Cigarette smoking, *N*-acetyltransferase genes and the risk of advanced colorectal adenoma

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Background: Cigarette use is associated with greater risk for colorectal adenoma, a colorectal cancer precursor. *N*-acetyltransferases, NAT1 and NAT2, are important enzymes involved in the metabolism of aromatic amine carcinogens present in cigarette smoke. Our interest is in the polymorphisms within the *NAT1* and *NAT2* genes that influence the tobacco–colorectal tumor relationship by impacting on the metabolic activation and detoxification of tobacco smoke-derived carcinogens. **Methods:** In the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial, we compared *NAT1* and *NAT2* gene variant distributions for 772 cases with left-sided advanced adenoma and 777 gender and age-matched controls. Individual *NAT1* and *NAT2* diplotypes were assigned and NAT2 acetylator phenotypes were derived. **Results:** Risks for advanced colorectal adenoma were significantly increased among recent smokers (current smokers or those who quit less than 10 years ago) (odds ratio [OR] = 2.3, 95% confidence interval [CI]: 1.7–3.1) and among those who smoked more than 20 cigarettes per day (OR = 1.7, 95% CI: 1.3–2.2), compared with nonsmokers. Risk decreased with increasing NAT2 phenotypic activity (0: slow, 1: intermediate, and 2: rapid) (OR trend: 0.8; 95% CI: 0.7–1.0, p-trend = 0.04) overall. When stratified by smoking status, significant phenotype-associated trends were observed among recent smokers (OR trend = 0.4, 95% CI: 0.3–0.7, p trend <0.001) (p-interaction = 0.02), but not among past or nonsmokers. Diplotypes most strongly associated with lower risks in smokers were *NAT2*4/*5B* (OR = 0.3, 95% CI: 0.1–0.8, p = 0.01) and *NAT2*4/*4* (OR = 0.2, 95% CI: 0.04–0.7, p = 0.02), categorized as intermediate and rapid acetylators, respectively. One *NAT1* diplotype, *NAT1*4/*10* (OR = 0.5, 95% CI: 0.3–0.9, p = 0.03), was also associated with a decreased risk in smokers. **Conclusions:** Our study indicated that *NAT2* gene variants associated with a slow acetylator phenotype were more susceptible to the effects of tobacco smoking with respect to adenoma risk, providing leads for disease prevention.

Keywords: cancer precursor, cigarette smoking, colorectal adenoma, *N*-acetyltransferases, NAT1, NAT2

Colorectal adenoma are recognized preneoplastic lesions; genetic and environmental factors contribute to the formation of these tumors, and their progression to colorectal carcinoma [1]. Cigarette smoking has consistently been associated with risk for colorectal adenoma [2,3]. Tobacco-associated adenoma risks have been particularly noted among recent smokers (i.e., current and who quit less than 10 years a go) [3]. Our interest is in genetic determinants that influence the tobacco–colorectal tumor relationship, by impacting on the metabolic activation and detoxification of tobacco smoke-derived carcinogens.

N-acetyltransferases are involved in the metabolism of aromatic amines derived from tobacco smoke [4]. NAT1 and NAT2 are important enzymes for the detoxification of certain aromatic amine carcinogens and activation of other amine proto-carcinogens to their ultimate carcinogenic form [5,6]. Humans have three *N*-acetyltransferase (*NAT*) loci: two expressed genes, *NAT1* and *NAT2*, and a pseudogene (*NATP*). *NAT1* is located at 8p21.3–23.1, 168 kilobase pairs (kb) upstream from *NAT2* [7]. *NAT1* [8] and *NAT2* [9] possess multiple exons and both encode proteins of 290 amino acids [9], with 81% nucleotide sequence identity in the coding regions [9]. Several *NAT2* single base pair substitutions, haplotypes and diplotypes (haplotype pairs) have been described [10,11] and related in human *in vitro* [12] and *in vivo* [13,14] studies to slow, intermediate, or rapid *N*-acetyltransferase enzymatic activity. While there is a clear consensus with respect to diplotype-imputed acetylator phenotypes for *NAT2* [15], such consensus has not yet been reached on deduced *NAT1* phenotypes.

Several studies have investigated the role of *NAT* genes in colorectal adenoma [16–20] and carcinoma [21–27]; however, the results are inconclusive, with sample size and design limitations possibly accounting for discrepancies between some of these published reports.

