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Angaben zur Veröffentlichung / Publication details:

Frank, B., B. Hoeft, M. Hoffmeister, Jakob Linseisen, L. P. Breitling, J. Chang-Claude, H. Brenner, and A. Nieters. 2010. "Association of hydroxyprostaglandin dehydrogenase 15-(NAD) (HPGD) variants and colorectal cancer risk." *Carcinogenesis* 32 (2): 190–96. https://doi.org/10.1093/carcin/bgq231.

Association of hydroxyprostaglandin dehydrogenase 15-(NAD) (*HPGD*) variants and colorectal cancer risk

Bernd Frank*, Birgit Hoeft[1], Michael Hoffmeister, Jakob Linseisen[1,2], Lutz P.Breitling, Jenny Chang-Claude[1], Hermann Brenner and Alexandra Nieters[1,3]

Division of Clinical Epidemiology and Aging Research, German Cancer Research Center, Heidelberg, Germany, [1]Division of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany, [2]Institute of Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany and [3]Molecular Epidemiology, Center of Chronic Immunodeficiency, University of Freiburg Medical Centre, Freiburg, Germany

*To whom correspondence should be addressed. Tel: +49 6221 548143; Fax: +49 6221 548142;
Email: b.frank@dkfz.de

A recent study examined associations of tagging single nucleotide polymorphisms (tagSNPs) in 43 fatty acid metabolism-related genes and risk of colorectal cancer (CRC), showing rs8752, rs2612656 and a haplotype [comprising both of the single nucleotide polymorphisms (SNPs)] in the hydroxyprostaglandin dehydrogenase 15-(NAD) (*HPGD*) gene to be positively associated with CRC risk. In the present study, we attempted to replicate these single marker and haplotype associations, using 1795 CRC cases and 1805 controls from the German Darmkrebs: Chancen der Verhütung durch Screening study (DACHS). In addition to rs8752 and rs2612656, *HPGD* tagSNPs rs9312555, rs17360144 and rs7349744 were genotyped for haplotype analyses. Except for a marginally significant inverse association of *HPGD* rs8752 with CRC risk [odds ratio (OR) = 0.85; 95% confidence interval (CI) = 0.74, 0.98; $P = 0.03$], none of the analyzed tagSNPs showed any association with CRC. Subset analyses for colon and rectal cancers yielded similar, yet non-significant risk estimates at all five loci. Also, none of the haplotypes was found to be associated with CRC, colon or rectal cancers. However, rs8752 was significantly associated with a decreased risk of CRC among individuals with a body mass index < 30 (OR = 0.82, 95% CI = 0.70, 0.95, $P = 0.01$) as well as among smokers (OR = 0.74, 95% CI = 0.61, 0.90, $P = 0.003$). Yet, our data do not support the previously reported associations of *HPGD* tagSNPs and risk of CRC.

Introduction

Colorectal cancer (CRC) is the third most common malignant neoplasm and the fourth leading cause of cancer deaths worldwide, with 1 million new cases in 2002 and age-standardized mortality rates of 10.2 and 7.6/100 000 in men and women, respectively (1–4). Familial and genetic segregation studies have consistently adduced evidence that genetic components, along with environmental influences, play a substantial role in colorectal carcinogenesis (5–7). Recent findings have suggested the arachidonic acid (AA)—or prostaglandin (PG) synthesis—pathway to be implicated in CRC development (8–10). This pathway metabolizes ϖ-6 (n-6) and n-3 polyunsaturated fatty acids into PGs and thromboxanes (8–11). The key regulatory steps of this process involve the oxygenation of AA to form prostaglandin endoperoxide H_2 and the subsequent conversion of prostaglandin endoperoxide H_2 to at least five bioactive prostanoids (PGD_2, PGE_2, PGF_2, PGI_2 and thromboxane A) (8–11). Prostanoids mediate a number of biological processes, regulating immune or inflammatory functions and gastrointestinal integrity (9,11). Most interestingly, PGE_2 appears to be implicated in tumor-associated angiogenesis, cell migration or invasion, inhibition of apoptosis and inflammatory responses (9–12). Prostanoids, such as PGE_2, are metabolized through oxidation of their 15(S)-hydroxyl group by NAD+-linked 15-hydroxyprostaglandin dehydrogenase (15-PGDH, encoded by *HPGD*) to inactive 15-keto products (8–13). 15-PGDH, a cytosolic enzyme, has been reported to act as bladder, breast, gastric, lung and colorectal tumor suppressor (11,14–23). As potent suppressor of the growth of human colon tumor cell lines in immunodeficient mice, 15-PGDH inhibits the development of murine intestinal neoplasias (17,23,24). These findings and the fact that 15-PGDH is abolished in various cancers, particularly in human colonic neoplasms (12,17,23,24), emphasize the oncogenic potential of the PG synthesis pathway.

