Colorectal Adenomatous and Hyperplastic Polyps: Smoking and *N*-Acetyltransferase 2 Polymorphisms

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Abstract

Arylamine *N*-acetyltransferase 2 (NAT2) is involved in both the detoxification and bioactivation of carcinogenic arylamines and other mutagens. This enzyme is polymorphic, and the fast and slow phenotypes are thought to be risk factors for colon and bladder cancer, respectively. Here, we report on a case-control study of adenomatous and hyperplastic polyps, with particular attention to tobacco smoking, a known risk factor for adenomas, and polymorphisms of *NAT2*. All participants underwent complete colonoscopy and were subsequently divided into case and control groups on the basis of pathology. Cases were diagnosed with confirmed adenomas ($n = 527$) or hyperplastic polyps ($n = 200$); controls ($n = 633$) had no history of colonic neoplasia and no polyps at colonoscopy. *NAT2* genotype was determined using an oligonucleotide ligation assay and fast, intermediate, or slow phenotype imputed. Multivariate-adjusted odds ratios (ORs) and 95% confidence intervals were computed using logistic regression adjusting for age, sex, nonsteroidal anti-inflammatory drug use, and hormone replacement therapy use. Smoking was associated with an increased risk of adenomas [current *versus* never smoking OR = 2.0 (95% confidence interval, 1.4–2.9)] and hyperplastic polyps [current *versus* never smoking OR = 4.1 (2.6–6.5)]. NAT2 status among adenomatous polyp patients and hyperplastic polyp patients, respectively, showed ORs of 1.1 (0.8–1.4) and 1.2 (0.8–1.6; intermediate *versus* slow) and 1.1 (0.6–1.9) and 0.9 (0.4–1.9; fast *versus* slow). There were no differences in risk when adenoma patients were stratified on multiplicity, size, or histopathological subtype of polyps. Never-smokers showed no variation in risk across acetylator status for either species of polyp, whereas current smokers showed ORs of 2.0 (1.2–3.2) and 2.3 (1.4–3.9) for adenomas and 3.9 (2.1–7.1) and 4.9 (2.6–9.4) for hyperplastic polyps for slow and intermediate/fast NAT2, respectively, compared with slow-NAT2 never-smokers. Risks of both multiple [OR = 4.3 (2.1–8.8)] and large [OR = 3.8 (1.9–7.5)] adenomas were somewhat elevated in current smokers with an intermediate/fast phenotype compared with smokers with a slow NAT2 phenotype, but the interaction was not statistically significant. Risk of hyperplastic polyps and adenomatous polyps is strongly related to smoking. There is little suggestion of interaction between NAT2 status and smoking and no relationship with *NAT2* genotype alone.

Introduction

The etiology of colorectal cancer is complex and multifactorial. A number of modulators of risk, including family history, diet, alcohol, physical activity, and hormone replacement therapy use, have been identified (1). Tobacco smoking, on the other hand, was only occasionally associated with increased risk of large bowel cancer in the early studies, and in some studies, this was confined to pipe and cigar (2, 3) rather than cigarette use. Nonetheless, there is now evidence that prolonged use of tobacco or early age at first use is associated with elevated risk (4–7).

Adenomatous polyps of the colon and rectum are widely held to be the precursor lesions in almost all large bowel cancer. Almost every study of adenomatous polyps has shown increased risk associated with tobacco smoking (4, 5, 8–19).

Although it is established that adenomatous polyps are the precursor lesions of most colorectal cancers, hyperplastic polyps are less clearly related to the development of cancer. Nonetheless, some risk factors are shared. For instance, Kearney *et al.* (20) noted an increase in risk of hyperplastic polyps associated with smoking, alcohol consumption, low folate, and, possibly, animal fat. Martinez *et al.* (21) reported higher risks in association with low fiber and high alcohol but not fat; they also reported that smoking was associated with an elevated risk and that aspirin was inversely associated with hyperplastic polyps. Hyperplastic polyps also frequently exhibit mutations of K-ras (22).

