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Original Article

Polymorphisms in heterocyclic aromatic amines metabolism-related genes are associated with colorectal adenoma risk

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Abstract: Colorectal adenoma (CRA) and colorectal cancer (CRC) risks have been linked to the intake of red and processed meat. Heterocyclic aromatic amines (HCA) formed herein during high temperature cooking, are metabolized by a variety of enzymes, and allelic variation in the coding genes could influence individual CRA risk. Associations of polymorphisms in *NAT1, NAT2, GSTA1, SULT1A1, CYP1A2, UGT1A7, UGT1A9, GSTP1* genes with colorectal adenoma risk were investigated in a nested case-control study of the EPIC-Heidelberg cohort including 428 cases matched by age, sex and year of recruitment with one or two controls (n=828) with negative colonoscopy per case. Genoyping was preformed with the Sequenom MassArray system and the LightCycler 480. Conditional logistic regression was used to compute odds ratios (OR) and corresponding 95% confidence intervals (CI). For rs15561 (*NAT1)* and rs1057126 (*NAT1*), the rarer allel was significantly inversely associated with adenoma risk OR=0.80 (95% CI 0.65-0.97) and (OR=0.81 (95% CI 0.65-0.99) and, respectively). For the combined *NAT2* alleles encoding for enzymes with medium (versus slow) activity we also observed a significantly inverse association with adenoma risk (OR=0.75; 95% CI 0.85-0.97). In addition, homozygous carriers of the A allele of rs3957357 (*GSTA1)*, i.e., those with a decreased enzyme activity, had a decreased risk of colorectal adenoma with an OR of 0.68 (95% CI 0.50-0.92; AA versus GG/GA). Polymorphisms in the other tested genes did not modify the risk of colorectal adenomas. In conclusion, polymorphisms in *NAT1, NAT2,* and *GSTA1* are related to colorectal adenoma risk in this German cohort.

Keywords: Colorectal adenoma, genetic polymorphisms, *NAT1, NAT2, GSTA1*

Introduction

High meat intake has been demonstrated to be associated with an increased risk of colorectal adenoma (CRA) in some [1, 2] but not all studies [3, 4]. In addition, a systematic review of the epidemiological studies has suggested that high consumption of red and processed meat is convincingly associated with an increased risk of colorectal cancer (CRC) [5]. One factor possibly explaining these associations is the observation that potent chemical carcinogens such as heterocyclic aromatic amines (HCAs) are generated when meat is cooked at high temperature [6, 7]. For HCAs to be carcinogenic, they must be activated by enzymes. In an initial step, they are N-hydroxylated by cytochrome P450 1A2 (CYP1A2), followed by conjugation of the resulting N-hydroxyl group by N-acetyltransferase (NAT) or sulfotransferase (SULT). In addition to bioactivation by NAT or SULT, enzymes such as UDP-glucuronyltransferases or glutathione S-transferases (GST) contribute to the detoxification of HCAs [8].

These enzymes are encoded by genes that are highly polymorphic in humans, thus, leading to interindividual differences in enzyme activities and inducibility. Such differences in bioactivating and detoxifying capacity may lead to variation in adenoma and cancer risk between individuals. Thus, polymorphisms might be used to

identify high risk persons [9, 10].

To date, only 5 case-control studies [4, 9, 11-13] have evaluated the associations between the polymorphisms in genes analyzed in the present article and CRA risk. A greater number of studies tried to clarify the relevance of such polymorphisms for colorectal cancer (e.g., [11, 14-16] and a meta-analysis [17]. Adenomas are considered to be precursor lesions that may develop to CRC, but risk modulation by polymorphisms may differ between colorectal adenoma and carcinoma, respectively. Overall, the evidence for main effects of single-nucleotide polymorphisms (SNPs) related to both colorectal adenoma and cancer is sparse and for the former based on only a few studies.

In the context of a case-control study nested within the Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC) study, we explored the association of polymorphisms in HCA metabolism-related genes with risk of colorectal adenomas. Interactions with heterocyclic amine intake, meat intake or smoking were not part of the present analysis.