Using a case–control study design nested within the international prospective population-based European Investigation into Cancer and Nutrition (EPIC) study, Hoeft *et al.* (25) have analyzed associations of 392 gene variants in 43 selected AA pathway genes with CRC risk in 1225 cases and 2032 control individuals. Notably, minor allele carriers of the *HPGD* rs8752 and rs2612656 tagging single nucleotide polymorphisms (tagSNPs) were associated with a 22 and 24% higher risk of CRC, respectively. Additionally, a *HPGD* five single nucleotide polymorphism (SNP) haplotype (AGGAG), containing the potentially risk increasing minor alleles of rs8753 and rs2612656, was found significantly associated with a 33% increased CRC risk (25).

The objectives of the present study were as follows: firstly, it aimed at replicating the above-named associations in a large German case–control study with 1775 CRC cases and 1805 controls, genotyping *HPGD* SNPs rs9312555, rs8752, rs2612656, rs17360144 and rs7349744. Besides, the results of the present study should elucidate the role and support the involvement of the PG synthesis pathway in CRC etiology, performing stratified analyses according to cancer site (colon and rectum), body mass index (BMI) and smoking status, thus helping to assess individual susceptibility and to target potential measures of cancer prevention.

Abbreviations: AA, arachidonic acid; BMI, body mass index; CI, confidence interval; CRC, colorectal cancer; DACHS, Darmkrebs: Chancen der Verhütung durch Screening; EPIC, European Investigation into Cancer and Nutrition; HPGD, hydroxyprostaglandin dehydrogenase 15-(NAD); OR, odds ratio; 15-PGDH, 15-hydroxyprostaglandin dehydrogenase; PG, prostaglandin; SNP, single nucleotide polymorphism.

Materials and methods

Study population

CRC cases and controls were drawn from the German DACHS (Darmkrebs: Chancen der Verhütung durch Screening) study, a large population-based case–control study carried out in the Rhine-Neckar-Odenwald and Heilbronn regions in the southwest of Germany (26–28). The analyses comprised 1795 unrelated male and female case patients (33–94 years of age, mean = 68.2 years) with a first histologically confirmed diagnosis of CRC (ICD-10 codes C18–C20) between January 2003 and December 2007 (Table I). Controls consisted of 1805 individuals (34–98 years of age, mean = 68.7 years) who were randomly selected from lists of residents supplied by population registries and frequency-matched to cases by 5-year age groups, sex and county of residence (Table I). Case patients and control individuals were eligible if they were ≥30 years of age, lived in the study region, were German-speaking and mentally and physically able to participate in a personal interview of ∼1 h. All of them gave written informed consent. The study was approved by the ethics committees of the Medical Faculty of Heidelberg and the State Medical Boards of Baden-Wuerttemberg and Rhineland-Palatinate, Germany.

Data collection

Details of the data collection procedures for the DACHS study are reported elsewhere (27,28). Briefly, the study subjects were asked to participate in an in-person interview and to donate a blood sample. In the rare cases in which blood samples were not available, a mouthwash was taken. Information on demographic factors, anthropometric measures, medical history and lifestyle factors was collected by trained interviewers, utilizing a standardized questionnaire.

Table I. Characteristics of the DACHS study population

Variable	Cases		Controls	
	N	%	*N*	%
Total (*N* = 3600)	1795	100	1805	100
Sex				
Male	1049	58.4	1073	59.4
Female	746	41.6	732	40.6
Age at diagnosis/interview (years)				
30–39	17	0.9	5	0.3
40–49	58	3.2	60	3.3
50–59	270	15.0	260	14.4
60–69	624	34.8	574	31.8
70–79	568	31.6	612	33.9
80+	258	14.4	294	16.3
Cancer localization				
Colon	1096	61.1	n/a	n/a
Rectum	699	38.9	n/a	n/a
Proximal[a]	573	31.9	n/a	n/a
Distal[a]	1204	67.1	n/a	n/a
Proximal + distal[a]	14	0.8	n/a	n/a
Missing	4	0.2	n/a	n/a
UICC stage at diagnosis				
I	435	24.2	n/a	n/a
II	539	30.0	n/a	n/a
III	566	31.5	n/a	n/a
IV	251	14.0	n/a	n/a
Missing	4	0.2	n/a	n/a
First-degree family history of CRC				
Yes	259	14.4	202	11.2
No/Unknown	1485	82.7	1549	85.8
Missing	51	2.8	54	3.0
Regular active smoking				
Ever	933	52.0	861	47.7
Never	860	47.9	943	52.2
Missing	2	0.1	1	0.1
BMI				
<30	1437	80.1	1451	80.4
≥30	348	19.4	353	19.6
Missing	10	0.6	1	0.1

n/a, not applicable; UICC, International Union Against Cancer.
[a]Distal cancer includes cancers of the splenic flexure, descending colon, sigmoid and rectum; proximal cancer includes all other locations.

