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Polymorphisms in the genes involved in the arachidonic acid-pathway, fish consumption and the risk of colorectal cancer

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The objective of this study on colorectal cancer was to investigate the associations between SNPs in the genes involved in the arachidonic acid (AA)-pathway, their haplotypes and colorectal cancer. Moreover, interactions between SNPs and fish consumption were considered. In this study, a total of 508 cases and 772 controls were included, originating from 2 prospective cohorts, the Monitoring Project on Cardiovascular Disease Risk Factors (PPHV) and Diagnostisch Onderzoek Mammacarcinoom (DOM). Genotypes of 23 SNPs in 7 candidate genes were determined and the modifying effect of fish consumption was considered. A protective effect of the minor allele of SNP *V102V* in *PTGS2* was observed (odds ratio (OR), 0.37; 95% confidence intervals (CI), 0.16–0.87). The haplotype representing this allele showed a weaker inverse association, indicating that 2 alleles are necessary to obtain this protective effect. Fish consumption data was available for 209 cases and 418 controls. Increased fish consumption was inversely associated with cancer, although not significant (OR, 0.83; 95% CI, 0.57–1.20). Despite the substantial reductions in cancer risk for some genotypes in combination with high fish intake, no significant interactions between any SNP studied and fish consumption were observed. We have previously described an association between colorectal adenomas and SNP *V102V* in *PTGS2* and have now confirmed this association for colorectal adenocarcinomas. Fish consumption of once a week or more might protect against colorectal cancer, but no significant interactions with SNPs in the genes involved in the AA-pathway could be detected within the study.

Key words: polymorphisms; haplotypes; arachidonic acid pathway; fish consumption; colorectal cancer

Colorectal cancer is one of the leading causes of death through cancer in the developed world, with over 9,500 new patients in The Netherlands in the year 2002 alone.[1] Epidemiological and experimental evidences indicate that both genetic and environmental factors are involved. Lipid metabolism, in particular the arachidonic acid (AA)-pathway, appears to play a critical role in colorectal tumor development, as reviewed by Jones *et al.*[2]

The evidence implicating several major genes within this pathway in colon tumorigenesis has been discussed previously,[3] but is recapitulated in short. Cyclooxygenase-1 and cyclooxygenase-2, also known as prostaglandin endoperoxide synthases (*PTGSs*), are 2 key genes in the AA-pathway, encoding enzymes that initiate the synthesis of biologically important prostanoids (PGs) and eicosanoids.[4] Both genes have been demonstrated to be involved in intestinal tumorigenesis, and proposed mechanisms include promoting tumor growth, cell proliferation, angiogenesis and inhibiting apoptosis. Phospholipase A_2 (PLA_2) enzymes are a family that, besides other conversions, catalyze the generation of free fatty acids, such as AA, from membrane-bound phospholipids. Most important in the AA-pathway are cytosolic PLA_2 (PLA2G4A) and secretory PLA_2 (PLA2G2A), which have both been demonstrated in mouse models to be involved in tumorigenesis.[5,6] Lipoxygenase (ALOX) is an enzyme for which AA is one of the substrates. Activation of the enzyme ALOX15 might inhibit carcinogenesis *via* the conversion of linoleic acid into 13-*S*-hydroxyoctadecadienoic (13-*S*-HODE) acid, which in turn downregulates PPARδ, thereby restoring apoptosis.[7] Peroxisome proliferator-activated receptors (PPARs) play an important regulatory role in lipid metabolism and cancer, and PPARs can be activated by a variety of eicosanoids.[8,9] There are 3 distinct types of PPARs, α, δ and γ. Both PPARδ and PPARγ have been implicated to play a role in colorectal tumorigenesis by transcriptionally controlling pathways involved in cell proliferation, differentiation and survival.[8] Moreover, recently the role of both PPARδ and PPARγ as focal points of cross-talk between prostaglandin and Wnt signaling pathways has been suggested.[10,11] Most data of PPARγ suggests a tumor suppressive role; however, there is still some controversy about the increase of intestinal polyps in Apc^{Min} mice by ligands of PPARγ.[12,13]

