CYP1A2 164 A→C Polymorphism, Cigarette Smoking, Consumption of Well-done Red Meat and Risk of Developing Colorectal Adenomas and Carcinomas

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Abstract. *Background: Genetic polymorphisms in metabolizing enzymes may modify the association of environmental exposure on colorectal cancer (CRC) and adenoma risk. Materials and Methods: One hundred and ninety-eight CRC cases, 422 adenomas (206 low-risk and 216 high-risk adenomas) and 222 controls were genotyped for the CYP1A2 164 A→C polymorphism and questionnaires were used to assess environmental exposure. Results: The smoking parameter "current smoking" was significantly associated with CRC risk, and all the smoking parameters related to current smoking, having ever smoked or high numbers of cigarette years were significantly associated with risk of adenomas. No association was detected between red meat consumption or how well red meat was cooked and colorectal carcinogenesis. When stratifying the case groups based on CYP1A2 genotype, all the smoking parameters yielded stronger risk association in carriers of the C allele. Conclusion: These findings may indicate that the association between cigarette smoking and colorectal carcinogenesis can be modified by the CYP1A2 genotype.*

Worldwide, colorectal cancer (CRC) is the fourth most frequent type of cancer, while in developed countries it comes second (1). Most CRCs develop through multiple mutations in the normal colonic mucosa and evolve through the adenoma-carcinoma sequence (2, 3). Development of sporadic colorectal adenomas and carcinomas has been associated with several lifestyle factors, including cigarette smoking (4, 5) and dietary items such as red meat (6-8).

Cigarette smoke is a major source of a wide variety of carcinogens, including nitrosamines, polycyclic hydrocarbons (PAHs), aromatic amines (AAs) and heterocyclic aromatic amines (HCAs) (9, 10). Carcinogens in cigarettes may reach the colorectal mucosa through the circulatory system (11, 12). Long-term, heavy cigarette smokers have a 2- to 3-fold elevated risk of colorectal adenoma and the vast majority of studies in the past several years show an association between cigarette use and CRC (4, 13). In addition, cigarette smoke has been shown to induce the expression of some metabolic genes (14). The risk-enhancing effect of red meat is not precisely known, but meat prepared at high temperatures may contain significant quantities of a number of HCAs (15).

The influence of exposures on cancer development may be affected by variation in biotransformation of carcinogens. The cytochrome P450 (CYP)-dependent monooxygenase (Phase I enzyme) represents the first line of defense against toxic chemicals (16). CYP1A2 is the major enzyme involved in the metabolism of HCAs and AAs (17, 18) and phenotype studies have detected large interindividual variation of CYP1A2 expression in the liver (19, 20). The distribution of CYP1A2 activity in humans is generally trimodal or bimodal (slow/intermediate/rapid or slow/rapid) (20-23). The expression of CYP1A2 is believed to be regulated by two mechanisms: one that controls constitutive expression and one that regulates inducibility (21). Variation in CYP1A2 activity in humans may be due to various environmental exposures, including cigarette smoke (9, 14, 21, 24-27), genetic differences (28) or gene-gene interaction (29).

In phenotype studies, rapid CYP1A2 in combination with reported preference for well-done red meat and smoking status (ever smoking) has been associated with increased CRC risk (30, 31). The hypothesis is that increased CYP1A2 activity increases CRC susceptibility (26, 31). The *CYP1A2*

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164 A→C (*CYP1A2*1F*) polymorphism (20), located in intron 1 is common in Caucasians. The functional significance of this polymorphism is not yet clear but it appears to affect enzyme inducibility. Some studies have reported that the *CYP1A2* 164 A/A genotype may represent a high inducibility genotype (26, 28, 32), but a recent Korean study found higher CYP1A2 activity in individuals carrying the *CYP1A2* C allele (33).

In this study, the association between cigarette smoking, intake of well-done red meat, the *CYP1A2* 164 A→C polymorphism and adenoma and CRC risk in a Norwegian population was investigated. We also investigated whether the association between environmental exposure and adenoma and CRC risk could be modified by the *CYP1A2* genotype.