Materials and methods

Study population

The study population consists of participants of the EPIC-Heidelberg cohort. EPIC-Heidelberg is part of the European Prospective Investigation into Cancer and Nutrition (EPIC), a European-wide cohort study with more than 500,000 participants. Participants in Heidelberg were recruited at random in a predefined age range from the general population in Heidelberg, Germany, and surrounding communities between 1994 and 1998. At recruitment, women were 35-65 and men 40-65 years old. The final cohort consists of 25,540 participants (38% of those originally invited) [18].

At the baseline examination, questionnaires and interviews were used to assess information on diet, lifestyle, education and medical history. Anthropometric data were assessed in the study center, where also a 30-ml blood sample was taken from 95.8% of the study participants [19]. Blood samples were aliquotted into 0.5 mL straws of serum, plasma, buffy coat, and erythrocytes, respectively, and stored in liquid nitrogen at -196°C.

Since the recruitment of the study participants, three rounds of follow-up have been conducted in the EPIC-Heidelberg cohort. The aim of the follow-up is to collect, among others, information on the occurrence of major chronic diseases. Within these follow-ups, the study participants were asked to report the diagnosis of benign tumors, such as colorectal polyps. After three follow-ups (between September 1997 and 2007), 960 participants had declared the diagnosis of a colorectal polyp. Colorectal polyps reported by the study participants were verified by a trained physician by means of medical records. The second version of the International Classification of Diseases for Oncology was applied to code incident adenoma cases. Through verification, 536 were identified as incident colorectal adenomas and 171 as hyperplastic polyps.

After exclusion of prevalent and incident cases of cancer (except for non-melanoma skin cancer), myocardial infarction and stroke, 444 cases of colorectal adenomas for which blood samples were available were included in the study. Two controls per case were selected from subjects who had reported a negative colonoscopy; from the group of eligible controls we excluded prevalent or incident cancer cases (besides non-melanoma skin cancer), subjects who reported prevalent colorectal adenoma, prevalent or incident hyperplastic polyps, and subjects with missing blood samples. Cases and controls were matched by sex, age (+/- 1 year), and year of recruitment (+/- 0.5 years). Controls had to be alive at the time the matched case was diagnosed with adenomas.

Of the 1332 participants included in our study, 50 (3.7%) were excluded because genotyping failed and 14 individuals of incomplete case sets (i.e., controls without corresponding case or cases without controls). The analytical dataset included 428 cases and 828 controls.

All study participants signed a consent form and the ethics committee of the Heidelberg Medical School approved the study.

Laboratory analyses

Genomic DNA was extracted from buffy coat with FlexiGene kit (Qiagen) in accordance with

the manufacturer's instructions. DNA was stored at 4°C until use. Genotyping for polymorphisms of the genes *NAT1* (C1095A, rs15561), *NAT2* (T341C, rs1801280; G590A, rs1799930), *GSTA1* (G-52A, rs3957357; which is fully linked to C-69T), *SULT1A1* (G638A, rs9282861), *CYP1A2* (A-164C, rs762551), *UGT1A7* (T387G, rs17868323; G392A, rs17868324; T622C, rs11692021), *UGT1A9* (A $(T)_{9/10}$AT, rs3832043), and *GSTP1* (A313G, rs1695), were done as multiplex on the MassArray system (Sequenom) applying the iPLEX method and matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry for analyte detection. The analysis was carried out by Bioglobe (Hamburg, Germany). All duplicated samples (quality-control repeats of 8% of the samples) to verify interexperimental reproducibility and accuracy delivered concordant genotype results by >95%. A LightCycler 480 (Roche) was used to determine two more polymorphisms in *NAT1* (T1088A, rs1057126, G560A, rs4986782) and one more in *NAT2* (G857A, rs1799931) with real time PCR and melting curve analysis using hybridization probes. Determination was done in triplicate and a SD of >10% led to repeated analysis. Five percent of the samples were repeated for quality-control reasons and concordance of the assigned genotypes was >95%. All laboratory analyses were carried out with the laboratory personnel blinded to the case-control status.