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We conducted a case–control study of advanced colorectal adenoma in a screening trial, investigating interrelationships of *NAT1* and *NAT2* and cigarette smoking on risk of this premalignant condition. We hypothesized that slow NAT2 acetylators were at an increased risk for colorectal adenoma because of their reduced capacity to detoxify (i.e., *N*-acetylate) the aromatic amine carcinogens present in cigarette smoke.

Materials & methods

The prostate, lung, colorectal & ovarian (PLCO) cancer trial

The Prostate, Lung, Colorectal, and Ovarian (PLCO) cancer screening trial, conducted by the National Cancer Institute (NCI), includes 77,483 individuals between the ages of 55–74, randomized to the screening arm (38,364 men, 39,119 women) and a similar number of non-screened controls, at ten US screening centers (Birmingham, AL, Denver, CO, Detroit, MI, Honolulu, HI, Marshfield, WI, Minneapolis, MN, Pittsburgh, PA, Salt Lake City, UT, St Louis, MO, and Washington, DC). Subjects eligible for the trial reported no prior personal history of prostate, lung, colorectal or ovarian cancer. Criteria for exclusion were:

- Current treatment for cancer (excluding basal cell and squamous cell skin cancer)
- Prior total colectomy, pneumonectomy, prostatectomy or bilateral oophorectomy (with bilateral oophorectomy dropped as an exclusion criterion beginning in 1996)
- Participation in another cancer screening or primary prevention study
- Recent use of finasteride (Proscar®, Merck & Co., Inc., NJ, USA) or tamoxifen (Nolvadex®, AstraZeneca Pharmaceuticals, DE, USA)

Beginning in April 1995, PLCO excluded men reporting more than one prostate-specific antigen (PSA) blood test and men and women reporting any lower gastrointestinal procedure (proctoscopy, sigmoidoscopy, barium enema or colonoscopy) within 3 years before study enrollment. The primary method for recruiting study subjects involved mailing informational brochures and letters of invitation to eligible persons identified on public, commercial, or screening center-specific mailing lists.

Physician and nonphysician examiners, all centrally registered, followed standardized procedures to perform and record results from an initial 60 cm flexible sigmoidoscopy examination, performed as soon after study entry as possible. Subjects with screen-detected abnormalities were referred to personal physicians for diagnostic follow-up. PLCO abstracted medical records pertaining to subsequent diagnostic work-ups. Information abstracted from medical records included the occurrence and date of follow-up flexible sigmoidoscopy and/or colonoscopy examinations, the anatomic location, size (visual estimate as recorded on clinical endoscopy reports), histology of polyps and masses observed on follow-up pathological examination and dates of diagnosis. Questionnaire data and biological samples were acquired from study participants. Participants provided written informed consent. The study was approved by the institutional review boards of the NCI and the ten screening centers.

Genetic studies population

Cases and controls for this study were drawn from screening-arm participants at the ten screening centers of the PLCO Trial between September 1993 and September 1999; to be eligible they had to fill out risk factor questionnaires, have a successful baseline sigmoidoscopy (insertion to at least 50 cm with >90% of mucosa visible or a suspect lesion identified), and provide a blood sample for use in etiological studies (n = 42,037). Of these participants, we excluded 4834 with a self-reported history of ulcerative colitis, Crohn's disease, familial polyposis, colorectal polyps, Gardner's syndrome, or cancer (except basal cell skin cancer). We randomly selected 772 of 1234 cases who had at least one advanced colorectal adenoma (adenoma ≥ 1 cm or containing high-grade dysplasia or villous, including tubulovillous, elements) in the distal colon (descending colon and sigmoid or rectum) detected at baseline screen and 777 of 26,651 control participants, with a negative baseline sigmoidoscopy screening (i.e., no polyp or other suspect lesion), frequency-matched to the cases by gender and ethnicity (white, black, Hispanic, other). Study subjects were predominantly white (94%). Among the 772 cases, 572 (74%) had a lesion ≥ 1 cm; 489 (63%) showed advanced histological features; and 245 (32%) had multiple adenoma. Also, 631 (82%) cases had an advanced adenoma of the descending colon or sigmoid and 232 (30%) had an advanced adenoma of the rectum, including subjects having lesions at both sites.