DNA preparation and genotyping

Genomic DNA was isolated from blood and mouthwash samples with FlexiGene DNA and QIAamp DNA Mini Kits (QIAGEN GmbH, Hilden, Germany), respectively (28). *HPGD* rs9312555, rs8752, rs2612656, rs17360144 and rs7349744 SNPs were genotyped by Pyrosequencing™ (29). Standard polymerase chain reaction was performed using isolated DNA, 10× Reddy Mix™ polymerase chain reaction buffer with $MgCl_2$ (15 mM), ThermoPrime *Taq* DNA Polymerase (5 U/μl) (Thermo Fisher Scientific, Worcester, MA), 20 mM dNTPs and 10 μM each primer (Invitrogen Ltd, Paisley, UK). Primer sequences and cycling conditions are available upon request.

A random selection of >5% of all samples was genotyped twice for quality control, and samples with ambiguous results were repeated. The successfully genotyped duplicate samples displayed a concordance rate of 100%.

Statistics

Each SNP was tested for deviation from Hardy–Weinberg equilibrium in controls by comparing observed and expected genotype frequencies, using Pearson's χ^2-tests with one degree of freedom. According to the NCBI Variation Database (dbSNP), expected minor allele frequencies of *HPGD* rs9312555, rs8752, rs2612656, rs17360144 and rs7349744 among Europeans are 0.14, 0.42, 0.25, 0.06 and 0.35, respectively.

Unconditional logistic regression was applied to estimate odds ratios (ORs) and corresponding 95% confidence intervals (CIs) adjusted for matching factors age and sex, using dominant and codominant genotype models (30). Tests for linear trend (additive genotype models) were also employed. Five-loci haplotypes were estimated using Phase 2.1.1 (31,32). For these analyses, only subjects without missing data at any of the loci were considered, and those for whom haplotypes could not be inferred with a probability > 80% were excluded. Haplotypes with a frequency < 5% were combined as 'rare haplotypes' (hRARE). The most common haplotype (AAAAA) served as reference in regression models. All tests were two-sided and considered statistically significant with $P < 0.05$. Subset analyses included stratifications according to cancer site, BMI and smoking status. Whereas BMI was calculated as weight divided by height squared (kilograms per square metre), smoking status was classified into ever and never regular active smoking (Table I).

The aforementioned analyses were carried out using Statistical Analysis Software version 9.1 (SAS Institute, Cary, NC). Power calculations were done with the power and sample size software PS version 3.0.7 (33).

Results

The majority of DACHS cases and controls were males between 60 and 79 years of age (median ages: 69 and 70 years, respectively). First-degree family history of CRC (CRC FH1) was slightly more common among cases than among controls. Two-thirds of cancers were located in the sigmoid colon and rectum, and slightly more than half were diagnosed at stage I or II. Main characteristics of the DACHS study population have been described previously (26).

The average call rate for the five analyzed SNPs was 99.5% (range: 99.2–99.8%), and it did not differ between cases and controls for any individual assay. Allele frequencies among controls were consistent with Hardy–Weinberg equilibrium for all SNPs ($P \geq 0.05$).

Table II shows the results for the five analyzed SNPs. Assuming a dominant model (comparing carriers of the minor allele with wild-type homozygous subjects), one of five SNPs attained evidence for association with CRC (rs8752, OR = 0.85, 95% CI = 0.74, 0.98, $P = 0.03$; Table II). While the remaining SNPs showed no evidence of an association with CRC risk, the association of rs8752 was marginal (unadjusted for multiple comparisons), and the estimated effect was reversed compared with the results by Hoeft *et al.* (25). Additional adjustment for CRC FH1 had no impact on the estimates.

The risk estimates for rs8752 among colon and rectal cancers (OR = 0.87 and OR = 0.83, respectively) as well as among proximal and distal cancers (OR = 0.83 and OR = 0.87, respectively) were consistent, yet not statistically significant (Table III). The previously observed association for rs2612656 with colon cancer (25) could not be confirmed in our study (Table III).

Strikingly, rs8752 was significantly associated with a decreased risk of CRC among individuals with a BMI < 30 (OR = 0.82, 95% CI = 0.70, 0.95, $P = 0.01$; Table IV) as well as among smokers (OR = 0.74, 95% CI = 0.61, 0.90, $P = 0.003$; Table IV).

ORs for all major haplotypes and risk of CRC are shown in Table V. Haplotype h3, representing the minor alleles of rs8752 and rs2612656 (in combination with the major alleles of rs9312555, rs17360144 and rs7349744), which was associated with a 33% increased CRC risk in the EPIC study (25), showed a non-significant OR of 1.05 (95% CI = 0.90, 1.21; Table V) in the DACHS study population. This result was consistent among the subgroups of colon and rectum cancers (Table VI). Nor did the remainder of the haplotypes show any association with CRC, colon or rectal cancers (Tables V and VI).