The role of genetics in the etiology of colorectal cancer is well established, with two discrete Mendelian syndromes, familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer, being associated with markedly elevated risk (23, 24). These syndromes, however, account for only a minor proportion of all cases, perhaps a total of 3% (25). It is possible that genetic variation in metabolizing enzymes, particularly those that plausibly play a role in the metabolism of suspected human colon carcinogens, account for a much larger proportion of risk across the population.

One of these enzymes, NAT2, can detoxify carcinogenic arylamines through *N*-acetylation or activate *N*-hydroxylated heterocyclic amines found in the diet and tobacco smoke by *O*-acetylation (26, 27). NAT2 is a polymorphic enzyme, in

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Table 1 Selected characteristics of patients in the Minnesota Cancer Prevention Research Unit Polyp Study

Characteristic	Adenomatous polyps (n = 527)	Hyperplastic polyps (n = 200)	Controls (n = 633)
% male	62.2	56.5	38.5
Age (yr)	58.1 ± 9.7	53.8 ± 10.0	52.7 ± 10.9
Ethnic origin (% white)	97.7	97.0	96.8
Cigarette smoking			
% never-smokers	33.2	22.0	47.7
% current smokers	21.1	30.5	15.5
% ex-smokers	45.7	47.5	36.8
Pack-years (mean ± SD)	19.5 ± 23.4	21.2 ± 22.3	12.9 ± 21.9
% alcohol users	73.4	79.4	71.7
% post-menopausal (women)	84.4	72.4	68.7
% HRT[a] users (women)	40.6	41.4	50.6
% NSAID users	15.1	20.4	23.5
% family history of colorectal cancer	16.6	20.7	27.3
Primary indication for colonoscopy			
% bleeding	38.4	34.9	51.1
% diagnostic	7.8	8.5	16.5
% follow-up	5.8	11.6	5.5
% positive family history	7.6	14.8	21.8
% screening	39.2	28.6	3.2
% other	1.2	1.6	1.8
NAT2-imputed phenotype			
% fast	6.1	5.0	6.6
% intermediate	37.2	39.0	34.5
% slow	56.7	56.0	58.9

[a] HRT, hormone replacement therapy; NSAID, nonsteroidal anti-inflammatory drug.

Table 2 *NAT2* allele[a] frequencies in the Minnesota Cancer Prevention Research Unit Polyp Study

Allele	% frequency
4 (wild type)	23.4
5A	3.2
5B,C	41.9
6A,B	27.4
7A,B	3.1
12A,B	0.9
14A,B	0.2

[a] Nomenclature according to Vatsis *et al.* (41).

Table 3 Distribution of genotypes and imputed phenotypes in the Minnesota Cancer Prevention Research Unit Polyp Study

NAT2 genotype and phenotype	Frequency	%
Fast		**6.2**
4/4	86	5.7
4/12A,B	7	0.5
Intermediate		**36.2**
4/5A	23	1.5
4/5B,C	314	20.6
4/6A,B	172	11.3
4/7A,B	23	1.5
14A,B/12A,B	2	0.1
5B,C/12A,B	13	0.9
6A,B/12A,B	4	0.3
Slow		**57.7**
14A,B/5B,C	2	0.1
14A,B/6A,B	1	0.1
5A/5A	1	0.1
5A/5B,C	40	2.6
5A/6A,B	30	2.0
5A/7A,B	3	0.2
5B,C/5B,C	260	17.1
5B,C/6A,B	344	22.6
5B,C/7A,B	43	2.8
6A,B/6A,B	132	8.7
6A,B/7A,B	18	1.2
7A,B/7A,B	3	0.2

which seven point mutations leading to amino acid changes and three silent point mutations have been identified (28–33). Alleles containing any of the missense mutations at nt[2] positions 191, 341, 590, and 857 produce an enzyme that metabolizes its substrate more slowly than the wild-type allele (34, 35). Individuals carrying two of these missense mutations are slow acetylators (29, 36, 37). The point mutation at nt 803, resulting in a Lys → Arg amino acid change, as well as the silent mutations do not seem to affect enzyme activity (35, 38). NAT2 phenotype has been shown to correlate closely with genotype (37).