Materials and Methods

The cohort in the KAM (Kolorektal Cancer, Arv og Miljø/CRC Inheritance and Environment) molecular epidemiological study is based on the screening group of the Norwegian Colorectal Cancer Prevention study (The NORCCAP study) in the county of Telemark (34). In addition, patients diagnosed with CRC operated on at Telemark Hospital (Skien) and Ulleval University Hospital (Oslo) were included. The KAM biobank has been described elsewhere (35). All of the participants completed a questionnaire on demographics, health status, dietary and smoking habits, alcohol consumption, physical exercise and occupation. The Regional Ethics Committee and the Data Inspectorate approved the KAM study.

The KAM cohort is based on an ethnic homogeneous group of Norwegian origin. The ID number for the NORCCAP study at Clinicaltrials.gov is -I NCT00119912 (36). The 424 adenoma cases (206 with low-risk and 216 with high-risk adenomas) and a control group of 222 individuals were drawn from the NORCCAP study. The high-risk adenoma, defined by high-grade dysplasia, size >1 cm or villous features, has a greater potential of developing into a cancerous tumor than a low-risk adenoma (37). All the controls were screen negative participants (negative flexible sigmoidoscopy). For CRC cases, all patients diagnosed with colorectal cancer who were mentally competent to complete the questionnaire were asked to participate in the KAM study. The questionnaire contained information on a family history of cancer and the selected CRC cases had no known personal history of cancer. In this study, 198 CRC cases were drawn from the KAM cohort.

Genomic DNA was isolated from blood samples according to standard procedures (38) with minor modifications as described elsewhere (35). The *CYP1A2* 164 A→C polymorphism (*CYPIA2*1F*) was genotyped according to the method described by Christiansen *et al*. (39). In short, the fragment was amplified using the sense primer 5'-GGAAGGTATCAGCAGAAAGCC-3'and the anti-sense primer 5'-GGCTCATCCTTGACAGTGCC-3' to produce a 626 bp product. The presence of the A/C polymorphism was detected using *Apa I*, which cleaves the C alleles into two products of 181 bp and 445 bp. A positive control was used in all runs and, in addition, 10% of the samples were retyped with identical results.

Differences in characteristics between groups were assessed using the χ^2 test for categorical variables and the Mann-Whitney test for continuous variables; *P*-values <0.05 were considered significant. Logistic regression was used to examine the association between cigarette smoking, red meat consumption, doneness level of red meat and the *CYP1A2* 164 A→C polymorphism, and colorectal carcinogenesis measured separately as odds ratio (OR) with 95% confidence interval (CI). The *CYP1A2* A allele was used as wild-type because this allele is more common in both Caucasian and Asian populations (33, 40). The international *CYP* allele nomenclature (http://imm.ki.se/CYPalleles/cyp1a2.htm) uses the C allele as wild-type. The genotypes were analysed based on the A/A genotype, or carriers of the C allele. Total intake of red meat was created from the dietary items in the questionnaire and the frequency and portion size were multiplied to obtain the amount in grams per day. Intake of red meat was divided into tertiles based on consumption among controls ($T_1 \leq 22.5$g (reference category), $22.5\text{g} > T_2 < 45.0$g and $T_3 \geq 45.0$g). The doneness level of red meat was divided into two categories rare/medium (reference category) and well-done. In addition to an overall case-control comparison, individuals were further stratified based on *CYP1A2* genotype (A/A genotype or carriers of the C allele) and separately examined for smoking and diet parameters. MiniTab Statistical Software, Release 13.1 Xtra (Minitab Inc. USA) was used for statistical calculations. The data were adjusted for age, sex and ever/never smoking status.

Results

Selected characteristics of cases and controls are presented in Table I. There were significant differences in the numbers of males and females among the control group and the low- and high-risk adenoma groups, $p<10^{-4}$, and between the control and CRC group, $p=0.014$, respectively. There were also significant differences in age between the control group and all of the case groups, $p<10^{-4}$. There were significantly more ever smokers in the low- and high-risk adenoma groups compared to the control group, $p<10^{-4}$, and between the control and CRC group, $p=0.019$, respectively. There was a significant difference in median red meat intake (grams, per day) between the control group and the CRC case group, $p=0.04$. The response rate regarding cigarette smoking (78% *vs*. 87%) and consumption of red meat (65% *vs*. 91%) were significantly different between CRC patients and the controls, $p=0.018$ and <0.001, respectively.