Genetic variants represented by Single Nucleotide Polymorphisms (SNPs) in genes encoding these key players of the AA-pathway may contribute to variation in susceptibility to colorectal cancer. Recently, the focus of attention has shifted from the use of single genetic variants in association studies to using haplotypes.[14] By using the information about the common SNPs in a particular population, combinations of SNP alleles (called haplotypes) can be estimated, after which differences in frequencies between cases and controls can be evaluated.[15,16] Haplotypes can also be used to select those SNPs that are representative for a certain haplotype, the so-called tagging SNPs.[15]

Among environmental factors, diet appears to play a major role in the risk of developing colorectal cancer. Several dietary components have been identified as risk factors, including red meat and alcohol, whereas others have been shown to reduce cancer risk, for example, fruit, vegetables and calcium.[17] Additionally, some (but not all) animal experimental studies and epidemiological studies have shown that fish consumption may decrease the risk of colorectal tumors.[18–20] The mechanism by which fish may exert its protective effect on colorectal tumors might include modulation of the AA-pathway, by increasing the n-3 polyunsaturated fatty acids (PUFAs) content in cellular membranes. The AA-pathway utilizes both dietary n-3 and n-6 PUFAs to produce eicosanoids. High fish consumption might therefore cause a shift in substrates from n-6 to n-3 PUFAs, resulting in a different class of eicosanoids. A high ratio of fish fatty acids (FAs) to AA in adipose tissue, as a marker for

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Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; PTGS, prostaglandin endoperoxide synthase; PG, prostanoids; PLA2, phospholipase A2; LOX, lipoxygenase; 13-*S*-HODE, 13-*S*-hydroxyoctadecadienoic; PPAR, peroxisome proliferator-activated receptor; SNP, single nucleotide polymorphism; PUFA, polyunsaturated fatty acid; UTR, untranslated region.

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TABLE I – CHARACTERISTICS OF STUDY POPULATION

	PPHV[1]		DOM[2]	
	Cases (n = 204)	Controls (n = 399)	Cases (n = 304)	Controls (n = 373)
Demographic				
Female (%)	44.1	44.9	100	100
Age at baseline, mean (SD)	51.2 (7.53)	52.6 (6.81)	57.3 (4.13)	56.2 (4.14)*
Height, cm, mean (SD)	171.5 (9.54)	170.2 (9.30)	162.7 (5.89)	162.1 (10.5)
Weight, kg, mean (SD)	77.5 (12.4)	75.0 (12.5)	69.9 (10.7)	68.5 (10.4)
BMI, kg/m^2, mean (SD)	25.9 (3.68)	25.5 (3.61)	26.5 (3.92)	25.9 (3.53)
Total cholesterol, mmol/l (SD)	5.97 (1.23)	6.03 (1.12)		
HDL cholesterol, mmol/l (SD)	1.22 (0.35)	1.24 (0.33)		
Total/HDL cholesterol ratio (SD)	5.28 (1.87)	5.24 (1.76)		
Lifestyle				
Diet, mean (SD)				
Fish, frequency per month	2.49 (2.96)	2.89 (3.12)		
Meat, frequency per day	0.67 (0.29)	0.69 (0.34)		
Fruit, frequency per day	0.96 (0.66)	1.07 (0.66)		
Vegetables, frequency per day	1.12 (0.62)	1.13 (0.57)		
Total energy, kJ/day	6,999 (1,877)	6,918 (2,135)		
Alcohol, g/day	10.1 (13.1)	10.4 (13.8)		
Calcium, mg/day	969 (387)	957 (405)		
β-carotene, mg/day	1.10 (0.56)	1.11 (0.60)		
Vitamin C, mg/day	55.9 (27.7)	57.4 (25.4)		
Vitamin E, mg/day	12.2 (5.22)	11.9 (5.18)		
Fiber, mg/day	17.2 (5.58)	16.4 (5.67)		
Other lifestyle, %				
Smoking, ever	69.3	71.2	26.8	26.4
Education, low	56.9	53.8		
Regular physical activity leisure time	63.4	71.4*		
Regular physical activity work	23.7	32.7*		
Use of insulin	2.45	1.00		
Use of aspirin	33.3	26.8		

[1]Monitoring Project on Cardiovascular Disease Risk Factors.–[2]Diagnostisch Onderzoek Mammacarcinoom.