Single nucleotide polymorphism selection & genotyping assay

DNA samples were obtained from stored blood samples using Qiagen (Hilden, Germany)

standard protocols (QIAamp® DNA Blood Midi or Maxi kit [101]). A total of four *NAT1* single nucleotide polymorphisms (SNPs) (C-344T, A-40T, T1088A and C1095A) and six *NAT2* SNPs (C282T, T341C, C481T, G590A, A803G, and G857A) were tested by TaqMan® (Applied Biosystems, CA, USA). All assays were validated and optimized at the NCI Core Genotyping Facility [102]. Genotyping for *NAT1* and *NAT2* SNPs was successfully completed for over 90% of study subjects. For quality control, replicate blinded samples were included (10%) with the study samples, showing more than 99% concordance for all SNPs.

Questionnaire data

Participants completed a baseline general risk factor questionnaire and a 137-item food frequency questionnaire. Detailed information on smoking history was collected, including ages started and stopped, total years of use, amount usually used, and type of tobacco used (cigarettes, pipes and cigars). Subjects who did not smoke cigarettes for more than 6 months or did not smoke pipes or cigars for more than a year were considered to be nonsmokers. For the evaluation of risks in relation to time period of cigarette use, cigarette users were classified as former smokers (quit ≥10 years before enrollment) or current and recent smokers (quit <10 years before enrollment). For risk evaluation with respect to the amount of cigarette use, smokers were classified as light to moderate smokers (≤20 cigarettes per day) and heavy smokers (>20 cigarettes per day).

Acetylator phenotype deduction

NAT1 and *NAT2* SNP genotyping data were used to determine the haplotypes and diplotypes. Subjects were classified as homozygote for the most frequent allele (0), heterozygote (1) or homozygote for the least frequent allele (2). The frequencies of the common *NAT1* and *NAT2* haplotypes in the entire pool of white controls were determined in Haploview [28] using an accelerated expectation maximization (EM) algorithm [103]. Figure 1 depicts the relationship between alleles at *NAT* SNPs and haplotype assignments. The assignment of *NAT* haplotypes based on alleles at *NAT* SNP loci have been reviewed extensively [11,15]. Individual *NAT* haplotypes and diplotypes were determined at the University of Louisville [29] and NAT2 acetylator phenotypes, associated with individual *NAT2* diplotype assignments, were then deduced based on previous *in vitro* [12] and *in vivo* [13,14] studies. Classification of acetylator phenotypes based on NAT2 diplotypes has been extensively reviewed elsewhere [15]. There is consensus on deduced NAT2 phenotypes (for brevity, subsequently referred to as 'phenotype') among the multiple laboratories that have used recombinant expressions of *NAT2* alleles to determine their phenotype [15]. However, such clear consensus has not yet been reached on deduced NAT1 phenotypes [21]; therefore, we did not attempt to assign phenotypes for the *NAT1* diplotypes in our study.

Statistical analysis

Allele frequencies at the *NAT1* and *NAT2* SNP loci among controls in our sample were similar to those reported for the US population [102]. Departure from Hardy–Weinberg equilibrium was assessed by the asymptotic Pearson's χ^2 test. The Haploview [28] program was used to assess the degree of linkage disequilibrium (LD) between the SNPs.