Discussion

In this large case–control study, we initially attempted to replicate genetic associations between rs8752, rs2612656 and the *HPGD* AGGAG haplotype that were previously reported to be associated with CRC risk (25). We could not confirm either finding and, unexpectedly, we even observed a marginal inverse association of rs8752 with CRC.

Recently, Hoeft *et al.* investigated the associations of 392 SNPs in 43 candidate genes with the risk of CRC, utilizing 1225 cases and 2032 controls nested in the prospective EPIC cohort that includes 23 subcohorts from 10 European countries (25,34). Given their substantial involvement in colorectal carcinogenesis (17,23,24), the biological rationale to select PG synthesis-related gene variants for analyses on CRC risk is manifest (25). Interestingly, homozygous mutations in *HPGD* have been described to cause cranio-osteoarthropathy,

Table II. HPGD polymorphisms and their associations with CRC

SNP ID	Localization	Genotype	CRC cases *N* (%)	Controls *N* (%)	OR[a] (95% CI)	*P*
rs9312555	Exon 7/3′ UTR	AA	1305 (72.9)	1275 (70.8)	1	
		AG	452 (25.3)	486 (27.0)	0.91 (0.78, 1.06)	0.22
		GG	33 (1.8)	40 (2.2)	0.81 (0.50, 1.29)	0.36
		GG + AG	485 (27.1)	526 (29.2)	0.90 (0.78, 1.04)	0.17
		MAF	0.14	0.16		
						$P_{trend} = 0.14$
rs8752	Exon 7/3′ UTR	AA	630 (35.3)	572 (31.8)	1	
		AG	835 (46.8)	912 (50.7)	0.83 (0.72, 0.97)	0.02
		GG	318 (17.8)	316 (17.6)	0.91 (0.75, 1.11)	0.35
		GG + AG	1153 (64.7)	1228 (68.2)	0.85 (0.74, 0.98)	0.03
		MAF	0.41	0.43		
						$P_{trend} = 0.16$
rs2612656	Intron 4	AA	1049 (58.9)	1058 (58.9)	1	
		AG	627 (35.2)	647 (36.0)	0.98 (0.85, 1.13)	0.77
		GG	105 (5.9)	91 (5.1)	1.15 (0.86, 1.55)	0.35
		GG + AG	732 (41.1)	738 (41.1)	1.00 (0.88, 1.14)	0.99
		MAF	0.23	0.23		
						$P_{trend} = 0.69$
rs17360144	Intron 4	AA	1536 (86.5)	1527 (85.0)	1	
		AC	226 (12.7)	260 (14.5)	0.86 (0.71, 1.05)	0.14
		CC	14 (0.8)	9 (0.5)	1.53 (0.66, 3.54)	0.32
		CC + AC	240 (13.5)	269 (15.0)	0.89 (0.74, 1.07)	0.21
		MAF	0.07	0.08		
						$P_{trend} = 0.34$
rs7349744	Intron 4	GG	864 (48.3)	857 (47.7)	1	
		AG	740 (41.4)	785 (43.7)	0.94 (0.82, 1.07)	0.35
		AA	185 (10.3)	153 (8.5)	1.20 (0.95, 1.51)	0.13
		AA + AG	925 (51.7)	938 (52.3)	0.98 (0.86, 1.12)	0.75
		MAF	0.31	0.30		
						$P_{trend} = 0.57$

MAF, minor allele frequency; SNP ID, single nucleotide polymorphism identification; UTR, untranslated region.
[a]ORs were adjusted for age and sex.

a variant of the rare primary hypertrophic osteoarthropathy, an autosomal-recessive condition, which is characterized by digital clubbing, arthropathy and periostosis (35). Yet, a most recent candidate and tagSNP approach to investigate correlations of PGE_2-related gene variants, including *HPGD* SNPs, with the risk of colorectal adenoma revealed no significant association (36).

Similarly, the observed associations of *HPGD* rs8752, rs2612656 and the AGGAG haplotype with CRC could not be replicated in the present study, and associations in our study opposed the risk increase as shown by Hoeft *et al.* (25). The inconsistent results in variant–disease association may be the consequence of multiple reasons. Indeed, EPIC case and control individuals were carefully selected, matching two controls to each case for most study centers following an incidence density matching protocol with a series of criteria, such as sex, age at blood donation, study center, fasting status, time of the day of blood collection, menopausal status for women and phase of menstrual cycle for premenopausal women (25). However, analyzed EPIC samples were collected from groups with different geographic regions (25). Whereas DACHS samples were exclusively of German origin, EPIC samples derive from 23 research centers in Denmark, France, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden and the United Kingdom (25,34), i.e. regions with different rates of cancer occurrence. Thus, one possible explanation for the reverse and negative replications may be population stratification, also involving differences in genetic ancestry and in linkage disequilibrium between the studied SNPs and a true causative variant elsewhere in the *HPGD* region (37–40). Another potential source of the variable findings is gene–gene or gene–environment interactions (41), given the different genetic and environmental backgrounds of the study populations (e.g. dietary habits and distributions of lifestyle factors, such as smoking status). Possibly, individual analyses of the five *HPGD* SNPs according to EPIC study center could help to explain the discrepant findings.

