

Null Results in Brief

No Modifying Effect of *NAT1*, *GSTM1*, and *GSTT1* on the Relation between Smoking and Colorectal Cancer Risk

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Introduction

Cigarette smoke contains potent carcinogenic compounds, including polycyclic aromatic hydrocarbons, heterocyclic amines, and nitrosamines. When entering the body, cigarette carcinogens have relatively weak mutagenic potential. After being metabolized, more potent mutagens are formed, which could result in DNA adduct formation. The genes involved in this metabolism include *NAT1*, *NAT2*, *GSTM1*, and *GSTT1*, and several polymorphisms could influence their enzyme activity. For *NAT2*, we found smoking to be a risk factor for colorectal cancer, especially in women with rapid *NAT2* imputed phenotype (1). No consistent association has been found between *NAT1* and colorectal cancer, as reported in a review (2). The glutathione *S*-transferases are a family of enzymes responsible for the detoxification of mutagenic electrophiles including polycyclic aromatic hydrocarbons. Homozygotes for the null alleles (deletion) of glutathione *S*-transferase μ (*GSTM1*) and glutathione *S*-transferase θ (*GSTT1*) lack activity of the respective enzymes. Because it seems likely that genetically determined variation in enzymatic (in)activation may modify colorectal cancer risk associated with smoking, the aim of the present study was to investigate the combined effect of smoking and genetic polymorphisms in each of the relevant metabolic genes (*NAT1*, *GSTM1*, and *GSTT1*).

Materials and Methods

A population-based screening program for early detection of breast cancer was started in 1974, in Utrecht, the Netherlands: the so-called "DOM" (Diagnostisch Onderzoek Mammacarcinoom) project. A total of 25,769 women, born between 1911 and 1945 attended the screening and provided a urine sample, which was stored at -20°C . The participation rate was 70% (1).

Follow-up from 1976 until 1987 revealed 56 deaths because of colon cancer and 8 deaths because of rectal cancer. From 1987 to January 1, 1996, 161 incident colon cancer cases

and 73 incident rectal cancer cases were identified. For the present study, a subcohort of 1000 women was randomly selected from the source population. The institutional review board for human studies of the University Medical Center Utrecht approved the study.

Urine samples of 1298 study participants were retrieved and thawed, and 50 ml were removed for DNA isolation. Four *NAT1* polymorphisms were detected in duplicate by radioactive allele-specific oligonucleotide hybridization. For genotype determination four X-ray films were read at the same time. The samples were classified in duplicate by two independent observers as *NAT1**3, *NAT1**4, *NAT1**10, or *NAT1**11 homozygote or heterozygote.

The presence or absence of the *GSTM1* and *GSTT1* gene was determined by multiplex PCR with an internal control. All laboratory analyses were performed blind with respect to the case or control status.

Smoking habits were assessed at baseline by self-administered questionnaire. For 10 women, the smoking status was not known; for 267 participants, DNA was insufficient or genotyping method failed (23% cases and 20% subcohort) for the *NAT1* gene, and for 306 women (26% cases and 23% subcohort), we could not determine *GSTM1* or *GSTT1* genotype.

Women with at least one *NAT1**10 allele were classified as rapid acetylators, and the remaining women were classified as slow acetylators. We used Poisson regression models to estimate incidence rate ratios and corresponding 95% CIs² for colorectal cancer. Because the 1000 women constitute a random sample of the total cohort, multiplication of the person-years in the reference group by 25.9 (the inverse of the subcohort non-cases sampling fraction) enabled us to analyze the data as a full cohort analysis, in which the person-years provide unbiased estimates of true person-years. Robust 95% CIs were calculated to account for additional variance introduced by sampling for the cohort.

Results

There were no differences between the genotyped and non-genotyped women in any of the following baseline characteristics: age; height; and weight. About one-third of the women reported ever having smoked. None of the three putative at risk genotypes (*NAT1**10 allele, *GSTM1*-null genotype, or *GSTT1*-null genotype) showed increased colorectal cancer risk. Women who had ever smoked were at increased risk for colorectal cancer (RR = 1.33; 95% CI, 1.00–1.79). This risk was equal for colon cancer (RR = 1.37; 95% CI, 0.99–1.90) and rectal cancer (RR = 1.26; 95% CI, 0.76–2.08), respectively. Compared with nonsmoking women with slow *NAT1* genotype, women with rapid *NAT1* genotype who smoked showed a

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² The abbreviations used are: CI, confidence interval; RR, incidence rate ratio.