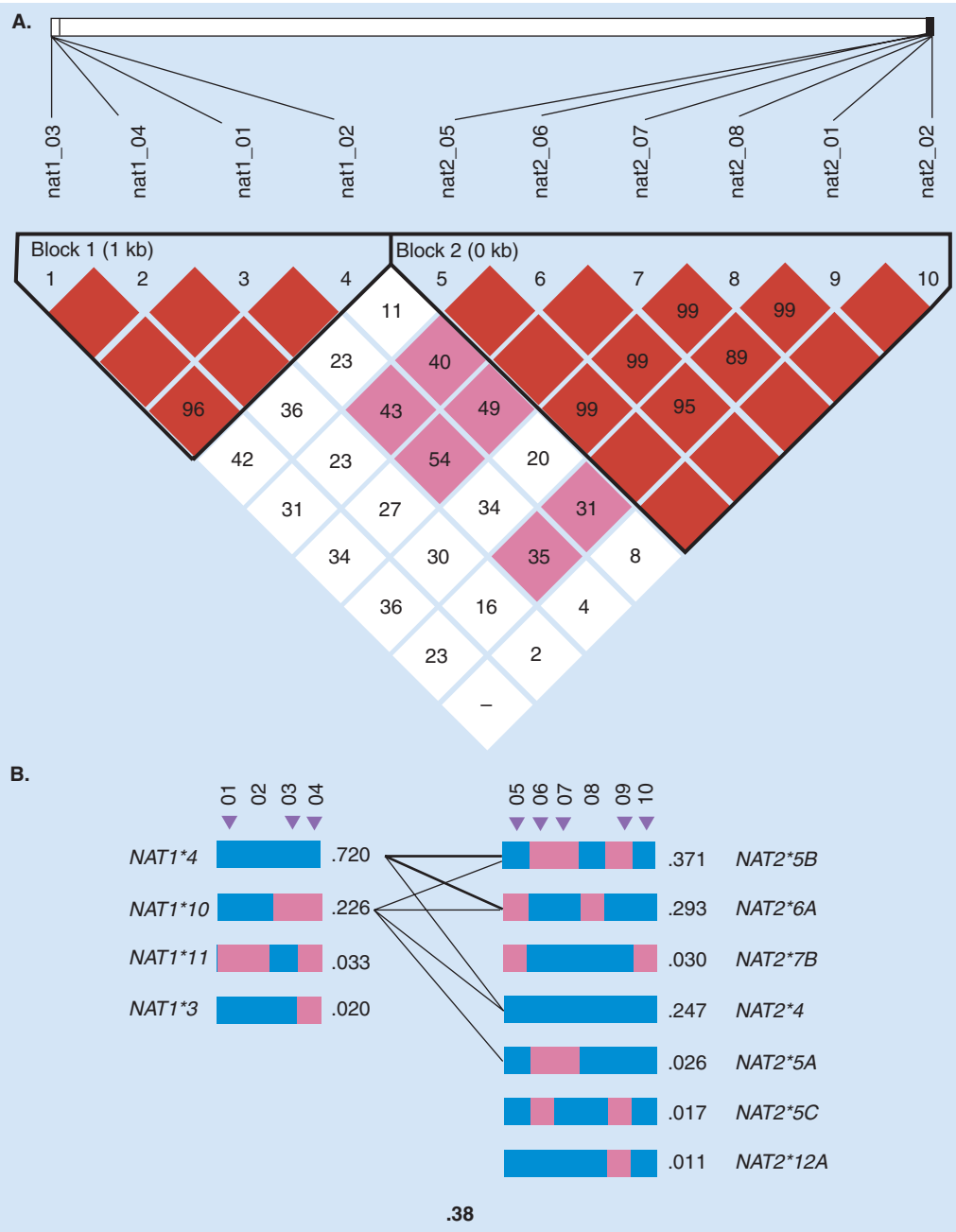
Odds ratios (OR) and 95% confidence intervals (CI) were obtained using unconditional logistic regression, adjusting for gender, ethnicity and age (55–59, 60–64, 65–69, 70–74). Multiplicative interactions between smoking and gene variants were tested using the Wald test for the interaction term in the logistic regression model. An omnibus test for multiplicative interaction between *NAT1* and *NAT2* diplotypes was performed using a likelihood ratio χ^2 statistic.

Results

The matching factors of gender and race were similarly distributed in cases and controls; however, cases were older than controls and were more likely to have reported having a first-degree relative with colorectal cancer. Cases also had less education, compared with the controls, and had a slightly higher body mass index (BMI) at the time of interview. The differences between cases and controls with respect to these variables were not statistically significant (Table 1).

Smokers had a significantly greater risk of advanced colorectal adenoma compared with nonsmokers, with the greatest risks seen in recent and heavy smokers (Table 2). Characteristics of the four *NAT1* and six *NAT2* polymorphic loci are described in Table 3. The haplotypes with a frequency greater than 1% among controls are shown in Figure 1. There was a strong LD pattern within each gene, while there was little LD between *NAT1* and *NAT2* (Figure 1). All SNPs evaluated appeared to be in Hardy–Weinberg equilibrium (Table 3).

Figure 1. Linkage disequilibrium map of *NAT1* and *NAT2* single nucleotide polymorphisms and haplotype frequencies among white controls.