The smoking parameter "current smoker or stopped smoking ≤10 years ago" was significantly associated with CRC risk OR=2.37 (CI 1.29-4.38) (see Table II). All the smoking parameters, except cigarette years <260 for high-risk adenomas, yielded significant associations for adenomas (see Table II). None of the diet parameters were associated with increased risk of adenoma or carcinoma (see Table II).

The genotype distribution for the *CYP1A2* 164 A→C polymorphism was in Hardy-Weinberg equilibrium and comparable to frequencies reported in other studies of Caucasian populations (28, 41). The *CYP1A2* 164 A→C

Table I. *Distribution of selected characteristics by case-control status.*

Characteristic	Controls n=222	Cases		
		CRC n=198	High-risk adenomas n=216	Low-risk adenomas n=206
Men	91 (41%)	105 (53%)	141 (65%)	126 (61%)
Women	131 (59%)	93 (47%)	75 (35%)	80 (39%)
Mean age at cohort entry (years)	54.8±3.7	67.7±11.9	57.2±3.5	59.0±3.1
Never smoked*	95 (49%)	59 (38%)	48 (26%)	53 (28%)
Ever smoked*	99 (51%)	97 (62%)	135 (74%)	140 (72%)
Median smoking dose (smoking years)*	260	296	350	350
Never smoked or stopped >10 years ago	145 (73%)	103 (66%)	80 (44%)	92 (48%)
Current smoker or stopped ≤10 years ago	49 (27%)	53 (34%)	103 (56%)	99 (52%)
Median red meat intake (g)*	28.5	24	27	27
Cooking preference well-done[1]*	121 (62%)	73 (62%)	109 (58%)	104 (54%)
CYP1A2 164 A→C				
Distribution in %, AA/AC/CC	55/38/7	49/44/7	47/44/9	52/40/8

[1]The cooking preference of red meat was divided into two categories rare/medium and well-done. *Missing values for smoking parameters, red meat intake and doneness level of red meat gave rise to diminished numbers of cases.

Table II. *Result of logistic regression analysis for CYP1A2-164 A→C polymorphism and smoking and diet parameters.*

	Controls	CRC	OR (95% CI)[a]	High-risk adenomas	OR (95% CI)[a]	Low-risk adenomas	OR (95% CI)[a]
CYP1A2-164 A→C*							
A/A	106	73	1(ref)	87	1(ref)	97	1(ref)
Any C	88	83	0.91 (0.52-1.60)	96	1.01 (0.64-1.59)	96	0.99 (0.60-1.62)
Smoking parameters*							
Never smoked	95	59	1(ref)	48	1(ref)	53	1(ref)
Ever smoked	99	97	1.61 (0.90-2.89)	135	2.57 (1.58-4.18)	140	2.48 (1.48-4.17)
Cigarette years <260	49	41	1.41 (0.69-2.89)	42	1.65 (0.91-2.99)	56	2.25 (1.21-4.19)
Cigarette years ≥260	50	54	1.66 (0.83-3.31)	93	3.50 (2.03-6.04)	84	2.70 (1.49-4.89)
Never smoked or stopped >10 years ago	145	103	1(ref)	80	1(ref)	92	1(ref)
Current smoker or stopped ≤10 years ago	49	53	2.37 (1.29-4.38)	103	4.51 (2.75-7.38)	99	4.83 (2.79-8.35)
Red meat (g/day) *							
1 (≤22.5)	74	58	1(ref)	74	1(ref)	78	1(ref)
2 (>22.5 and ≤ 45.0)	77	48	1.07 (0.54-2.14)	78	1.25 (0.74-2.11)	66	1.14 (0.64-2.04)
3 (>45.0)	50	23	1.58 (0.71-3.47)	45	1.05 (0.57-1.92)	50	1.47 (0.75-2.85)
Doneness level*							
Rare/medium	75	45	1(ref)	85	1(ref)	89	1(ref)
Well-done	121	73	0.69 (0.36-1.32)	111	0.69 (0.43-1.10)	104	0.70 (0.42-1.17)