Statistical analysis

Three SNPs in *NAT1* [T1088A (rs1057126), G560A (rs4986782), and C1095A (rs15561)] were used to identify carriers of the *NAT1*4* (wild type) allele and the *NAT1*10* (T1088A and C1095A), NAT1*11 (C1095A) and NAT1*14 allele (T1088A, C1095A and G560A).

To distinguish carriers of the *NAT1*10* allele from the *NAT1*14* allele, we used the G560A polymorphism. Other genotypes were not accounted for in our study. We determined 3 SNPs in *NAT2* [T341C (*NAT2*5*) (rs1801280), G590A (*NAT2*6*) (rs179930), and G857A (*NAT2*7*) (rs1799931)], which were used to construct three phenotypes: Fast acetylators were defined as carriers of two *NAT2*4* alleles (no polymorphisms at any of the three sites); intermediate acetylators were carriers of one *NAT2*4* allele and one mutant allele, and slow acetylators had no NAT2*4 allele. *GSTA1* activity was defined as normal when having GG or GA allele at rs3957357; AA has decreased activity [20]. Carriers of the GG and GA alleles of rs9282861 (*SULT1A1)* were defined as having normal enzyme activity, the AA alleles as having decreased activity [21]. Homozygous carriers of the A allele in rs762551 (*CYP1A2)* were classified as having normal enzyme activity, carriers of the GA or GG alleles as having enhanced activity [22]. Three SNPs in *UGT1A7* [T387G (rs17868323), which is related to the Asp129Lys amino acid change, G392A (rs17868324; related to Arg131Lys), and T622C (rs11692021; related to Trp208Arg)] were used to identify carriers of the *UGT1A7*1* allele (wild type), the allele *UGT1A7*2* (Asp129Lys & Arg131Lys), the allele *UGT1A7*3* (Asp129Lys, Arg131Lys & Trp208Arg) allele *UGT1A7*4* allele (Trp208Arg). These four alleles were used to determine phenotypes with low, intermediate, and high enzyme activity: high *(*1/*1, *1/*2, *2/*2*), intermediate *(*1/*3, *1/*4, , *2/*3*), and low *(*3/*3, *3/*4, *4/*4*) [23].

For *UGT1A9*, individuals with deletions at rs3832043 (i.e., A$(T)_9$AT) were classified as having normal activity, whereas DEL/T or TT (i.e., A$(T)_{10}$AT) variants lead to enhanced activity [24].

Genotype frequencies for the selected polymorphisms were computed and deviations from Hardy-Weinberg equilibrium (HWE) were determined by $\chi 2$ test. Conditional logistic regression was used to examine the associations between genotypes of the above mentioned genes and colorectal adenomas stratified by matched case set. Tests for linear trend (additive genotype models) were also employed. Similarly, we examined the associations between phenotypes and adenoma risk. In most cases, carriers of the homozygous common allele were used as reference category; exceptions were *UGT1A7*_129 and *UGT1A7*_131. All statistical analyses were conducted using SAS version 9.2 (SAS Institute, Inc., Cary, NC).

Results

Baseline characteristics of the participants in this nested case-control study including adenoma size and location and the main risk factors are summarized in **Table 1**. Cases with colorectal adenoma and controls did not differ by age at recruitment, sex and year of recruitment

Table 1. Baseline characteristics of colorectal adenoma cases and controls (with a negative colonoscopy) in the EPIC-Heidelberg study.