Here, we describe a study of adenomatous and hyperplastic polyps with particular attention to the roles of smoking and genetic variation in arylamine NAT2 activity. We hypothesized that smoking would be associated with an increased risk of both hyperplastic and adenomatous polyps and that individuals with an intermediate or fast NAT2 phenotype would be particularly at risk as a result of smoking.

Materials and Methods

This case-control study was conducted between April 1991 and April 1994 as part of the Minnesota Cancer Prevention Research Unit, an NCI-funded program project that combined several units within the University of Minnesota and DH, a large multiclinic private gastroenterology practice. DH conducts colonoscopies in 10 hospitals and, at the time of this study, undertook ~60% of all colonoscopies in the Minneapolis metropolitan area. This study was approved by the internal review boards of the University of Minnesota and each DH endoscopy site. Written informed consent was obtained from each study participant.

Study Participants. DH staff initiated study recruitment at the time of scheduling colonoscopy appointments. The initial eligibility assessment evaluated whether patients were aged 30–74 years, residents of the Twin Cities metropolitan area, English speaking, free of known genetic syndromes associated with predisposition to colonic neoplasia and of individual history of ulcerative colitis, Crohn's disease, adenomatous polyps, and cancer (except nonmelanoma skin cancer). Patients were recruited at all 10 DH endoscopy sites. Two to 5 days later, a DH nurse called the potential participant, confirmed the arrival of study materials, and sought verbal permission for the patient to be contacted by University of Minnesota staff. If permission was granted, the participant was called, and further information on the study was provided.

At the colonoscopy visit, the signed consent form and completed questionnaires were collected, and blood was drawn. Colonoscopists recorded findings on standardized forms. Polyp size was determined *in vivo* by comparison of the polyp with a set of fully opened standard-sized flexible colonoscopy forceps.

[2] The abbreviations use are: nt, nucleotide(s); NAT2, *N*-acetyltransferase 2; DH, Digestive Healthcare PA; CI, confidence interval; OR, odds ratio.

Table 4 Percentage of patients within each imputed NAT2 phenotype with specified pathological characteristics of adenomatous polyps: the Minnesota Cancer Prevention Research Unit Polyp Study

Polyp characteristics	Imputed NAT2 phenotype			
	Fast (n = 32)	Intermediate (n = 196)	Slow (n = 299)	Total (n = 527)
No. of adenomas				
1	65.6	65.8	72.9	69.8
2–3	34.4	29.6	21.7	25.4
≥4	0.0	4.6	5.4	4.7
Size of largest adenoma (cm)				
≤0.5	50.0	49.5	50.8	50.3
0.6–0.9	21.9	15.8	16.1	16.3
≥1.0	28.1	34.7	33.1	33.4
Subtype: most severe adenoma				
Tubular	71.9	68.9	62.2	65.3
Villous	28.1	25.0	32.4	29.4
Tubulo-villous	0.0	6.1	4.7	4.9
Dysplasia: most severe adenoma				
None to mild	53.1	46.9	39.8	43.3
Moderate	34.4	44.4	52.8	48.6
Severe/carcinoma *in situ*	12.5	8.7	7.4	8.2
Shape: most severe adenoma				
Pedunculated	18.8	22.4	25.8	24.1
Sessile	43.8	54.6	50.5	51.6
Location: most severe adenoma				
Colon	90.6	83.2	84.0	84.1
Proximal[a]	9.4	15.8	18.1	16.7
Distal[a]	81.2	67.4	65.9	67.4
Rectum[a]	9.4	16.8	16.0	15.9

[a] Proximal colon = cecum to hepatic flexure; distal colon = transverse colon to sigmoid; rectum includes rectosigmoid.

Table 5 Cigarette smoking: multivariate-adjusted[a] ORs (95% CI) for adenomatous and hyperplastic polyps *versus* controls

Smoking variables	Adenomatous polyps	Hyperplastic polyps
Smoking status		
Never-smoker	1.0	1.0
Ex-smoker	1.4 (1.1–1.9)	2.4 (1.6–3.6)
Current smoker	2.0 (1.4–2.9)	4.1 (2.6–6.5)
Pack years		
0	1.0	1.0
1–19	1.4 (1.0–2.0)	2.4 (1.6–3.7)
≥20	1.9 (1.4–2.6)	3.2 (2.1–4.9)

[a] Adjusted for age, sex, nonsteroidal anti-inflammatory drug use, and hormonal replacement therapy use.