*$p \leq 0.05$ Wilcoxon rank test (continuous variables) or Fisher's exact test (categorical variables).

fatty acid intake, has been associated with a lower risk of colorectal adenomas.[21] SNPs in the genes involved in AA-pathway may interact with fish consumption by influencing the conversion of these PUFAs into eicosanoids.

In this association study, 23 SNPs in genes encoding 5 enzymes and 2 nuclear receptors have been used. The objective of this study on colorectal cancer was to investigate whether SNPs, previously found to play a role in adenoma formation, might be important in the progression of adenoma to carcinoma, and to evaluate the role of fish consumption in this process.[3] In addition, a previously reported association between a SNP in the 3′ UTR of *PTGS2* and colorectal cancer is investigated.[22]

Material and methods

Study population

We conducted a nested case–control study using 2 Dutch studies. The first prospective study called Monitoring Project on Cardiovascular Disease Risk Factors (PPHV) conducted in 3 Dutch towns between 1987 and 1991 included over 36,000 participants. A detailed description of the study was published previously.[23] Follow-up for incident cancer for the period from 1987 to mid-2003 was achieved *via* computerized record linkage with the Netherlands Cancer Registry and with the 3 regional cancer registries. In total, 209 colorectal cancer cases (46 prevalent and 163 incident) could be identified. A random sample of controls was drawn from the same cohort, frequency matched on age (5-year intervals), gender and center, to include 418 subjects.

The second so-called ''DOM'' (Diagnostisch Onderzoek Mammacarcinoom) project was conducted between 1976 and 1978, wherein all women born between 1911 and 1925 living in the city of Utrecht were invited to participate in a population-based screening program for the early detection of breast cancer.[24] Each participant provided an overnight urine sample which was stored at −20°C. Follow-up for incident cancer from 1976 through linkage to the regional cancer registry resulted in a total of 355 colorectal cancer cases. A random selection of controls was drawn frequency matched on age at intake (5-year intervals) and all were of postmenopausal status, to include 426 subjects.

Fish consumption and other lifestyle factors

For the PPHV cohort, information about fish consumption and other lifestyle factors was obtained using a self-administered questionnaire. Dietary habits were estimated using a validated semi-quantitative food-frequency method.[25] Frequency of fish consumption was assessed in 6 categories: never, less than once a month, 1–3 times monthly, once per week, 2–4 times weekly and more than 4 times a week.

The intake of total energy from this questionnaire was calculated with the computerized Dutch food composition table.[26]

Genetic analysis

For participants of the PPHV cohort, genomic DNA was extracted from buffy coats by digestion with proteinase K, followed by salting out with potassium acetate and chloroform/isoamyl alcohol extraction.[27] For participants of the DOM cohort, DNA was isolated from 100-ml frozen urine by alcohol precipitation as described earlier.[28]

TABLE II – *PPARγ AND PTGS2 VARIANTS AND COLORECTAL CANCER IN THE PPHV AND DOM COHORTS*

Genotype (rs number)[49]	PPHV cohort		DOM cohort		OR (95%CI)[1]	Power
	Cases (n = 204)	Controls (n = 399)	Cases (n = 304)	Controls (n = 373)		
PPARγ						
P12A (*rs1801282*)						
CC	160	325	387	596	1.00 (ref)	
CG	40	71	92	146	0.96 (0.72–1.28)	0.05
GG	1	2	8	8	1.34 (0.50–3.62)	0.08
H477H (*rs3856806*)						
CC	155	307	380	555	1.00 (ref)	
CT	42	79	92	162	0.82 (0.61–1.10)	0.28
TT	4	4	7	9	1.14 (0.42–3.11)	0.04
PTGS2						
c.-1329A>G (*rs689466*)						
AA	127	243	283	422	1.00 (ref)	
AG	59	128	132	226	0.87 (0.67–1.13)	0.20
GG	10	20	19	41	0.68 (0.39–1.20)	0.31
V102V (*rs5277*)						
GG	142	287	339	521	1.00 (ref)	
GC	56	100	133	195	1.04 (0.80–1.35)	0.05
CC	5	11	7	28	0.37 (0.16–0.87)	0.78
c.2242T>C (*rs5275*)						
TT	97	190	216	339	1.00 (ref)	
TC	83	163	171	281	0.97 (0.75–1.25)	0.05
CC	20	35	55	73	1.14 (0.77–1.69)	0.11
c.3618A>G (*rs4648298*)						
AA	194	368	461	699	1.00 (ref)	
AG+GG	5	21	13	36	0.56 (0.29–1.06)	0.49

[1]Univariate adjustment for cohort, analysis of the two cohorts added up.