*Missing values for smoking parameters, red meat intake and doneness level of red meat gave rise to diminished numbers of cases. [a]All data are adjusted for age and gender, and in addition genotyping and diet parameters are adjusted for ever smoking.

polymorphism was not associated with increased adenoma or CRC risk (see Table II). When stratifying the CRC case groups based on *CYP1A2* genotype, all the smoking parameters yielded stronger risk association in carriers of the C allele than in cases with the A/A genotype (see Table III). None of the case groups showed any significant association with consumption of red, or well-done red meat, even after stratification by the *CYP1A2* genotype (results not shown).

Table III. *Effects of smoking parameters on adenoma and CRC case groups stratified by CYP1A2 genotype.*

	CYP1A2 – 164 A→C polymorphism					
	Colorectal cancer[a]		High-risk adenomas[a]		Low-risk adenomas[a]	
Smoking parameter	A/A	Any C	A/A	Any C	A/A	Any C
Never smoked	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
Case/control	33/52	26/43	30/52	18/43	29/52	24/43
Ever smoked						
Case/control	40/54	57/45	57/54	78/45	68/54	72/45
OR (95% CI)	1.02 (0.47-2.21)	2.92 (1.15-7.41)	1.68 (0.87-3.24)	4.43 (2.09-9.38)	2.15 (1.04-4.43)	2.98 (1.40-6.35)
Cigarette years <260*						
Case /control	16/28	25/21	21/28	21/21	24/28	32/21
OR (95% CI)	0.59 (0.21-1.64)	3.84 (1.25-11.78)	1.23 (0.55 -2.78)	2.55 (1.03-6.29)	1.57 (0.63-3.89)	3.13 (1.31-7.48)
Cigarette years ≥260*						
Case /control	24/26	30/24	36/26	57/24	44/26	40/24
OR (95% CI)	1.56 (0.63-3.86)	1.89 (0.61-5.83)	2.15 (1.01 - 4.54)	6.15 (3.71-13.97)	2.67 (1.17-6.06)	2.85 (1.19-6.83)
Never smoked or stopped >10 years ago*	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
Case/control	52/77	51/68	39/77	47/68	48/77	44/68
Current smoker or stopped ≤10 years ago*						
Case/control	21/29	32/20	48/29	59/20	49/29	50/20
OR (95% CI)	1.42 (0.61-3.27)	4.61 (1.79-11.87)	3.36 (1.71-6.60)	6.29 (3.00-13.16)	3.72 (1.75-7.91)	6.53 (2.91-14.64)

*Missing values for smoking parameters, gave rise to diminished numbers of cases. [a]All data are adjusted for age and gender.

Discussion

Sporadic CRC is a complex disease and several environmental and genetic factors may have an impact on its development. We detected significant associations for cigarette smoking and adenoma and carcinoma risk, which is in agreement with the majority of studies in the past several years (4, 13). We detected no association between consumption of red meat or the doneness level of red meat and risk of colorectal carcinogenesis. A number of studies have focused on the relationship between red meat consumption and CRC risk, but the results remain controversial (6-8, 42-45). The most convincing evidence of a positive association between meat intake and risk of colorectal carcinogenesis comes from case-control studies (46, 47), and the risk is more consistent for red and processed meat than for white meat. Production of HCAs during preparation of meat at high temperature has been suggested as a potential mechanism, but epidemiological evidence is sparse (48, 49). Sinha *et al.* (50) showed that the level of some HCAs in cooked white meat exceeds the levels in cooked red meat, but studies have shown that diets high in white meat (poultry, fish) are not associated with an increased risk (8).

Information regarding exposure may be influenced by recall bias and there is also the chance that CRC patients have changed their diet (more vegetables). In our study group, the median daily intake of red meat was significantly lower among CRC (24.0 g) cases compared to controls (28.5 g) and there was no difference between the groups in the doneness level of consumed red meat.