A. Sequence of SNPs along the top represents their relative order on the chromosome and within the *NAT1* and *NAT2* genes, 5′ to 3′. The red squares indicate a statistically significant (LOD>2) allelic association between the pair of SNPs. The thick line indicates SNPs in linkage disequilibrium (LD) using the 'spine of LD' algorithm in Haploview. White and pink squares indicate lack of linkage between the SNPs. Numbers inside the square are the value of D′ multiplied by 100. D′ = 1 indicates complete linkage disequilibrium while D′ = 0 indicates complete linkage equilibrium.

B. The blue bar represents the most common allele and the pink bar represents the variant allele. Marker numbers are shown across the top for each gene with the tag SNPs identified by a triangular pointer. Frequencies are shown besides each haplotype for the entire pool of white controls. Thick lines between haplotypes signify the most common crossings from one block to the next; thinner lines signify the less common crossings. The number beneath the crossing line is the multilocus D′ (0.38), which is a measure of LD between two blocks.

LD: Linkage disequilibrium; NAT1: *N*-acetyltransferase 1; NAT2: *N*-acetyltransferase 2; SNP: Single nucleotide polymorphism.

Table 1. Characteristics of study subjects.

Characteristics	Cases n = 772		Controls n = 777	
	n	%	n	%
Gender				
Male	535	69.3	536	69.0
Female	237	30.7	241	31.0
Race				
White	725	93.9	729	93.8
Black	22	2.8	23	3.0
Other*	25	3.2	25	3.2
Age category at interview (years)				
55–59	257	33.3	363	46.7
60–64	244	31.6	200	25.7
65–69	172	22.3	140	18.0
70–74	99	12.8	74	9.5
First degree family history of colorectal cancer				
Yes	97	12.6	70	9.0
No	675	87.4	707	91.0
Education				
≤11 years	72	9.3	50	6.4
12 year/high school equivalent	191	24.7	176	22.7
Some college	276	35.7	247	31.8
College and above	232	30.0	303	39.0
	(1 unanswered)		(1 unanswered)	
Body mass index at interview (kg/m²)				
≤18.5	5	0.6	2	0.3
>18.5 ≤25	200	25.9	219	28.2
>25 ≤30	349	45.2	357	45.9
>30	215	27.8	188	24.2
	(3 unanswered)		(11 unanswered)	

**American Indian/Alaskan Native (two controls, two cases); Pacific Islander (three controls & three cases); Asian (13 controls & 13 cases); Hispanic (seven controls & seven cases).*

NAT2 & colorectal adenoma

Risks of advanced colorectal adenoma decreased with increasing NAT2 phenotype activity, due to trends among recent smokers, but not among past smokers or nonsmokers (Table 4). The test of statistical interaction between NAT2 metabolic phenotype was significant with recency of smoking (p = 0.02) (Table 4), but not with amount of cigarettes smoked (p = 0.1) (data not shown). Excluding nonwhite participants from these analyses did not change the odds ratio estimates.

The *NAT2* diplotype most strongly associated with a lower risk among all subjects was *NAT2*4/*5B* (intermediate acetylator phenotype), using *NAT2*5B/*5B* (most common homozygote slow acetylator-associated diplotype) as the reference category (Table 5). In recent smokers only, significantly decreased risks of colorectal adenoma were found with *NAT2*4/*5B* and *NAT2*4/*4* (rapid acetylator phenotype) (Table 5). The tests for statistical interaction between *NAT2*4/*4* and recency of smoking were significant (p = 0.02) (data not shown). Haplotype analysis, assuming an additive mode of effect [30], tended to confirm these findings, with reduced risks associated with *NAT2*4* haplotype only in recent smokers (OR = 0.6, 95% CI: 0.4–1.0, p = 0.05) (data not shown).

NAT1 & colorectal adenoma

*NAT1*4/*10* was associated with a decreased risk of advanced colorectal adenoma among recent

Table 2. Cigarette use and the risk of advanced colorectal adenoma.