Twenty-one of the 23 SNPs have been selected on the basis of an inventory of the genetic variation in the Dutch population of the selected genes, in which 58 polymorphisms were identified. The SNP selection was based on allele frequency (with some exceptions, only those SNPs with a minor allele frequency of 5% or higher were considered), position in the gene (when possibly evenly distributed across the gene), possible impact on protein function (amino acid changes) and linkage between the SNPs in one gene (of 2 or more tightly linked SNPs, only one was selected). One SNP was selected on the basis of a population study on *PTGS1* variants,[29] and one on a previous association between a SNP in *PTGS2* and colorectal cancer risk.[22]

PCR and genotyping of 21 SNPs has been described previously.[3] In short, all samples were genotyped using a technique known as Pyrosequencing™,[30] using a biotinylated single-stranded PCR product. Two additional SNPs were genotyped using the restriction fragment length polymorphism (RFLP) method. SNP *c.136-37G > A* in *ALOX15* was genotyped using 2 units of HaeIII (New England Biolabs) and buffer provided, per 10 μl PCR product. SNP *c.3618A>G* in *PTGS2* was genotyped using 1 unit of Alu and buffer provided, per 10 μl PCR product. Both mixtures were incubated at 37°C for 2 h. Each PCR plate contained 92 DNA samples, 1 negative (TE) control, and 3 positive controls, one for each genotype if available.

Primers for 21 SNP are described previously[3] and primers for 2 additional SNPs are given in supplementary table. All SNPs were genotyped in the PPHV cohort from which DNA was available for 603 participants (204 cases and 399 controls). Based on (*i*) these PPHV results, (*ii*) previously found associations[3] and (*iii*) limited available DNA from the DOM cohort, the SNPs in *PPARγ* and *PTGS2* were selected for additional genotyping in the DOM cohort. Because of insufficient DNA or failed genotyping, which is not unusual for DNA extracted from urine,[28] genotypes were determined for 677 participants from de DOM cohort (304 cases and 373 controls).

Data analysis

Analyses were performed on all genotyped participants. Logistic regression analysis was performed to calculate odds ratios (ORs) and 95% confidence intervals (95% CI) of separate genotypes when possible. If the numbers were too small, analyses were performed using pooled heterozygote and homozygote minor genotypes. The matching variables age, gender and cohort or center were considered as potential confounding factors. Only the covariate cohort changed the β-estimate by more than 10%, therefore for the genotype analysis with pooled cohorts, this was included into the model as a covariate. All other factors were assumed to be unrelated to genotype.

Haplotypes were estimated and ORs calculated using the Hplus program, available online at http://qge.fhcrc.org/hplus. Hplus is a SNP analysis tool for performing haplotype estimations, according to the distribution of unphased genotypes in a population. It is able to handle datasets that include case–control status as well as covariates and SNP location variables.[31]

Multiple logistic regression analysis was performed to evaluate the modifying effect of fish consumption in the PPHV cohort. Fish consumption, in 6 categories, was divided in low fish consumption (less than once a week) and high fish consumption (once a week or more frequent). The low fish consumption group in combination with a homozygote major allele for the SNP of interest was considered as reference group. Analysis was performed on incident cases only, since prevalent cases could have changed their eating habits after first diagnosis. This resulted in 161 cases for analysis.

The variables age, gender, smoking, aspirin use (ever/never), physical activity at work, physical activity in leisure time, education (high/low), insulin use (yes/no), fruit, vegetables, meat (all quartiles), total energy intake, alcohol, calcium, β-carotene, vitamin C, vitamin E, fiber, total cholesterol, HDL cholesterol and BMI (all continuous) were considered as potential confounding factors. No variables were included into the model, since none of these factors changed the β-estimates by more than 10%.

To test whether the combination of genotypes and fish consumption deviated from multiplicativity, we calculated p-values for interaction in an exploratory way by inclusion of a numerical term for genotype, multiplied by fish consumption in 2 categories, low or high fish consumption, into our multivariate models.

The analyses were conducted using Statistical Analysis Software (SAS) for Windows, version 8.