To our knowledge, the present case–control study is the first to attempt to replicate observed associations of *HPGD* SNPs and haplotypes with CRC risk (25). Strengths include a population-based design, a well-defined homogeneous study population and a sound sample size. With the latter, we had a power of 80% at a significance level of 0.05 to detect ORs $\geq$ 1.22 (rs9312555), 1.23 (rs8752), 1.21 (rs2612656), 1.29 (rs17360144) and 1.20 (rs7349744), respectively (26).

In conclusion, we found rs8752 to be significantly associated with a decreased CRC risk both among individuals with a BMI $<$ 30 and among smokers. Nonetheless, we could neither confirm previously reported associations between *HPGD* rs8752 and rs2612656 nor between the AGGAG haplotype and increased CRC risk (25). Quite contrary to the results by Hoeft *et al.* (25), rs8752 was marginally, yet inversely associated with risk of CRC. The findings of the present study may elucidate the role and support the involvement of the PG synthesis pathway in CRC, providing useful information for the assessment of individual susceptibility, and they may help to target potential measures of cancer prevention, which should be the subject of future research.

Funding

German Research Council (Deutsche Forschungsgemeinschaft) (BR 1704/6-1, BR 1704/6-3, BR 1704/6-4, CH 117/1-1); German Federal Ministry for Education and Research (Bundesministerium für Bildung und Forschung) (01 KH 0404, 01 GS 08181); German Cancer Aid (Deutsche Krebshilfe) (106812).

Table III. HPGD polymorphisms and their associations with colon and rectal as well as with proximal and distal cancers