	Cases (n = 772*)		Controls (n = 777*)		Risk‡ OR (95% CI)
	n	%	n	%	
Smoking status					
Nonsmokers	260	33.8	315	40.9	1.0
Ever cigarette smokers	473	61.3	419	53.9	1.4 (1.2–1.8)
Pipe/cigar smokers	39	5.1	43	5.6	1.2 (0.7–1.9)
Time period					
Past smokers (quit ≥10 years)	272	35.4	302	39.2	1.1 (0.9–1.4)
Recent smokers (quit <10 years)	198	25.7	111	14.1	2.3 (1.7–3.1)
Trend statistic§					1.4 (1.2–1.6)
p-trend					<0.001
Amount					
Light smokers (≤20 cigarettes/day)	261	33.9	254	32.9	1.3 (1.0–1.6)
Heavy smokers (>20 cigarettes/day)	212	27.6	165	21.4	1.7 (1.3–2.2)
Trend statistic§					1.3 (1.1–1.5)
p-trend					<0.001

**Numbers in variable categories may not add up to this total due to missing values of individual variables.*

‡Adjusted for gender, age and race.

§Reference group: nonsmokers.

CI: Confidence interval; OR: Odds ratio.

smokers, compared with *NAT1*4/*4* (most common diplotype); weaker effects were noted among all subjects (Table 6). Tests for statistical interactions of *NAT1* diplotypes with recency or amount of cigarette use were not statistically significant (data not shown). Haplotype analysis, assuming an additive mode of effect [28], did not yield significant associations between any *NAT1* haplotype and risk of advanced adenoma (data not shown).

Joint-effects analysis

Examining the joint effects of *NAT1* and *NAT2* in recent smokers, decreased risks of advanced adenoma were associated with the *NAT2*4/*5B* and *NAT1*4/*10* diplotype combination (OR = 0.05, 95% CI: 0.01–0.28, p = 0.001) and the *NAT2*4/*4* and *NAT1*4/*10* combination (OR = 0.1, 95% CI: 0.01–0.8, p = 0.03), compared with the most common reference category (*NAT2*5B/*5B* and *NAT1*4/*4* diplotype combination), in a manner consistent with the multiplicative model (p-value for the omnibus test of multiplicative interaction between *NAT1* and *NAT2* diplotypes was 0.29) (data not shown).

Discussion

Our study of 772 cases with left-sided advanced colorectal adenoma and 777 gender and age-matched controls showed that *NAT2* gene variants related to intermediate and rapid acetylator activity were associated with decreased risks for advanced colorectal adenoma, particularly among recent smokers, and especially for carriers of the *NAT2*4* haplotype. The findings that slow NAT2 acetylators who smoked carried the greatest risk for advanced colorectal adenoma suggests a role for NAT2 in the detoxification of aromatic amines found in cigarette smoke, rendering these compounds less carcinogenic for colonic epithelial tissue. In our study, we also found risk differentials with respect to *NAT1* gene variants – phenotype relationships for this and other *NAT1* gene variants have not been clearly elucidated. Furthermore, with the *NAT1* SNPs analyzed in our study, we were not able to distinguish between *NAT1*10* and *NAT1*14A* haplotypes. However, based on the relative frequencies of *NAT1*10* and *NAT1*14A* haplotypes (approximately 20% vs 2%, respectively) reported among the general US population [31,32], overestimation of *NAT1*10* haplotype in our study would be approximately 10%.

Tiemersma and colleagues reported marginal evidence of an increased risk of colorectal adenoma among smokers who were carriers of genotypes associated with combined NAT2 slow and sulfotransferase family, cytosolic 1A, phenol-preferring, member 1 (SULT1A1) rapid enzymatic

Table 3. *NAT1* and *NAT2* genetic polymorphisms analyzed.

Nucleotide substitution	Location	Type of mutation	Amino acid substitution	Minor allele frequency among controls*	HWE (p-values)*
NAT2					
[NAT2_05] C282T‡	Exon 2	Silent	NA	0.32	0.29
[NAT2_06] T341C‡	Exon 2	Missense	I114T	0.43	0.83
[NAT2_07] C481T‡	Exon 2	Silent	NA	0.41	0.84
[NAT2_08] G590A‡	Exon 2	Missense	R197Q	0.29	0.09
[NAT2_01] A803G‡	Exon2	Missense	K268R	0.41	0.95
[NAT2_02] G857A‡	Exon 2	Missense	G286E	0.03	0.57
NAT1					
[NAT1_03] C-344T‡	5' UTR	(Noncoding)	NA	0.03	0.40
[NAT1_04] A-40T‡	5' UTR	(Noncoding)	NA	0.03	0.60
[NAT1_01] T1088A‡	3' UTR	(Noncoding)	NA	0.23	0.21
[NAT1_02] C1095A‡	3' UTR	(Noncoding)	NA	0.26	0.86

*Based on analyses in white controls.