	Controls		Cases	
	N	Percent	N	Percent
Adenoma size				
≤ 1cm			178	41.6
> 1 cm			140	32.7
Missing			110	25.7
Adenoma location*				
Right colon & transversum			118	27.6
Left colon			155	36.2
Rectum			93	21.7
Missing			19	4.4
Sex				
Male	540	65.2	279	65.2
Female	288	34.8	149	34.8
Education, university degree	261	31.5	125	29.2
Smoking status				
Never	328	39.6	145	33.9
Former	378	45.7	196	45.8
Current	122	14.7	87	20.3
Family history of colorectal cancer	92	11.1	71	16.6
	Mean	std	Mean	std
Age at recruitment (years)	54.6	6.2	54.5	6.2
Age at diagnosis (years)			60.3	6.6
BMI (kg/m^2)	26.4	3.5	26.4	3.4
Total energy (kcal/d)	1978	638	2011	697
Alcohol (g/d)	20.4	23.7	24.4	27.3
Dietary fiber (g/d)	20.5	7.2	20.0	6.9
Processed meat (g/d)	51.5	40.4	56.2	42.5

*The colon was divided based on ICD-10 into right colon & transversum (C180, C181, C182, C183, C184, C1841, C1842, C1843, and C185), left colon (C186 and C187), and rectum (C199, C209, C2091, C2092, and C2093).

(matching factors). Cases were more likely to have a family history of colorectal cancer, to be current smokers, to drink more alcohol and to have a higher intake of processed meat. They less often had a university degree. There were no differences in mean BMI and intake of dietary fiber and energy.

All cases and controls were genotyped for polymorphisms in eight genes of phase I and phase II carcinogen-metabolizing enzymes being of importance in the activation or detoxification of HCAs. The corresponding genotype frequencies of *NAT1, NAT2, GSTA1, SULT1A1, CYP1A2, UGT1A7, UGT1A9, GSTP1* in case and control subjects are shown in **Table 2**. All genotype distributions in the control group were in Hardy-Weinberg equilibrium with the exception of *UGT1A7*_129, *UGT1A7*_131 and *UGT1A9*. For *NAT1*_560 and *NAT2*_857 the equilibrium could not be tested because the number of subjects was less than five in at least one category of the chi-squared test.

In **Tables 2** and **3**, the associations between genetic variants and derived phenotypes and colorectal adenoma risk are summarized.

A significantly decreased risk was observed between colorectal adenoma risk and *NAT1*_1095 (rs15561) CA versus CC genotypes; with each rare allele, risk decreased by 20% (95% CI = 0.65-0.97). A similar result was obtained per A allele of rs1057126 (*NAT1)* (OR = 0.81; 95% CI = 0.65-0.99). Genetic polymorphisms related to *NAT1*_1088 (rs1057126) and *NAT1*_560 (rs4986782) did not reveal statistically significant associations with colorectal adenoma

Table 2. Association between polymorphisms in HCA-metabolizing genes and risk of colorectal adenomas in EPIC-Heidelberg