Table 6 Imputed NAT2 phenotype: multivariate-adjusted[a] ORs (95% CI) for various polyp pathology characteristics *versus* controls

Polyp characteristics	Intermediate *vs.* slow NAT2	Fast *vs.* slow NAT2
Hyperplastic polyp	1.2 (0.8–1.6)	0.9 (0.4–1.9)
Adenoma	1.1 (0.8–1.4)	1.1 (0.6–1.9)
Single adenoma	1.0 (0.7–1.3)	0.9 (0.5–1.7)
Multiple adenomas	1.3 (0.9–2.0)	1.6 (0.7–3.4)
<1 cm adenoma	1.1 (0.8–1.4)	1.2 (0.6–2.1)
≥1 cm adenoma	1.1 (0.7–1.6)	1.0 (0.4–2.2)
Tubular adenoma	1.2 (0.9–1.6)	1.2 (0.7–2.2)
Tubulovillous adenoma	0.9 (0.6–1.3)	0.9 (0.4–2.0)

[a] Adjusted for age, sex, nonsteroidal anti-inflammatory drug use, hormone replacement therapy use, and smoking.

All polyps were removed and examined histologically by a single study pathologist using the diagnostic criteria established for the National Polyp Study (39). If polyps had been removed during a sigmoidoscopy prior to the colonoscopy, the relevant slides were also evaluated by the study pathologist.

On the basis of the colonoscopy and pathology findings, participants were assigned to one of three groups. To be eligible as a hyperplastic polyp or adenoma case or a colonoscopy-negative control, the participant must have had a complete colonoscopy reaching the cecum, all polyps removed, no new diagnosis of ulcerative colitis or Crohn's disease, and no polyps showing invasive carcinoma. Adenoma cases had at least one adenomatous polyp (defined as either adenomatous or mixed pathology). Hyperplastic cases had at least one hyperplastic polyp and no adenomas. Controls were polyp free at colonoscopy.

Data Collection. Study participants provided detailed information on demographic characteristics, personal medical history, usual physical activity, anthropometric measurements, reproductive history (women only), and family history of polyps and cancer. Smoking history included current and past smoking status, age at starting smoking, and average number of cigarettes smoked per day; for quitters, years since quitting and average cigarettes smoked per day when smoking were also recorded. Pack-years of smoking were calculated as years smoked multiplied by current (or past, in the case of former smokers) cigarettes smoked per day divided by 20. Study staff telephoned participants to retrieve data when information was incomplete.

Genotyping. Genomic DNA was extracted from peripheral WBCs using Puregene (Gentra Systems, Minneapolis, MN). *NAT2* genotyping at nt 191, 341, 590, 803, and 857 was performed using an oligonucleotide ligation assay, as described previously (40). We did not analyze for the silent mutations at nt 282 and 481. This assay allows the use of 96-well microplates and a robotic workstation. A single PCR with an input of

Table 7 Cigarette smoking and imputed NAT2 phenotype: multivariate-adjusted[a] ORs (95% CI) for various polyp pathology characteristics *versus* controls