‡Minor allele.

HWE: Hardy-Weinberg equilibrium; NA: Not applicable; NAT: N-acetyltransferase; UTR: Untranslated region.

activities [16]. Potter and colleagues found no main effect of *NAT2* and no statistical interaction between NAT2 acetylator status and smoking in the risk for colorectal adenoma [17]. Probst-Hensch and colleagues also reported an overall null effect of *NAT2* genotypes and phenotypes on the prevalence of colorectal adenoma; however, smoking was not considered [18].

Several studies have investigated the association between *NAT2* acetylator genotypes and/or phenotypes and colorectal cancer, as reviewed in 2000 [21], and as more recently reported [22–25,27]. Some findings suggest that *NAT2* gene variants associated with more rapid acetylation activity may be related to increased risk of colorectal cancer [23–26]; however, the overall evaluation shows no consistent association between acetylator phenotype or genotype and colorectal cancer [21]. Interpretation of these colorectal cancer studies is further complicated by the uncertainty of an association between smoking and risk for colorectal cancer [21]. A study reporting association between smoking and risk of rectal cancer among men did not find a main or modifying effect of NAT2 imputed phenotype [27].

The comparison of studies on NAT2 and colorectal tumors is further complicated by the range of assays that have been used. Early investigations relied on drug probes, which have the advantage of integrating genetic complexity, but may be limited in their specific relevance for metabolism of the carcinogenic agents of interest in colorectal carcinogenesis. Later on, genotype probes were used, but until recently the specific markers studied and their categorization as phenotype analogs had often varied between studies. As our understanding of gene variation and phenotype has developed [11,33], more recent studies have tended to capture the major determinants of variation, and a consensus has been reached on NAT2 deduced phenotypes [15]. Another potential cause for discrepancies between the previous studies could be the variation in the composition of cigarettes as reviewed previously [34,35].

Our findings suggest that recent smoking is related to adenoma risk, consistent with a relatively short time span from early tobacco-related initiation to the development of an advanced adenomatous lesion [24,36]. Cancers deriving from adenomas likely take more than a decade to evolve, consistent with indications that tobacco-associated colorectal cancer is potentially primarily related to smoking several decades in the past [2]. Our finding that *NAT2* gene variants modified adenoma risk only in recent smokers is consistent with a genetic impact during the time period when its effect would be relevant for tumor formation. Future studies of NAT2 effects on colorectal cancer related to smoking several decades in the past would help to fill out this picture.

The dual role of NAT2 in both detoxification and activation of various amine carcinogens makes the interpretation of colorectal adenoma and colorectal cancer studies more complex. Theoretically, if aromatic amines are the relevant colorectal carcinogens, then NAT2 slow acetylators would be at increased risk. In contrast, rapid acetylators would be at increased risks if the

Table 4. Risk of colorectal adenoma, deduced NAT2 metabolic phenotype, and recency of smoking.

Phenotype	All subjects			Nonsmokers			Past smokers (quit ≥10 years ago)			Recent smokers (quit <10 years ago)			Trend statistics‡	
	CON (%)	CAS (%)	OR* (95% CI)	CON (%)	CAS (%)	OR* (95% CI)	CON (%)	CAS (%)	OR* (95% CI)	CON (%)	CAS (%)	OR* (95% CI)	OR* (95% CI)	p trend
Slow	376 (54.3)	413 (60.3)	1.0	158 (22.8)	140 (20.4)	1.0	153 (22.1)	134 (19.6)	1.0 (0.7–1.4)	42 (6.1)	115 (16.8)	3.4 (2.2–5.2)	1.7 (1.4–2.0)	*<0.001*
Intermed.	271 (39.1)	234 (34.1)	0.8 (0.6–1.0)	108 (15.6)	71 (10.4)	0.7 (0.5–1.1)	98 (14.1)	96 (14.0)	1.2 (0.8–1.7)	43 (6.2)	56 (8.2)	1.7 (1.0–2.7)	1.5 (1.2–1.9)	*0.002*
Rapid	46 (6.6)	38 (5.6)	0.8 (0.5–1.2)	16 (2.3)	21 (3.1)	1.4 (0.7–2.9)	18 (2.6)	12 (1.7)	0.8 (0.4–1.8)	9 (1.3)	4	0.4 (0.1–1.5)	0.5 (0.3–1.0)	*0.05*
p trend§			*0.04*			0.7			0.8			*<0.001*		

p-interaction¶ 0.02.