SNP	rs number	allele	cases	controls	OR	CI
NAT1_1088	rs1057126	TT	278	501	1	Ref.
NAT1_1088	rs1057126	TA	121	264	0.82	(0.63, 1.06)
NAT1_1088	rs1057126	AA	16	44	0.62	(0.34, 1.12)
Per allele					*0.81*	*(0.65, 0.99)*
HWE*				0.24		
NAT1_560	rs4986782	GG	396	748	1	Ref.
NAT1_560	rs4986782	GA	15	39	0.73	(0.40, 1.34)
NAT1_560	rs4986782	AA	0	0	--	--
HWE*				-**		
NAT1_1095	rs15561	CC	256	436	1	Ref.
NAT1_1095	rs15561	CA	144	328	0.74	(0.58, 0.96)
NAT1_1095	rs15561	AA	26	60	0.72	(0.44, 1.17)
Per allele					*0.80*	*(0.65, 0.97)*
HWE*				0.88		
NAT2_341	rs1801280	TT	127	276	1	Ref.
NAT2_341	rs1801280	CT	214	396	1.19	(0.90, 1.57)
NAT2_341	rs1801280	CC	86	153	1.25	(0.89, 1.78)
Per allele					*1.13*	*(0.95, 1.34)*
HWE*				0.60		
NAT2_590	rs1799930	GG	211	418	1	Ref.
NAT2_590	rs1799930	GA	177	337	1.03	(0.80, 1.32)
NAT2_590	rs1799930	AA	39	71	1.04	(0.67, 1.60)
Per allele					*1.02*	*(0.85, 1.23)*
HWE*				0.79		
NAT2_857	rs1799931	GG	387	745	1	Ref.
NAT2_857	rs1799931	GA	24	44	1.10	(0.66, 1.83)
NAT2_857	rs1799931	AA	0	1	--	--
HWE*				-**		
GSTA1	rs3957357	GG	140	256	1	Ref.
GSTA1	rs3957357	GA	222	395	1.01	(0.78, 1.32)
GSTA1	rs3957357	AA	66	175	0.68	(0.48, 0.97)
Per allele					*0.85*	*(0.72, 1.01)*
HWE*				0.32		
SULT1A1	rs9282861	GG	183	389	1	Ref.
SULT1A1	rs9282861	AG	193	354	1.15	(0.90, 1.48)
SULT1A1	rs9282861	AA	48	76	1.31	(0.88, 1.96)
Per allele					*1.15*	*(0.96, 1.37)*
HWE*				0.72		
CYP1A2	rs762551	AA	207	403	1	Ref.
CYP1A2	rs762551	CA	171	348	0.97	(0.75, 1.24)
CYP1A2	rs762551	CC	46	70	1.24	(0.82, 1.87)
Per allele					*1.06*	*(0.88, 1.26)*
HWE*				0.67		
UGT1A7_129	rs17868323	GG	161	305	1	Ref.
UGT1A7_129	rs17868323	GT	202	425	0.90	(0.70, 1.16)
UGT1A7_129	rs17868323	TT	62	93	1.23	(0.85, 1.78)
Per allele					*1.05*	*(0.88, 1.26)*
HWE*				0.002		
UGT1A7_131	rs17868324	AA	161	305	1	Ref.
UGT1A7_131	rs17868324	GA	203	425	0.91	(0.52, 1.05)
UGT1A7_131	rs17868324	GG	62	93	1.24	(0.56, 1.16)
Per allele					*1.06*	*(0.89, 1.26)*
HWE*				0.002		
UGT1A7_208	rs11692021	TT	153	318	1	Ref.
UGT1A7_208	rs11692021	CT	210	396	1.11	(0.86, 1.43)
UGT1A7_208	rs11692021	CC	65	114	1.18	(0.82, 1.69)
Per allele					*1.09*	*(0.92, 1.29)*
HWE*				0.60		
UGT1A9	rs3832043	DEL	158	306	1	Ref.
UGT1A9	rs3832043	T/DEL	206	428	0.94	(0.73, 1.21)
UGT1A9	rs3832043	TT	62	91	1.29	(0.89, 1.86)
Per allele					*1.08*	*(0.91, 1.29)*
HWE*				0.001		
GSTP1	rs1695	AA	196	365	1	Ref.
GSTP1	rs1695	GA	182	359	0.93	(0.72, 1.20)
GSTP1	rs1695	GG	49	99	0.91	(0.62, 1.34)
Per allele					*0.95*	*(0.80, 1.13)*
HWE*				0.46		

*P value of $\chi 2$ test for deviation from HWE in controls; ** not computed because n<5 in at least one category

Table 3. Association between phenotypes of HCA-metabolizing genes and risk of colorectal adenomas in EPIC-Heidelberg.

Gene	Phenotype/enzyme activity	cases	controls	OR	CI
NAT1	*4/*4	239	411	1	Ref.
NAT1	*10/other	96	210	0.76	(0.56, 1.03)
NAT1	*10/*10	11	34	0.52	(0.26, 1.06)
NAT1	Other	53	121	0.75	(0.52, 1.08)
NAT2	Slow	248	421	1	Ref.
NAT2	Medium	146	328	0.75	(0.58, 0.97)
NAT2	Fast	16	41	0.65	(0.35, 1.20)
GSTA1	Normal (GG&GA)	362	651	1	Ref.
GSTA1	Decreased (AA)	66	175	0.68	(0.50, 0.92)
SULT1A1	Normal (GG&GA)	376	743	1	Ref.
SULT1A1	Decreased (AA)	48	76	1.22	(0.84, 1.78)
CYP1A2	Normal (AA)	207	403	1	Ref.
CYP1A2	Enhanced (CC&CA)	217	418	1.01	(0.80, 1.28)
UGT1A7	High	153	318	1	Ref.
UGT1A7	Medium	207	391	1.09	(0.84, 1.41)
UGT1A7	Low	65	114	1.16	(0.81, 1.67)
UGT1A9	Normal (Del)	158	306	1	Ref.
UGT1A9	Enhanced (Del/T & TT)	268	519	1.00	(0.79, 1.27)