SNP ID genotype	Controls *N* (%)	Colon cancer *N* (%)	OR[a] (95% CI)	*P*	Rectal cancer *N* (%)	OR[a] (95% CI)	*P*	Proximal cancer[b] *N* (%)	OR[a] (95% CI)	*P*	Distal cancer[b] *N* (%)	OR[a] (95% CI)	*P*
rs9312555													
AA	1275 (70.8)	784 (71.9)	1		521 (74.5)	1		427 (73.2)	1		886 (72.8)	1	
AG	486 (27.0)	287 (26.3)	0.96 (0.81, 1.14)	0.67	165 (23.6)	0.83 (0.68, 1.02)	0.08	146 (25.0)	0.90 (0.72, 1.11)	0.32	308 (25.3)	0.91 (0.77, 1.08)	0.29
GG	40 (2.2)	20 (1.8)	0.82 (0.48, 1.42)	0.48	13 (1.9)	0.82 (0.43, 1.55)	0.54	10 (1.7)	0.75 (0.37, 1.52)	0.42	23 (1.9)	0.83 (0.49, 1.40)	0.48
GG + AG	526 (29.2)	307 (28.1)	0.95 (0.81, 1.13)	0.57	178 (25.5)	0.83 (0.68, 1.02)	0.07	156 (26.8)	0.89 (0.72, 1.09)	0.25	331 (27.2)	0.91 (0.77, 1.07)	0.24
				P_{trend} = 0.48			P_{trend} = 0.08			P_{trend} = 0.22			P_{trend} = 0.22
rs8752													
AA	572 (31.8)	382 (35.1)	1		248 (35.7)	1		211 (36.3)	1		421 (34.8)	1	
AG	912 (50.7)	507 (46.6)	0.84 (0.71, 0.99)	0.04	328 (47.2)	0.83 (0.68, 1.01)	0.07	266 (45.7)	0.80 (0.65, 0.99)	0.04	576 (47.6)	0.86 (0.73, 1.02)	0.07
GG	316 (17.6)	199 (18.3)	0.96 (0.77, 1.19)	0.69	119 (17.1)	0.84 (0.65, 1.09)	0.20	105 (18.0)	0.92 (0.70, 1.21)	0.57	214 (17.7)	0.91 (0.73, 1.12)	0.37
GG + AG	1228 (68.2)	706 (64.9)	0.87 (0.74, 1.02)	0.08	447 (64.3)	0.83 (0.69, 1.00)	0.06	371 (63.7)	0.83 (0.68, 1.01)	0.07	790 (65.2)	0.87 (0.75, 1.02)	0.08
				P_{trend} = 0.40			P_{trend} = 0.11			P_{trend} = 0.31			P_{trend} = 0.22
rs2612656													
AA	1058 (58.9)	646 (59.4)	1		403 (58.1)	1		347 (59.4)	1		706 (58.5)	1	
AG	647 (36.0)	374 (34.4)	0.95 (0.81, 1.12)	0.57	253 (36.5)	1.01 (0.84, 1.22)	0.92	204 (34.9)	0.98 (0.80, 1.19)	0.81	429 (35.5)	0.99 (0.85, 1.16)	0.90
GG	91 (5.1)	67 (6.2)	1.22 (0.87, 1.69)	0.25	38 (5.5)	1.05 (0.70, 1.56)	0.83	33 (5.7)	1.13 (0.74, 1.72)	0.57	72 (6.0)	1.15 (0.83, 1.60)	0.39
GG + AG	738 (41.1)	441 (40.6)	0.99 (0.85, 1.15)	0.86	291 (41.9)	1.01 (0.85, 1.21)	0.88	237 (40.6)	1.00 (0.82, 1.21)	0.96	501 (41.5)	1.01 (0.87, 1.17)	0.89
				P_{trend} = 0.73			P_{trend} = 0.84			P_{trend} = 0.85			P_{trend} = 0.65
rs17360144													
AA	1527 (85.0)	948 (87.5)	1		588 (85.0)	1		507 (87.1)	1		1036 (86.0)	1	
AC	260 (14.5)	129 (11.9)	0.80 (0.64, 1.01)	0.06	97 (14.0)	0.96 (0.75, 1.24)	0.76	73 (12.5)	0.85 (0.64, 1.13)	0.26	156 (13.0)	0.88 (0.71, 1.09)	0.25
CC	9 (0.5)	7 (0.6)	1.27 (0.47, 3.43)	0.64	7 (1.0)	2.00 (0.73, 5.43)	0.18	2 (0.3)	0.66 (0.14, 3.08)	0.60	12 (1.0)	1.91 (0.80, 4.57)	0.14
CC + AC	269 (15.0)	136 (12.5)	0.82 (0.65, 1.02)	0.07	104 (15.0)	1.00 (0.78, 1.28)	0.98	75 (12.9)	0.85 (0.64, 1.11)	0.23	168 (14.0)	0.92 (0.75, 1.13)	0.42
				P_{trend} = 0.11			P_{trend} = 0.79			P_{trend} = 0.22			P_{trend} = 0.68
rs7349744													
GG	857 (47.7)	523 (47.9)	1		341 (48.9)	1		279 (47.7)	1		593 (48.8)	1	
AG	785 (43.7)	464 (42.5)	0.98 (0.83, 1.15)	0.78	276 (39.6)	0.88 (0.73, 1.07)	0.19	255 (43.6)	1.01 (0.83, 1.23)	0.90	487 (40.1)	0.90 (0.77, 1.05)	0.17
AA	153 (8.5)	105 (9.6)	1.12 (0.85, 1.47)	0.41	80 (11.5)	1.31 (0.97, 1.77)	0.08	51 (8.7)	1.02 (0.72, 1.45)	0.89	134 (11.0)	1.26 (0.98, 1.63)	0.07
AA + AG	938 (52.3)	569 (52.1)	1.00 (0.86, 1.16)	0.99	356 (51.1)	0.95 (0.80, 1.14)	0.59	306 (52.3)	1.02 (0.84, 1.22)	0.88	621 (51.2)	0.96 (0.83, 1.11)	0.55
				P_{trend} = 0.67			P_{trend} = 0.56			P_{trend} = 0.87			P_{trend} = 0.57

SNP ID, single nucleotide polymorphism identification.
[a]ORs were adjusted for age and sex.
[b]Distal cancer includes cancers of the splenic flexure, descending colon, sigmoid and rectum; proximal cancer includes all other locations.

Table IV. HPGD polymorphisms and their associations with CRC among smokers, non-smokers and individuals with BMI $<$ or $\geq$ 30