Power calculations were conducted using an online software program provided by the UCLA department of statistics.[32]

Results

Table I shows the characteristics of both study populations. All genotypes of the SNPs studied were in Hardy–Weinberg equilibrium, in each cohort separately as well as in the combined set.

Tables II and III show the ORs for each genotype or pooled genotypes and colorectal cancer. Subjects with the CC genotype of SNP *V102V* in *PTGS2* have a 63% reduction in colorectal cancer risk as compared to those with the GG genotype (OR, 0.37; 95% CI, 0.16–0.87). None of the other risk estimates were significant, although the pooled minor genotypes of 2 SNPs in *PLA2G4A* (*c.1336+3G>A* and *c.2605G>A*) showed ORs of over 1.50. Analysis with only incident cases did not change the results.

TABLE III – *PPARS*, *PTGS1*, *PLA2G4A*, *PLA2G2A* AND *ALOX15* VARIANTS AND COLORECTAL CANCER IN THE PPHV COHORT

Genotype (rs number)[49]	Cases (n = 204)	Controls (n = 399)	OR (95%CI)	Power
PPARS				
c.-789C>T				
CC	179	338	1.00 (ref)	
CT+TT	22	39	1.07 (0.62–1.86)	0.04
N163N (*rs2076167*)				
TT	123	250	1.00 (ref)	
TC	68	122	1.14 (0.79–1.65)	0.11
CC	8	24	0.68 (0.30–1.57)	0.16
c.2021T>C (*rs3734254*)				
TT	129	261	1.00 (ref)	
TC	65	114	1.16 (0.80–1.69)	0.12
CC	10	20	0.89 (0.41–1.92)	0.05
c.2589G>A (*rs1053046*)				
GG	178	347	1.00 (ref)	
GA+AA	22	50	0.86 (0.51–1.47)	0.08
c.2806C>G (*rs9794*)				
CC	145	279	1.00 (ref)	
CG+GG	49	91	1.04 (0.70–1.56)	0.04
PTGS1				
W8R (*rs1236913*)				
CC	169	335	1.00 (ref)	
CT+TT	33	55	1.18 (0.74–1.88)	0.10
L237M (*rs5789*)				
CC	183	348	1.00 (ref)	
CA+AA	8	31	0.49 (0.22–1.10)	0.49
PLA2G4A				
c.918+23C>T (*rs2307200*)				
CC	137	279	1.00 (ref)	
CT	57	99	1.15 (0.78–1.70)	0.11
TT	9	16	1.15 (0.49–2.66)	0.05
c.1336+3G>A (*rs6661772*)				
GG	178	364	1.00 (ref)	
GA+AA	25	34	1.51 (0.88–2.61)	0.32
R651K (*rs2307198*)				
AA	196	378	1.00 (ref)	
AG+GG	8	20	0.78 (0.34–1.79)	0.09
c.2605G>A (*rs12720707*)				
GG	163	347	1.00 (ref)	
GA+AA	38	50	1.58 (0.99–2.51)	0.49
PLA2G2A				
c.-180C>G (*rs11573156*)				
CC	128	239	1.00 (ref)	
CG	55	133	0.76 (0.52–1.11)	0.31
GG	18	18	1.87 (0.94–3.71)	0.45
T32T (*rs2236771*)				
GG	167	330	1.00 (ref)	
GC+CC	36	68	1.05 (0.68–1.64)	0.04
c.665C>T (*rs11677*)				
CC	164	314	1.00 (ref)	
CT+TT	39	84	0.89 (0.59–1.37)	0.08
ALOX15				
c.-217G>C (*rs2664592*)				
GG	126	257	1.00 (ref)	
GC	64	112	1.18 (0.81–1.71)	0.14
CC	9	14	1.32 (0.56–3.14)	0.10
c.136-37G>A (*rs11568141*)				
GG	157	319	1.00 (ref)	
GA+AA	45	77	1.20 (0.79–1.81)	0.13
T485T (*rs743646*)				
AA	146	300	1.00 (ref)	
AG+GG	45	78	1.19 (0.79–1.81)	0.13

The ORs for all major haplotypes and colorectal cancer risk are shown in Tables IV and V. The haplotype representing the minor alleles of *c.1336+3G>A* and *c.2605G>A* in *PLA2G4A* (haplotype 1101, also containing the minor allele of *c.918+23C>T* and the major allele of *R651K*) showed an OR of over 1.50, in line with the pooled genotypes of the separate SNPs (OR, 1.54; 95% CI, 0.87–2.71). No significant ORs for any of the haplotypes were observed.