Table 1 Genotype, smoking, and colorectal cancer risk

Genotype	Smoking	Cases		Subcohort		Colorectal cancer RR ^a
		N	%	N	%	
<i>NAT1</i>						
Non *10 (slow)	Never	101	47	380	47	1.00
Non *10	Ever	53	24	169	21	1.40 (0.94–2.08)
*10 (rapid)	Never	40	18	166	21	0.88 (0.58–1.34)
*10	Ever	24	11	89	11	1.26 (0.75–2.11)
Total		218		804		
<i>GSTM1</i>						
Present	Never	73	34	271	35	1.00
Present	Ever	51	24	125	16	1.82 (1.18–2.81)
Null genotype	Never	65	31	257	34	1.00 (0.68–1.46)
Null genotype	Ever	23	11	112	15	0.95 (0.55–1.64)
Total		221		765		
<i>GSTT1</i>						
Present	Never	104	49	385	50	1.00
Present	Ever	50	24	156	20	1.37 (0.93–2.04)
Null	Never	34	16	143	19	0.93 (0.60–1.45)
Null	Ever	24	11	81	11	1.44 (0.83–2.51)
Total		212		765		

^a Adjusted for age (continuous) and Diagnostisch Onderzoek Mammacarcinoom cohort (categorical).

nonsignificant increase of 26% (RR = 1.26; 95% CI, 0.75–2.11; Table 1).

Discussion

The present study shows an increased colorectal cancer risk for women who reported ever having smoked; however, modification by *NAT1*, *GSTM1*, or *GSTT1* genotype was not observed.

According to a recent review regarding smoking, a long latency period (30–40 years) is suggested to be necessary before colorectal cancer develops (3). Women started smoking in the Netherlands in the late 1950s, and sufficiently long follow-up time up to 1990 is necessary to observe carcinogenic effects of smoking on the colon and rectum in women.

Because the cohort started as a population-based screening program for early detection of breast cancer, colorectal cancer registry started only after 1987. However, a mortality registry was set up from the beginning and identified 64 deaths due to colorectal cancer. Restriction of the analyses to only the incident cases led to the same results. Recently, it was also reported that colorectal cancer survival was not associated with *GSTM1*- or *GSTT1*-null genotype (4). We therefore think that the absence of interaction is real. The power to detect a doubling of risk associated with smoking, *NAT1* rapid genotype, *GSTM1*-null genotype, or *GSTT1*-null genotype is more than 95%, and the power to detect a 1.5× increased risk is 70%, 70%, 73%, and 61%, respectively.

We did not observe effects of *GSTM1*- or *GSTT1*-null genotype, which is in accordance with the hypothesis and a meta-analysis in which no association of either *GSTM1* (10 studies) or *GSTT1* (8 studies) deficiency with colorectal cancer risk was observed (5). The hypothesized higher colorectal cancer risk in women who smoked and who have a lower detoxification capacity, due to *GSTM1*- or *GSTT1*-null genotype, is

not supported by our data. Because all of the enzymes examined in this study are involved in carcinogen metabolism, but with low substrate specificity, it is possible that inadequate activation or detoxification due to polymorphisms in one gene is compensated for by another gene. An interaction between *NAT2* slow acetylator and *GSTT1*-null status was reported by Welfare *et al.* (Ref. 6; OR = 2.33; 95% CI, 1.1–5.0). We therefore classified women into categories with increasing number of putative risk genotype. However, no effect on colorectal cancer risk was observed, and numbers became too small to study a modifying effect on smoking.

In conclusion, this study supports smoking to be a risk factor for colorectal cancer, but no evidence that *NAT1**10 genotype, *GSTM1*-null genotype, and *GSTT1*-null genotype increase this risk was found.

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