Polyp characteristics	Smoking status			*P*-interaction	
	Never-smoker	Ex-smoker	Current smoker	Slopes (1 df)[b]	NAT2 × smoking (2 df)[b]
Hyperplastic polyp					
Slow	1.0	2.6 (1.5–4.3)	3.9 (2.1–7.1)	0.82	0.81
Intermediate/fast	1.1 (0.6–2.1)	2.5 (1.4–4.4)	4.9 (2.6–9.4)		
Adenoma					
Slow	1.0	1.5 (1.0–2.2)	2.0 (1.2–3.2)	0.97	0.87
Intermediate/fast	1.1 (0.7–1.7)	1.5 (1.0–2.2)	2.3 (1.4–3.9)		
Multiple adenomas					
Slow	1.0	1.5 (0.8–2.7)	2.5 (1.2–5.2)	0.44	0.76
Intermediate/fast	1.1 (0.6–2.3)	1.9 (1.1–3.5)	4.3 (2.1–8.8)		
≥1 cm adenoma					
Slow	1.0	1.9 (1.1–3.4)	2.4 (1.2–4.8)	0.73	0.28
Intermediate/fast	1.2 (0.6–2.3)	1.5 (0.8–2.7)	3.8 (1.9–7.5)		
Tubulovillous adenoma					
Slow	1.0	1.9 (1.1–3.3)	2.6 (1.3–4.9)	0.51	0.41
Intermediate/fast	1.2 (0.6–2.2)	1.3 (0.7–2.3)	2.4 (1.2–5.0)		

[a] Adjusted for age, sex, nonsteroidal anti-inflammatory drug use, hormone replacement therapy use.
[b] df, degree(s) of freedom.

50–100 ng of genomic DNA provides sufficient amplified *NAT2* fragments to analyze the five missense mutations. Briefly, primers 5′-GGAACAAATTGGACTTGG-3′ and 5′-TCTAGCATGAATCACTCTGC-3′ (30) were used to amplify the *NAT2* coding region from 100 ng of genomic DNA in 50-μl reactions containing 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin (Perkin-Elmer, Foster City, CA), 50 μg/ml BSA, 0.2 μM primers, 0.2 mM dNTPs, and 1 unit of Amplitaq DNA polymerase (Perkin-Elmer). The cycling conditions were: 4 min at 94°C, followed by 40 cycles at 94°C for 30 s, 57°C for 45 s, and 72°C for 90 s and a final extension at 72°C for 5 min (30). For the ligation, the PCR was diluted with 80 μl of 0.1% Triton X-100. The 20-μl ligation reactions consisted of 10 μl of diluted PCR product, 20 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 12.5 mM KCl, 1 mM DTT, 1 mM NAD, 0.1% Triton X-100, 8 fmol/μl biotinylated wild-type or mutant primer, 8 fmol/μl digoxigenin-tailed common primer (for primer sequences, see Ref. 40), and 0.015 units of thermostable ligase (Epicentre Technologies, Madison, WI). The cycling conditions for the ligation for all of the mutations were: 15 cycles at 93°C for 30 s and 58°C for 2 min. The reaction was stopped with 10 μl of a buffer containing 0.1 M EDTA (pH 8.0) and 0.1% Triton X-100.

The ligation reactions were transferred into streptavidin-coated 96-well plates. After incubation for 60 min at room temperature, the plates were washed twice with 10 mM NaOH and 0.05% Tween 20, followed by two washes with 200 μl of 100 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.05% Tween. The plates were then incubated with 40 μl of a 1000-fold dilution of antidigoxigenin Fab-fragment alkaline phosphatase conjugate (0.75 units/μl; Boehringer Mannheim, Indianapolis, IN) for 30 min at room temperature. After four washes with 100 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.05% Tween 20, the Life Technologies, Inc., ELISA amplification system was applied for the color reaction according to the manufacturer's recommendations. $A_{495\ nm}$ was recorded using a SpectraMax 250 plate reader (Molecular Devices, Sunnyvale, CA).

NAT2 genotypes were translated to imputed phenotypes according to the allele nomenclature described in Vatsis *et al.* (41) as fast (**4/*4* and **12AB/*12AB*), intermediate (*x/*4* and *x/*12AB*), and slow (other genotypes). Data were analyzed using all three imputed phenotypes or collapsing across fast and intermediate.

Data Analysis. Unconditional logistic regression models were used to obtain maximum likelihood estimates and 95% CIs for measures of association relating case-control status and exposure variables while controlling for potentially confounding variables, including age, sex, use of nonsteroidal anti-inflammatory drugs and, among women, use of hormone replacement therapy; we have shown these to be important modulators of risk of adenomatous polyps in this population (42). The group of nonsmokers was referent. ORs and 95% CIs were calculated. All tests of statistical significance were two-sided. Separate analyses were performed for adenoma cases and hyperplastic cases with colonoscopy-negative controls. Interactions between NAT2 phenotype and smoking were assessed by the introduction of multiplicative terms into the model and by comparing slopes. All analyses were performed using SAS Version 6.12 (SAS Institute Inc., Cary, NC).