NAT1: 5 subjects with missing information

(**Table 2**). For NAT1 phenotypes derived from these polymorphisms (**Table 3**), adenoma risk decreased, although results were not statistically significant.

No association could be established between adenoma risk and single polymorphisms in NAT2. However, a statistically significant inverse association (OR = 0.75; 95% CI = 0.85-0.97) was observed for the combined alleles encoding for enzymes with medium versus slow activity (**Table 3**). For fast metabolizers (versus slow metabolizers) the risk estimate was even lower but did not reach statistical significance (OR = 0.65; 95% CI = 0.35-1.20).

Homozygous carriers of the A allele of rs3957357 (*GSTA1*), i.e., those with a decreased enzyme activity, showed a significantly decreased risk of colorectal adenoma with an OR of 0.68 (95% CI = 0.50-0.92; AA versus GG/GA).

Genetic polymorphisms in *CYP1A2* (rs762551), *GSTP1* (rs1695), *SULT1A1* (rs9282861), *UGT1A7* (rs11692021), *UGT1A7* (rs17868323), *UGT1A7* (1786324), and *UGT1A9* (rs3832043) genes did not reveal any statistically significant association with colorectal adenoma risk (**Table 2**). This was also true for combinations of genotypes in these genes, encoding for enhanced or impaired enzymatic activity versus normal activity (**Table 3**).

Discussion

Polymorphisms in genes encoding for enzymes in the metabolic activation and detoxification of HCA are believed to result in variation in metabolism, potentially affecting colorectal adenoma and cancer risk [9]. In a nested case-control study, we investigated the role of polymorphisms in the following HCA metabolism-related genes in the development of colorectal adenomas. By this we add new findings to the very limited or missing results of previous studies on colorectal adenomas. Interactions with heterocyclic amine intake, meat intake or smoking will be presented in another article.

NAT1, NAT2

N-acetyltransferase (NAT) enzymes are responsible for metabolizing HCAs via N-acetylation

(detoxification) or via O-acetylation (activation), possibly depending on HCA concentration. Of the two polymorphic NAT enzymes, the *NAT2* genotype-phenotype relationship is well-established, whereas for *NAT1* it remains rather unclear [8, 25-27].

In the present analysis, a significant inverse association was observed between colorectal adenoma risk and the *NAT1* (rs15561) CA versus CC genotype whereas for NAT1 phenotypes, no significant results were observed. Per A allele of rs1057126 (*NAT1)* risk decreased statistically significantly by nearly 20% With respect to *NAT2,* a statistically significant inverse association was observed for the combined alleles encoding for medium versus slow metabolizers; only few cases and controls were classified as fast metabolizers. These results are in contrast to two previous case-control studies on CRA [4, 12]. In these studies, rapid acetylator *NAT1* genotypes were associated with a significantly elevated risk of colorectal adenomas compared to slow acetylator genotypes. There is no clear consensus for imputing NAT1 phenotypes. Therefore, our and these two studies differ from what is considered as slow, intermediate and rapid alleles. The study by Tiemersma et al. [4] grouped *NAT1* acetylators as follows: slow (at least 1 **11* allele), normal (no **10* or **11* alleles) and fast (at least 1 **10*, no **11* allele). Shin et al. [12] considered any participants possessing slow alleles (NAT1*14, *15, *17, *19, *22) to be a slow acetylators, any participants homozygous for rapid alleles (NAT1*10, *11) or in combination with a NAT1*3 and *4 allele to be a rapid acetylator, and all others as intermediate acetylators. In the same two studies, *NAT2* polymorphisms did not show any significant association. Concerning colorectal cancer risk, a meta-analysis including three studies on *NAT1* and 10 studies on *NAT2* observed a non-significant association with colorectal cancer risk for *NAT1*10* (OR = 1.25; 95% CI = 0.96–1.63), and no association for the *NAT2*-rapid acetylator genotype [28].