SNP ID	Genotype	CRC cases BMI $<$ 30	Controls BMI $<$ 30	OR[a] (95% CI)	*P*	CRC cases BMI $\geq$ 30	Controls BMI $\geq$ 30	OR[a] (95% CI)	*P*	CRC cases Smokers	Controls Smokers	OR[a] (95% CI)	*P*	CRC cases Non-smokers	controls Non-smokers	OR[a] (95% CI)	*P*
rs9312555	AA	1056 (73.7)	1029 (71.1)	1		245 (70.6)	245 (69.6)	1		667 (71.6)	596 (69.2)	1		636 (74.2)	678 (72.2)	1	
	AG	351 (24.5)	388 (26.8)	0.88 (0.75, 1.04)	0.14	96 (27.7)	98 (27.8)	0.99 (0.71, 1.38)	0.95	246 (26.4)	244 (28.3)	0.90 (0.73, 1.11)	0.34	206 (24.0)	242 (25.8)	0.91 (0.73, 1.13)	0.37
	GG	26 (1.8)	31 (2.1)	0.82 (0.48, 1.39)	0.45	6 (1.7)	9 (2.6)	0.66 (0.23, 1.88)	0.43	18 (1.9)	21 (2.4)	0.77 (0.41, 1.46)	0.43	15 (1.8)	19 (2.0)	0.83 (0.42, 1.65)	0.59
	GG + AG	377 (26.3)	419 (28.9)	0.88 (0.75, 1.03)	0.12	102 (29.4)	107 (30.4)	0.96 (0.69, 1.33)	0.81	264 (28.4)	265 (30.8)	0.89 (0.73, 1.09)	0.28	221 (25.8)	261 (27.8)	0.90 (0.73, 1.11)	0.33
					P_{trend} = 0.11				P_{trend} = 0.66				P_{trend} = 0.24				P_{trend} = 0.32
rs8752	AA	514 (36.0)	456 (31.5)	1		114 (33.0)	115 (32.7)	1		335 (36.1)	254 (29.5)	1		295 (34.5)	317 (33.8)	1	
	AG	652 (45.7)	742 (51.3)	0.78 (0.66, 0.92)	0.002	176 (51.0)	170 (48.3)	1.05 (0.75, 1.47)	0.78	433 (46.7)	442 (51.3)	0.74 (0.60, 0.92)	0.006	400 (46.8)	470 (50.1)	0.92 (0.75, 1.14)	0.45
	GG	262 (18.3)	249 (17.2)	0.93 (0.75, 1.15)	0.49	55 (15.9)	67 (19.0)	0.83 (0.53, 1.29)	0.41	159 (17.2)	165 (19.2)	0.73 (0.55, 0.95)	0.02	159 (18.6)	151 (16.1)	1.15 (0.87, 1.51)	0.33
	GG + AG	914 (64.0)	991 (68.5)	0.82 (0.70, 0.95)	0.01	231 (67.0)	237 (67.3)	0.99 (0.72, 1.36)	0.93	592 (63.9)	607 (70.5)	0.74 (0.61, 0.90)	0.003	559 (65.5)	621 (66.2)	0.98 (0.80, 1.19)	0.81
					P_{trend} = 0.18				P_{trend} = 0.52				P_{trend} = 0.008				P_{trend} = 0.52
rs2612656	AA	834 (58.4)	842 (58.3)	1		208 (60.5)	215 (61.4)	1		555 (59.8)	497 (58.0)	1		494 (58.0)	560 (59.7)	1	
	AG	504 (35.3)	533 (36.9)	0.95 (0.82, 1.11)	0.54	120 (34.9)	114 (32.6)	1.09 (0.79, 1.50)	0.60	325 (35.0)	317 (37.0)	0.92 (0.75, 1.12)	0.38	300 (35.3)	330 (35.2)	1.04 (0.86, 1.27)	0.68
	GG	89 (6.2)	70 (4.8)	1.26 (0.91, 1.75)	0.16	16 (4.7)	21 (6.0)	0.77 (0.39, 1.53)	0.46	48 (5.2)	43 (5.0)	0.98 (0.64, 1.51)	0.94	57 (6.7)	48 (5.1)	1.38 (0.92, 2.07)	0.12
	GG + AG	593 (41.6)	603 (41.7)	0.99 (0.85, 1.15)	0.88	136 (39.5)	135 (38.6)	1.04 (0.77, 1.41)	0.80	373 (40.2)	360 (42.0)	0.92 (0.77, 1.12)	0.41	357 (42.0)	378 (40.3)	1.09 (0.90, 1.31)	0.40
					P_{trend} = 0.65				P_{trend} = 0.91				P_{trend} = 0.51				P_{trend} = 0.20
rs17360144	AA	1233 (86.8)	1224 (84.6)	1		295 (85.3)	302 (86.5)	1		807 (87.6)	724 (84.5)	1		727 (85.2)	802 (85.5)	1	
	AC	174 (12.3)	215 (14.9)	0.80 (0.65, 0.99)	0.04	50 (14.5)	45 (12.9)	1.12 (0.72, 1.73)	0.61	105 (11.4)	129 (15.1)	0.73 (0.55, 0.96)	0.02	121 (14.2)	131 (14.0)	1.03 (0.79, 1.34)	0.86
	CC	13 (0.9)	7 (0.5)	1.81 (0.72, 4.55)	0.21	1 (0.3)	2 (0.6)	0.52 (0.05 5.78)	0.59	9 (1.0)	4 (0.5)	1.93 (0.59, 6.31)	0.28	5 (0.6)	5 (0.5)	1.12 (0.32, 3.89)	0.86
	CC + AC	187 (13.2)	222 (15.4)	0.83 (0.68, 1.03)	0.09	51 (14.7)	47 (13.5)	1.09 (0.71, 1.68)	0.68	114 (12.4)	133 (15.5)	0.77 (0.58, 1.00)	0.05	126 (14.8)	136 (14.5)	1.03 (0.79, 1.34)	0.83
					P_{trend} = 0.20				P_{trend} = 0.77				P_{trend} = 0.13				P_{trend} = 0.82
rs7349744	GG	687 (47.9)	684 (47.4)	1		172 (49.6)	172 (49.0)	1		427 (45.9)	409 (47.8)	1		435 (50.8)	448 (47.7)	1	
	AG	587 (41.0)	634 (43.9)	0.92 (0.79, 1.08)	0.31	150 (43.2)	151 (43.0)	1.00 (0.73, 1.37)	1.00	407 (43.8)	387 (45.3)	1.00 (0.82, 1.22)	1.00	333 (38.9)	398 (42.4)	0.86 (0.71, 1.05)	0.13
	AA	159 (11.1)	125 (8.7)	1.27 (0.98, 1.64)	0.07	25 (7.2)	28 (8.0)	0.89 (0.50, 1.59)	0.69	96 (10.3)	59 (6.9)	1.55 (1.09, 2.21)	0.01	89 (10.4)	93 (9.9)	0.99 (0.72, 1.36)	0.93
	AA + AG	746 (52.1)	759 (52.6)	0.98 (0.85, 1.13)	0.78	175 (50.4)	179 (51.0)	0.98 (0.73, 1.33)	0.91	503 (54.1)	446 (52.2)	1.07 (0.89, 1.29)	0.46	422 (49.2)	491 (52.3)	0.88 (0.73, 1.06)	0.19
					P_{trend} = 0.44				P_{trend} = 0.80				P_{trend} = 0.09				P_{trend} = 0.40