To investigate the association between variables and polyp characteristics, adenoma cases were classified into subgroups based on multiplicity, size, and pathological subtype. If a participant had more than one adenoma, the adenoma with the highest degree of dysplasia was used for this classification.

Results

The overall response rate among those who were colonoscoped was 68.2%. Of the participants, 574 were diagnosed with adenomatous polyps, 219 were diagnosed with hyperplastic polyps, and 707 were designated as colonoscopy-negative controls. Genotyping was completed on 527 adenomatous polyp patients, 200 hyperplastic polyp patients, and 633 controls; blood was not obtained from 92 participants, and DNA was not extracted successfully from 48 samples. Table 1 shows some relevant population characteristics for the two case groups and the control group. There were more males in the two case groups than in the control group. Controls were more likely to be never-smokers than either of the two case groups.

The allele frequencies and genotype distribution are shown in Tables 2 and 3, respectively. There were no differences in the distributions of imputed NAT2 phenotypes across the three groups. This was true even in the specific genotypes. We found an overall population distribution consistent with that reported in other populations with a similar ethnic background (26, 29, 34) and with our own data for colon cancer (43).

Table 8 Pack-years of cigarette smoking and imputed NAT2 phenotype: multivariate-adjusted[a] ORs (95% CI) for various polyp pathology characteristics versus controls

Polyp characteristics	Pack-years			*P*-interaction	
	0	1–19	20+	Slopes (1 df)[b]	NAT2 × smoking (2 df)[b]
Hyperplastic polyp					
Slow	1.0	2.4 (1.4–4.2)	3.5 (2.0–6.1)	0.88	0.94
Intermediate/fast	1.2 (0.6–2.2)	2.7 (1.5–4.9)	3.6 (2.0–6.5)		
Adenoma					
Slow	1.0	1.4 (0.9–2.1)	2.0 (1.3–2.9)	0.98	0.94
Intermediate/fast	1.1 (0.7–1.6)	1.5 (1.0–2.4)	2.0 (1.3–3.0)		
Multiple adenomas					
Slow	1.0	1.3 (0.7–2.6)	2.2 (1.2–4.0)	0.95	0.55
Intermediate/fast	1.1 (0.6–2.3)	2.5 (1.3–4.8)	2.6 (1.4–5.0)		
≥1 cm adenoma					
Slow	1.0	2.3 (1.3–4.1)	2.1 (1.2–3.8)	0.84	0.42
Intermediate/fast	1.3 (0.7–2.4)	1.6 (0.8–3.2)	2.6 (1.4–4.7)		
Tubulovillous adenoma					
Slow	1.0	2.2 (1.2–3.9)	2.2 (1.2–3.8)	0.98	0.29
Intermediate/fast	1.2 (0.6–2.2)	1.2 (0.6–2.4)	2.0 (1.1–3.7)		

[a] Adjusted for age, sex, nonsteroidal anti-inflammatory drug use, and hormone replacement therapy use.
[b] df, degree(s) of freedom.

The pathological characteristics of the adenomatous polyps, stratified by imputed NAT2 phenotype, are shown in Table 4. The majority of study patients had only one polyp. The polyps were largely distal (distal colon and rectum) and had tubular histopathology. There were no notable differences across NAT2 phenotypes.

Table 5 shows that tobacco smoking, a major source of heterocyclic amines, is associated with statistically significantly increased risks of both hyperplastic and adenomatous polyps for all smokers whether measured by current behavior or lifetime consumption. Additionally, there was a marginal difference in the elevated risk of tubular adenomas [OR = 1.5 (95% CI, 1.0–2.3)] *versus* tubulo-villous adenomas [OR = 2.3 (1.4–3.8)] for current *versus* nonsmokers.