There was an inverse association between fish consumption of once a week or more and colorectal cancer, as compared to less than once a week, although not significant (OR, 0.83; 95% CI, 0.57–1.20). The distribution of fish consumption in 6 categories among incident cases and controls is shown in Table 6. In an exploratory analysis of the interaction between genotypes and fish, divided into low and high consumption, no significant interactions were observed. The homozygous minor genotypes of 2 SNPs in *PPAR*δ (*N163N* and *c.2021T>C*), however, showed a decrease of 80% in colorectal cancer risk for those with high fish intake only, as compared to the major genotypes and low fish intake, although not significant. A similar reduction in risk was observed for the pooled CA+AA genotypes of *L237M* in *PTGS1* and high fish consumption, as compared to the CC genotype and low fish consumption (OR, 0.19; 95% CI, 0.03–1.45). Nearly 75% risk reduction was seen for the AG+GG genotypes of *c.3618A>G* in *PTGS2* only for those with high fish intake, as compared to the AA genotype and low fish intake (OR, 0.26; 95% CI, 0.03–2.06) (data not shown).

Discussion

A number of associations between a variety of SNPs in *PTGS2* and colorectal tumors has been reported.[3,22,34–36] Together with the results from this study, this underlines the importance of this gene and the pro-inflammatory AA-pathway in the development of both colorectal adenomas and carcinomas. We have previously shown that the heterozygote genotype of SNP *V102V* in exon 3 of *PTGS2* has a protective effect on colorectal adenomas.[3] Even though in the present study the protective effect is only evident when 2 C alleles are present, this result can still be considered as an indication of the importance of this SNP in colorectal tumor risk. Just 1 C allele appears to be sufficient for a reduction in risk of colorectal adenomas of 35%, whereas for the later stages of tumor development 2 alleles are needed to confer a risk reduction of 63%. These 2 inverse associations make it likely that there is either some functional effect of this SNP, for example on splicing,

TABLE IV – HAPLOTYPES OF *PPARγ* AND *PTGS2* AND COLORECTAL CANCER IN THE PPHV AND DOM COHORT

Haplotype[1]	Frequency cases	Frequency controls	OR (95%CI)[2]	Power
PPARγ				
00	0.849	0.847	1.00 (ref)	
11	0.071	0.079	0.88 (0.64–1.21)	0.13
01	0.038	0.044	0.89 (0.57–1.38)	0.08
10	0.041	0.030	1.33 (0.85–2.08)	0.25
PTGS2				
0000	0.333	0.308	1.00 (ref)	
0010	0.297	0.276	0.97 (0.79–1.21)	0.05
1000	0.195	0.222	0.81 (0.63–1.03)	0.55
0100	0.153	0.163	0.85 (0.67–1.08)	0.30
0011	0.014	0.024	0.51 (0.23–1.13)	0.51
1010	0.005	0.004	0.96 (0.17–5.53)	0.03

[1]0 represents major allele, 1 represents minor allele. SNP order according to Table II.–[2]Adjusted for cohort.

TABLE V – HAPLOTYPES OF *PPARS*, *PTGS1*, *PLA2G4A*, *PLA2G2A* AND *ALOX15* AND COLORECTAL CANCER RISK IN THE PPHV COHORT

