune antitumor surveillance and stimulated angiogenesis via $PGE_2$–PTGER2 activity (42–45). *PTGER2* gene disruption in adenomatous polyposis coli$^{\Delta 716}$ knockout mice decreased the number and size of intestinal polyps, suggesting that *PTGER2* is important in mediating intestinal polyposis (46). PTGER activation was also reported to increase ß-catenin/T-cell factor transcriptional activity, secondary to loss of adenomatous polyposis coli tumor suppressor gene function and thereby is affecting a crucial pathway implicated in the initiation of colon cancer (47). We found a significantly lower CRC risk in association with an intergenic, downstream *PTGER2* gene variant (rs17831718) and a *PTGER2* haplotype (GGG). Polymorphisms in *PTGER2* were previously associated with the pathogenesis of inflammatory conditions (48–50), but we present the first epidemiological evidence of an association with colon carcinogenesis.

A change in the concentration of intracellular free calcium is often a key component in the intracellular signaling pathway that follows the stimulation of lymphocytes and other cells by various agents. There is evidence that free fatty acids such as docosahexaenoic acid and eicosapentaenoic acid influence calcium concentrations by blocking its entry into cells (51). Another interesting finding from this study is that a synonymous-coding SNP in *TRPV3* (rs11078458) may be a risk factor for CRC.

Although the molecular mechanisms are not fully understood, growing evidence is accumulating that foods and dietary patterns influence the risk for tumors of the left- (distal) and right (proximal)-sided colon differently (52). For example, increased calcium intake was associated with reduced risk of distal colon cancers in particular (53,54). Furthermore, there are molecular and functional differences in proximal and distal colon cancer etiology, one site being more susceptible to specific exposures, such as diet, than the other (55,56). Thus, it is plausible that different genes may be implicated in the various CRC sites.

In the present study, we found two genes (*PLA2G6* and *SCD*) with noteworthy risk differences for colon and rectum. When associations for distal and proximal colon cancer risk were examined separately, three variants in two genes (*ALOX5* and *TBXA2R*) were associated with significantly reduced distal colon cancer risk, one SNP in *PLA2G6* with higher distal colon risk and one *PPARGC1A* SNP with higher proximal colon cancer risk. For gender-specific analyses, we found *TRPV3* rs6502729 significantly associated with higher CRC risk particularly in women. However, replication in independent studies is required to verify whether the here reported associations are site and gender specific.

Strengths of our study include the population-based design as nested case–control study within EPIC, the accurate ascertainment of disease through cancer registries and clinical records with histological confirmation and the sample size to detect main effects of common SNPs and haplotypes with adequate statistical power. However, several aspects of the current study warrant attention. With the pairwise tagging approach, we captured 94% of common SNP variation in European populations (57), >80% of all existing haplotype information (58), and recent work showed that insertion–deletion variants (indels) are often in strong LD with neighboring SNPs and may also be tagged (59,60). By using Tag SNPs, we focused on indirect genetic association mapping by detection of causative disease variants via their non-random association with genotyped SNPs. This approach targets at common SNPs, as they allow statistically powered studies in contrast to rarer variants. Although we aimed to capture the majority of gene variants, based upon the concept of LD, it is possible that in tagging only common SNPs we excluded the chance of discovering any substantial effect associated with low-frequency SNPs (MAFs $< 0.05$), which could be functional. Additionally, we may have missed untyped variants in the investigated genes or SNPs, which are not in LD with the here investigated variants. Differences in allele frequencies between cases and controls due to different ancestry could cause confounding by population stratification (61,62). It has been proposed that the effects of stratification can be controlled by careful matching of controls and cases according to ancestry and geographic origin (63). Cases and controls of the present study were systematically matched for country, region and recruitment center and regression analysis was adjusted for each country of recruitment. Additionally, for each SNP, allelic heterogeneity between cases and controls was checked. No significant difference was observed after stratification by EPIC country (data not shown). Heterogeneity in haplotype frequencies between the study populations did not impact upon the results of our haplotype association analysis in terms of spurious associations, i.e. false-positive results. However, we cannot exclude a loss of power due to heterogeneity.

Genome-wide association scans identified several SNPs with impact on CRC. However, the biological background of most variants identified through Genome-wide association scan and their contribution on CRC is not fully explained. Information from studies with more complete coverage and using larger numbers of patients and controls together are needed. The novel findings reported here have not been mentioned previously within a Genome-wide association scan, but our results are supported by the biological and medical knowledge and plausibility of the various functions of the here reported genes, their direct or indirect role in the fatty acid metabolism as well as their importance for the biological reactions including hemostasis, immune response and inflammation. Clearly, the replication of our findings in further independent studies is of great importance. Future analysis of the EPIC data regarding recently identified at-risk loci for CRC is needed to better estimate combined risks and the overall contribution of genetic variance in the fatty acid pathway in colon carcinogenesis. Furthermore, joint analyses with biomarker data of dietary fatty acid intake are needed to assess the contributing role of individual genetic susceptibility in modulating the risks of disease related to heterogeneity in fatty acid intake.

Overall, within this large case–control study, we found evidence for an association of *HPGD*, *PLA2G3*, *PLA2G6*, *TRPV3* and *PTGER2* with CRC risk. Particularly, this first report of an association of genetic variability in *HPGD*, a suspected tumor suppressor gene, warrants special consideration in large-scale replication projects of CRC.

## Supplementary material

Supplementary material can be found at http://carcin.oxfordjournals.org/

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