Table 6 shows that NAT2 status is not related to the risk of polyps, either by species or by aspects of adenoma pathology.

Tables 7 and 8 show that estimated ORs after stratification on imputed NAT2 phenotype are only marginally and not statistically significantly higher for either adenomas or hyperplastic polyps in smokers [whether data are stratified by current status (Table 7) or lifetime consumption (Table 8)] with an intermediate or fast phenotype than in smokers with a slow phenotype. The difference is somewhat more marked when multiple and large polyps are considered, but again, the tests for interaction are not statistically significant. There were no differences in the risk of smokers developing tubulo-villous as opposed to tubular adenomas by acetylator status.

Discussion

We found that the risk of developing both hyperplastic and adenomatous polyps is positively associated with tobacco smoking but that there is no independent association with NAT2 status. Furthermore, we have uncovered only a suggestion that an interaction between smoking tobacco and NAT2 status is determining the risk of developing both adenomatous and hyperplastic polyps.

As with all case-control studies, there are issues of bias and confounding to consider. Clearly, with nonreportable conditions such as those considered here, there is the problem of who gets colonoscoped. The use of a colonoscopy-negative control group provides a comparison with a group with similar socioeconomic backgrounds who have passed through similar filters in the medical referral system. Furthermore, we have evidence that, for smoking and some other relevant risk factors (42),[3] similar estimates of risk are derived from comparisons with both the colonoscopy-negative control group and a second control group chosen from the community. This suggests that there are no major biases in the self-reported measures used here. The biological measures are not subject to such reporting biases; all genotypes were performed blind to case-control status. The estimates derived from simple age- and sex-adjusted models and multivariate-adjusted models were quite similar. There were no plausible unmeasured confounders.

Some small studies have suggested that NAT2 phenotype is itself a risk factor for colon cancer (44, 45) but others have not (46). Some have suggested that those with the rapid phenotype are perhaps especially at risk in association with higher meat consumption (47) but that, paradoxically, smoking may be a greater risk for those who are slow acetylators (48).

There have been few studies of adenomas (49, 50) and, as far as we know, none of hyperplastic polyps. Probst-Hensch *et al.* (50) found no association between risk of polyps and *NAT2* genotype but noted an elevated risk among white current smokers who were fast acetylators (OR = 2.25; 95% CI = 1.00–5.08) compared to slow acetylating nonsmokers as the reference group. For currently smoking slow acetylators, the OR was 1.68 (0.81–3.48); this finding does not itself suggest an interaction between smoking and NAT2 status, but the marginally higher risk in the fast compared to slow acetylators among the smokers is consistent with the observations we present here for large and multiple polyps. The most recent findings of Lin *et al.* (51) suggest that neither NAT1 nor NAT2 is associated with risk of adenomas.

Our findings are not entirely helpful in resolving the differences in the strength of the relationship between smoking and adenomas and smoking and colorectal cancer. If we had found clear evidence that the risk of adenomas is elevated in those smokers who are fast acetylators, then this association

[3] Unpublished data.

would be diluted out when colon cancer is examined in relation to smoking, both because there would be only a subset at risk and because there are other risk factors that influence the progression to cancer. However, the difference between fast and slow acetylators in their smoking-associated risk of adenomas is essentially null and probably does not explain the difference in adenomas and cancer, even when we note the impact of both smoking and NAT2 phenotype on large and multiple polyps.

The highest risk associated with smoking was seen for hyperplastic polyps; it is largely agreed that these lesions, even if they act as markers of a higher-risk colon (about which there is much disagreement), do not seem to be part of the pathway to cancer. So, although smoking may initiate a hyperplastic response in the colonic epithelium, many of the lesions generated may have no malignant potential.

Fully characterizing the interplay among various risk pathways and their relationships to the somatic lesions that characterize colonic neoplasia will take some time. It is possible that studies, even those of the size presented here, will not be sufficient for the purpose. From this study, risks of both hyperplastic and adenomatous polyps are increased in the presence of smoking but *NAT2* genotype is unrelated to risk and appears not to modify the risk associated with smoking.

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