Haplotype[1]	Frequency cases	Frequency controls	OR (95%CI)	Power
PPARS				
00000	0.776	0.777	1.00 (ref)	
01101	0.137	0.131	1.05 (0.75–1.48)	0.05
11110	0.040	0.041	0.97 (0.53–1.77)	0.03
01110	0.023	0.027	0.84 (0.38–1.85)	0.07
11000	0.010	0.014	0.72 (0.23–2.30)	0.07
PLA2G4A				
0000	0.791	0.798	1.00 (ref)	
1000	0.091	0.102	0.92 (0.62–1.37)	0.06
1101	0.059	0.039	1.54 (0.87–2.71)	0.34
0010	0.014	0.026	0.79 (0.36–1.73)	0.09
1001	0.025	0.020	1.37 (0.61–3.09)	0.12
0001	0.011	0.009	1.22 (0.38–3.93)	0.05
PLA2G2A				
000	0.639	0.647	1.00 (ref)	
100	0.224	0.213	1.06 (0.78–1.43)	0.06
011	0.064	0.065	1.00 (0.61–1.64)	0.03
001	0.042	0.045	0.91 (0.48–1.74)	0.05
010	0.031	0.025	1.24 (0.60–2.56)	0.08
ALOX15				
000	0.684	0.690	1.00 (ref)	
100	0.115	0.101	1.14 (0.03–8.77)	0.10
010	0.076	0.094	0.85 (0.03–28.7)	0.11
101	0.060	0.078	0.78 (0.02–33.6)	0.18
001	0.029	0.033	0.89 (0.00–52.4)	0.05
111	0.035	0.005	8.29 (0.00–112.8)	0.99
PTGS1				
00	0.894	0.879	1.00 (ref)	
10	0.085	0.079	1.05 (0.69–1.58)	0.04
01	0.021	0.042	0.50 (0.23–1.07)	0.50

[1]0 represents major allele, 1 represents minor allele. SNP order according to Table III.

TABLE VI – DISTRIBUTION OF FISH CONSUMPTION AMONG INCIDENT CASES AND CONTROLS

Fish consumption category	Cases (n = 160)[1]	Controls (n = 397)[1]
Never	13	44
<once a month	41	82
1–3 times monthly	46	104
Once a week	54	144
2–4 times weekly	6	19
>4 times weekly	0	4

[1]Three cases and two controls had missing data on fish consumption.

or that this SNP is in LD with another functional variant. We have so far not been able to demonstrate any functionality of this SNP either *in silico* or experimentally. Our results, however, are not in line with another study on *PTGS2* variants, in which this SNP in exon 3 was also tested and no association with colorectal cancer was evident.[22] This study was performed in a Spanish population who may have a slightly different genetic background compared to our Dutch cohorts, which may be one of the factors accounting for the different results. When we analyzed the haplotype representing the minor allele of this SNP (0100), a slight reduction in risk was apparent (OR, 0.85; 95% CI, 0.67–1.08). This illustrates that 2 copies of this allele are necessary to obtain the strong protective effect seen for the homozygous individuals. However, it must be pointed out that these haplotypes are estimated and not measured through parent data, which results in less reliable information.

Although in this study the association between *PTGS2* and colorectal cancer was only evident for a SNP in the coding region of the gene and one other study has shown an association between a coding SNP (*V511A*) and colorectal cancer,[34] most other studies on associations between SNPs in *PTGS2* and disease observed an effect for SNPs in regulatory regions of the gene, indicating that the effect of *PTGS2* on the development of colorectal tumors is due to changes in expression levels. This is in line with recent findings that the protective effect of NSAIDs might be due to inhibition of NF-κB, which is associated with down-regulation of *PTGS2* expression.[37] A SNP in the 3′ UTR of *PTGS2* (*c.2242T>G*) has previously been associated with colorectal adenomas[3] and lung cancer.[38,39] The involvement of this SNP in colorectal cancer, however, could not be demonstrated in this study. A possible explanation could be that this SNP is only important in the early stages of tumor formation but does not play a role in the development from polyps to malignant colorectal tumors. This is in line with another study in which no association was found between this 3′ UTR SNP and colorectal cancer.[22] In this same study, a rare SNP in the 3′ UTR (*c.3618A>G*) did show a positive association between the minor allele and colorectal cancer risk. This association was not observed in this study. Our results even suggest an inverse relation between the minor allele and cancer risk (OR, 0.56; 95% CI, 0.29–1.06). However, the low minor allele frequency of this SNP (0.02 in controls) and therefore the small number of subjects with the minor genotypes might result in chance findings. Several studies have investigated the role of SNPs in the promoter of *PTGS2* in disease risk and several associations have been found,[35,36,40–43] indicating direct involvement of changes in gene expression on disease etiology. Some null results, however, have also been reported.[22,44]