Case-control comparisons of the *CYP1A2* 164 A→C polymorphism yielded no significant association for any of the case groups. This finding is in agreement with previous studies on colorectal cancer risk in Caucasians (41, 51). A recent Korean study detected a stronger association in CRC patients with the *CYP1A2* 164 C allele in individuals ≥55 years of age after adjusting for smoking habits (33). Moonen *et al.* (52) suggested that individuals carrying the *CYP1A2* A/A genotype may be at higher risk for developing high-risk adenomas compared to those with the any C allele, but this study was small with only 19 controls and 74 adenomas (37 adenomas <1 cm and 38 adenomas ≥1 cm and carcinoma *in situ*) (52).

In our study, we stratified the case groups based on the *CYP1A2* genotype (A/A or any C allele). All the smoking parameters yielded stronger risk association in carriers of the C allele than in cases with the A/A genotype. This may

indicate that the risk associated with cigarette smoking may be modified by the *CYP1A2* genotype. The smoking parameter "ever smoking" yielded significant associations only in carriers of the C allele for CRC and high-risk adenomas, but for low-risk adenomas both *CYP1A2* genotypes yielded significant results. This may imply that smoking can initiate carcinogenesis regardless of genotype, but that progression is more dependent on genotype.

The prevailing hypothesis is that increased CYP1A2 activity increases the likelihood of CRC development (26, 31). In which way the C allele affects inducibility and enzyme activity is not clear. Studies of the *CYP1A2* 164 A→C polymorphism and protein activity in humans have reported conflicting evidence. Both the A/A and any C allele had either no effect (53-55), or increased, or decreased activity (26, 28, 32, 33). Various markers have been used to assess protein activity (urinary caffeine metabolites, plasma metabolic ratio, urinary PhIP metabolites, clozapine serum concentrations) which makes it difficult to compare results from different studies. A Korean study used the urinary caffeine challenge test to analyze the genotype phenotype association and found that the CYP1A2 activity in healthy smokers with the C allele was significantly higher than that in individuals with the A/A genotype (33). The genotype frequencies of the *CYP1A2* 164 A→C polymorphism in the Korean study (33) were comparable to the result in this study and other Caucasian studies (28, 41). To clarify the effect of *CYP1A2* 164 A→C polymorphism on activity, identical methods for measuring activity should be used in additional studies to enhance our understanding of the genotype–phenotype associations.

Most CRCs evolve through the adenoma–carcinoma sequence (56) and high-risk adenomas have a greater potential of developing into a cancerous tumor (37). The fact that we detected a stronger effect modification for smoking parameters and the *CYP1A2* genotype in high-risk adenomas and the CRC case group compared to low-risk adenomas strengthens our findings. This may imply that the modifying effect of the *CYP1A2* genotype in combination with smoking is more strongly associated with progression than initiation.

The *CYP1A2* 164 A→C polymorphism is located in intron 1 and variation in activity may be due to both environmental exposures and gene–gene interactions (29). A shortcoming of this study is the lack of genotyping in other genes that may influence the biotransformation of carcinogens in cigarette smoke, such as *CYP1A1*, *N*-acetyltransferase (*NAT*) and glutathione *S*-transferases (*GSTs*). The analysis was also limited by a relatively small sample size. This is a pilot study, so we did not commit all our samples to the genotype analysis. The response rate for both cigarette smoking and consumption of red meat were significantly different between CRC patients and the controls. This may affect the result for overall association for cigarette smoking and red meat consumption. However, in this study we wanted to see if the *CYP1A2* genotype could modify the association of environmenetal exposure. There is no risk of bias in relation to genotype and the difference in response rate should therefore not affect the outcome.

Our results show an association between cigarette smoking and colorectal carcinogenesis. This association may be modified by the *CYP1A2* genotype since all the smoking parameters yielded stronger risk association in carriers of the C allele, than among cases with the A/A genotype. These results highlight the importance of considering genetic susceptibility when evaluating external exposure. A larger study would be required to clarify this issue.

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