GSTA1, GSTP1

GSTs catalyze reactions between glutathione and lipophilic compounds with electrophilic centres, resulting in neutralisation of toxic compounds, etc. Among others, *GSTA1* variants (*GSTA1*B*) and the Ile105Val variant of *GSTP1* might be associated with decreased enzymatic activities [17].

In the present analysis, a significantly decreased colorectal adenoma risk was observed for AA versus GG genotypes in rs3957357 (*GSTA1)*. The association remained significant for AA versus the combined GG and GA genotypes, such that participants with decreased activity had a lower adenoma risk compared with those who have enzymes with normal activity. These results seem to be in contradiction with the role of GSTA in the detoxification process of carcinogens. To our knowledge, no comparable studies in CRA cases and controls have been published so far.

In a study with similar results for gastric cancer [29], the authors pointed out that lower expression of the GSTA1 protein due to the *GSTA1* polymorphism may be compensated by an overexpression of the GSTA2 enzyme [30]. In a meta-analysis [17] including four case-control studies, no significant associations were demonstrated regarding heterozygous or homozygous *GSTA1* status and CRC risk.

Moreover, in the present study, no significant associations between *GSTP1* Ile105Val (rs1695) and CRA risk were detected. These findings are in accordance with the results from a case-control study by Northwood et al. [13], the only other survey on this topic published so far.

In relation to CRC risk in the already mentioned meta-analysis by Economopoulous et al. [17] (based on nineteen case-control studies), no overall statistically significant associations between *GSTP1* Ile105Val (rs1695) and CRC risk were detected neither in Caucasians nor in Chinese people.

CYP1A2

The phase I enzymes CYP1A1 and CYP1A2 play a role in HCA activation, and the resulting N-hydroxy-HCA metabolites can directly react with DNA to form covalent adducts. Of the polymorphic *CYP1A2* alleles, besides others, *CYP1A2*1F* has been associated with enhanced inducibility of expression and enhanced enzyme activity [8, 19].

In the present study, no association between the *CYP1A2*1F* polymorphism and colorectal

adenoma was observed. This result is in accordance with the null findings of two other studies on colorectal adenoma with 2495 and 604 participants, respectively [12, 13]. A small survey of 94 individuals [9] on the other hand, found a statistically significant direct association between the *CYP1A2*1F* polymorphism CC & CA vs. AA and colorectal adenoma risk, but the high -risk group (n = 38) included not only CRA cases but also individuals with carcinoma in situ.

In relation to CRC risk, one study showed no association with *CYP1A2*1F* polymorphism [31], another study suggested an increased risk for CRC among those with non-AA-genotypes [32]. In a Japanese study, a negative association for the CC genotype versus AA was seen, but the association disappeared for the combined CC and CA genotypes [16]. All of them were case-control studies.

UGT1A7, UGT1A9

UDP-glucoronyltransferases (UGTs) catalyze the glucuronidation and elimination of various compounds, including HCAs [8]. Of these, the UGT1A7 isoform is mainly expressed in the GI tract. In relation to *UGT1A9* polymorphisms, it was observed [24] that the transcriptional activity of the promoter region containing A(T)10AT was increased by 2.6-fold compared to that of A(T) 9AT, suggesting that the UGT1A9 protein level in vivo is increased in subjects carrying the variant allele.