We did not find associations between any other SNP and colorectal cancer. The effects we have previously shown for genotypes and haplotypes of SNPs in *PPARγ*, *PLA2G2A* and *ALOX15* on adenomas[3] could not be demonstrated in this study on adenocarcinomas. This could be due to the difference in study populations, adenoma *versus* colorectal cancer patients, indicating that the SNPs and these genes may only affect the first stages of tumor development. Since ~5% of adenomas develop further into malignant tumors, the association found for adenoma risk might not be evident when the endpoint is adenocarcinomas. This is in line with an inverse association found between SNP *P12A* in *PPARγ* and colorectal adenomas.[45] However, the association was stronger for more advanced adenomas, and moreover, an inverse association was observed in a colorectal cancer population for the minor allele of this SNP,[46] although this is not confirmed by our data. No clear data is available about the exact stage of tumor development in which *PLA2G2A* and *ALOX15* play a role, but our data suggests that they may be most important in the early stages.

We hypothesized that increased fish consumption, as a proxy of n-3 fatty acid intake, can influence the risk of colorectal cancer by modulation of the AA-pathway. We found an inverse association, although not significant, between fish consumption and colorectal cancer (OR, 0.83; 95% CI, 0.57–1.20) for subjects with fish consumption of once a week or more frequent as compared to less than once a week. This is in line with a strong protective effect of increased fish consumption shown recently in a large European study.[20] This association between fish consumption and colorectal cancer might be modified by polymorphisms in genes involved in the conversion of free fatty acids into prostaglandins. We have previously demonstrated the association between SNPs in *PPARδ* and *PTGS2* and fish consumption in relation to colorectal adenomas.[3] In this study, however (using only the PPHV cohort), we have not been able to show any significant interactions with any SNP and fish consumption, even though some substantial reductions in cancer risk were observed. This might be the cause of insufficient statistical power due to the relatively small population size. There are also some other possible explanations for the lack of significant interactions. First, the consumption of fish might be too low in our population to be able to measure an effect. We considered fish consumption of once a week or more as high fish intake. A large study on the effect of fish consumption on prostate cancer detected an inverse association for 4 or more servings of fish a week, which was significant.[47] Second, the associations were calculated for total fish intake because no distinction was made between the different types of fish in the food frequency questionnaire. If n-3 fatty acids are considered the bioactive agents in fish, it is likely that fish high in n-3 fatty acids, for example,

salmon, mackerel and herring, are more strongly associated with cancer than lean fish like cod and haddock. Also, other sources of n-3 fatty acids that have not been considered in the analysis, since no data was available, can influence the results. It has also been suggested that a low ratio n-6/n-3 fatty acids is more important than the total amount of n-3 fatty acids,[21,48] confirmation of which is awaiting ongoing FA analysis.

Our PPHV cohort included both incident and prevalent cases. We repeated the analysis for genotypes with only incident cases, but this did not change our results. However, prevalent cases might have changed their dietary habits after the first time they developed cancer, therefore these cases where excluded in the gene–diet interaction analysis.

Consideration must be given to some potential limitations of the study. First, the use of self-administered questionnaires can give rise to uncertainties, especially since the answer categories were limited to 6 options. The dietary intake data is therefore less accurate than when a more open questionnaire is used. Measurements of specific fatty acid content in plasma samples might be more accurate and is currently ongoing. Second, for the gene–diet interactions several potential confounders were tested. None of the variables tested effected the association, but it cannot be ruled that other factors for which no data was collected could cause confounding. Third, the lack of interaction between the SNPs tested and fish consumption does not rule out any interaction between these genes and fish consumption. It is possible that there are other SNPs present in the genes, which have low minor allele frequencies and have therefore not been tested in this study, that modify the association. Fourth, we were not able to investigate the gene–gene interactions because of small sample size, therefore associations between a combination of SNPs within 2 genes and colorectal cancer would not have been detected by our study if the effect of the single SNPs were negligible. Fifth, because of the small sample size, which is especially the case for the fish consumption data, there is a substantial lack of power to detect interactions. This is also the case for some main effects when allele or haplotype frequencies are low. And last, chance findings can never be excluded, especially when multiple testing is considered. However, we opted not to correct for multiple testing, since at this stage and with this sample size, Bonferroni corrections were considered punitively conservative.[49]

In conclusion, we have confirmed a previously found inverse association between SNP *V102V* in *PTGS2* and colorectal adenomas, in this study on colorectal cancer. Although the association between increased fish consumption and colorectal cancer was inverse, there were no significant interactions between the SNPs investigated and the fish consumption.

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