SNP ID, single nucleotide polymorphism identification.
[a]ORs were adjusted for age and sex.

Table V. HPGD haplotype frequencies and their associations with colorectal cancer

HPGD haplotypes[a]	Frequencies (*N*)		OR[b] (95% CI)	*P*	Overall frequencies	
	Cases	Controls			Present study	Hoeft *et al*. (25)
h1 = A-A-A-A-A	0.31 (1073)	0.30 (1069)	1		0.30	0.30
h2 = A-A-A-A-G	0.28 (970)	0.27 (946)	1.02 (0.90, 1.16)	0.71	0.27	0.28
h3 = A-G-G-A-G	0.16 (554)	0.15 (527)	1.05 (0.90, 1.21)	0.55	0.15	0.15
h4 = A-G-G-C-G	0.07 (248)	0.08 (274)	0.90 (0.74, 1.09)	0.28	0.07	0.07
h5 = G-G-A-A-G	0.14 (503)	0.16 (558)	0.90 (0.77, 1.04)	0.16	0.15	0.15
hRARE	0.04 (150)	0.05 (178)	0.83 (0.66, 1.05)	0.13	0.05	n/a

h, haplotype; n/a, not applicable.
Haplotypes with a frequency < 5% were combined as hRARE.
[a]SNP order: rs9312555-rs8752-rs2612656-rs17360144-rs7349744.
[b]ORs were adjusted for age and sex.

Table VI. HPGD haplotype frequencies and their associations with colon and rectal cancers

HPGD haplotypes[a]	Frequencies (*N*)		OR[b] (95% CI)	*P*	Frequencies (*N*)	OR[b] (95% CI)	*P*
	Controls	Colon cancer			Rectal cancer		
h1 = A-A-A-A-A	0.30 (1069)	0.31 (654)	1		0.31 (419)	1	
h2 = A-A-A-A-G	0.27 (946)	0.28 (588)	1.01 (0.87, 1.16)	0.93	0.28 (382)	1.05 (0.89, 1.24)	0.59
h3 = A-G-G-A-G	0.15 (527)	0.17 (353)	1.10 (0.93, 1.29)	0.27	0.15 (201)	0.95 (0.78, 1.16)	0.64
h4 = A-G-G-C-G	0.08 (274)	0.07 (140)	0.83 (0.67, 1.05)	0.11	0.08 (108)	1.00 (0.77, 1.28)	0.97
h5 = G-G-A-A-G	0.16 (558)	0.15 (315)	0.93 (0.78, 1.10)	0.38	0.14 (188)	0.86 (0.70, 1.05)	0.15
hRARE	0.05 (178)	0.04 (86)	0.78 (0.59, 1.03)	0.08	0.05 (64)	0.91 (0.67, 1.24)	0.55

h, haplotype.
Haplotypes with a frequency < 5% were combined as hRARE.
[a]SNP order: rs9312555-rs8752-rs2612656-rs17360144-rs7349744.
[b]ORs were adjusted for age and sex.

Acknowledgements

We wish to thank the clinicians who supported the study, all patients and control individuals who participated in the study and Ute Handte-Daub and Belinda-Su Kaspereit for excellent technical assistance.

Conflict of Interest Statement: None declared.

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