In this study, *UGT1A7* (rs11692021, rs17868323, rs1786324) genes did not reveal any statistically significant association with colorectal adenoma. No previous epidemiological study has examined *UGT1A7* alleles in relation to colorectal adenoma risk. With respect to CRC, one case-control study [33] observed a significant positive association and another [14] found a statistically significant association only after combination of all variant alleles. Furthermore, an increased risk was shown for patients with distal colon cancer who had the variant *UGT1A7* alleles compared with patients who had the most common alleles (OR = 3.0; 95% CI = 1.5-6.2).

In the present study, a genetic polymorphism related to *UGT1A9* (rs3832043), leading to enhanced enzyme activity, did not reveal any statistically significant associations with colorectal adenoma. To our knowledge, this is the first study to evaluate this association.

SULT1A1

SULTs are involved both in activation and detoxification of HCAs [8]. Therefore, it is difficult to predict the effect of polymorphic enzymes on CRA risk following exposure to HCAs. *SULT1A1,* a member of the *SULT* gene family, is polymorphic with the common allele, *SULT1A1*2*, where G is changed to A at nucleotide 638. Carriers of the GG and GA allele of *SULT1A1* are defined as having normal enzyme activity, the AA allele as having decreased activity [21].

In the present study, genetic polymorphisms related to *SULT1A1* (rs9282861) did not reveal any statistically significant associations with colorectal adenoma. This is also true for the AA genotype compared with the combined group of GG and AG genotypes. In the already mentioned Dutch case-control study [4], significant results were only found for interaction between *SULT1A1* and *GSTT1* genes. In an additional study [11], individuals with *SULT1A1* 638AA genotype and *NAT1* and *NAT2* intermediate/fast phenotypes had a 3.5-fold increased risk of colorectal adenoma (95% CI = 1.2-10.3) compared with individuals with *SULT1A1* 638GG genotype and NAT1 and NAT2 slow phenotypes.

The absence of a main effect of *SULT1A1* genotype on colorectal adenoma in the present study is in line with findings from several previous case-control studies on colorectal cancer [34-40]. In contrast, in a Swedish case-control study a significantly increased risk of CRC was observed for *SULT1A1*2* carriers, based on 109 patients [41].

Conclusions

Out of the polymorphisms in HCA metabolism-related genes evaluated in the present study only a few were associated with colorectal adenoma risk. These findings might be due to the complex relationship between genotype and phenotype of these enzymes, especially for NAT1. Besides the effect of genetic constellation, the enzymatic activity can be influenced by environmental factors such as smoking and diet [14], but also by gene–gene interactions [42]. On the other hand, in the absence of exposure to a genotoxin, the genetic polymorphism is not

necessarily being expected to be manifested as a risk factor. Since the same enzymes are involved both in activation and in detoxification of HCAs and the enzymes possess overlapping substrate-specificity, it is difficult to predict the consequences of these enzymes in individual susceptibility. Even though we investigated a considerable number of genes coding for phase I and II enzymes, additional enzymes and polymorphisms, respectively, are involved in the bioactivation and detoxification of HCAs. Furthermore, the location of the adenoma in the colon or rectum might be of importance.

In addition, there are issues of chance, bias and confounding to consider. The number of hypotheses tested in the present study urges caution in the interpretation of significant results.

Cases and controls were adequately matched by age, sex and year of recruitment, but not by family history of CRC (data not available). Including only participants with a negative colonoscopy in our control group might have affected the results, because this subgroup might have been in need of a colonoscopy and, thus, might not have been completely healthy. On the other hand, this subgroup might have been more health-conscious and, thus, more likely to participate in the colorectal screening program. This choice of controls is nevertheless justified by the fact that most individuals do not know that they have colorectal adenomas [43]. The agreement of allele frequencies detected in control subjects with published data [12] gives reassurance that no selection of genotypes occurred by the definition of the control group. With respect to the outcome, all self-reported diagnoses were medically confirmed, allowing the separation of adenoma cases from other types of colorectal polyps.

In conclusion, our findings suggest that some polymorphisms in HCA metabolism-related genes are associated with a decreased risk of colorectal adenoma, which could ultimately develop to CRCs. These include polymorphisms in NAT1, NAT2, and GSTA1.

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