**Adjusted for gender, age and race.*

‡*Trend statistics within each phenotype subgroup (Non-Smokers: 0, Past Smokers: 1, Recent Smokers (current or quit <10 years ago): 2, treated as a continuous variable).*

§*Trend statistics within all subjects and within each smoking variable subgroup (Slow: 0, Intermediate: 1, Rapid: 2, treated as a continuous variable).*

¶*Multiplicative interaction tested using the Wald test for the interaction term in the logistic regression model.*

CAS: Case; CI: Confidence interval; CON: Control; NAT: N-*acetyltransferase; OR: Odds ratio.*

carcinogens involved are the heterocyclic amines (i.e., from diet), which do not undergo *N*-acetylation very well, but rather undergo *O*-acetylation, which is an activation mechanism. Sinha and colleagues recently reported a positive association between high risk of colorectal adenoma and red meat intake [37].

Two smaller studies showed no main effect association between *NAT1* and colorectal adenoma; interactions with tobacco use were not considered [19,20]. Several colorectal cancer studies also investigated the role of *NAT1* genotypes [21], with one reporting an increased risk in association with the *NAT1*10* haplotype [37]; however, most of the studies of *NAT1* and cancer did not consider potential interrelationships with smoking.

Our study is the first to report on the effect of the combination of *NAT1* and *NAT2* diplotypes on the risk of colorectal adenoma, with *NAT1*4/*10/NAT2*5B/*6A* and *NAT1*4/*10/NAT2*4/*4* diplotypes being associated with decreased risks in recent smokers. Several studies investigated *NAT1* and *NAT2* combined effects on colorectal cancer [21]; however, no clear patterns were identified.

Conclusion & outlook

In summary, our large study in a well characterized population showed a decreased risk for advanced colorectal adenoma among individuals with intermediate to rapid genotype-based imputed NAT2 enzymatic phenotypes, and a significant statistical interaction with recent cigarette use. These results suggest a role for NAT2 in the detoxification of aromatic amines found in cigarette smoke. Although, our results also indicate a protective effect conferred by a *NAT1* diplotype (*NAT1*4/*10*), the functional significance of the *NAT1* variants remains unclear. Thus, in our study, carriers of certain *NAT2* gene variants associated with slow acetylator phenotype were more susceptible to the effects of tobacco smoking with respect to adenoma risk, providing leads for disease prevention.

As our understanding of genetic polymorphisms that influence the activity and expression of *N*-acetyltransferases and other proteins involved in the metabolic pathway of aromatic amine carcinogens evolves, we will be able to determine the impact of these polymorphisms on the risk of colorectal adenoma and cancer in the presence of other genetic and environmental modifiers of risk, leading in the direction of genomic-based personalized medicine and public health.

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Highlights

- Risks of advanced colorectal adenoma decreased with increasing *N*-acetyltransferase 2 (NAT2) phenotypic activity (0: slow, 1: intermediate, 2: rapid) (odds ratio [OR] trend = 0.8; 95% confidence interval [CI]: 0.7–1.0; p-trend = 0.04).
- NAT2 phenotype-associated trends were seen among recent smokers (current smokers or who quit smoking <10 years a go) (OR trend = 0.4; 95% CI: 0.3–0.7; p-trend <0.001), but not among past smokers (quit >10 years a go) (OR trend = 1.0; 95% CI: 0.8–1.4; p-trend = 0.8) or nonsmokers (OR trend = 0.9; 95% CI: 0.7–1.3; p-trend = 0.7).
- There was a significant statistical interaction between NAT2 acetylation phenotype-associated advanced colorectal adenoma risk and the recency of cigarette use (p = 0.02).
- Our results indicated a possible role for NAT2 in the detoxification of aromatic amines found in cigarette smoke.
- Our findings may provide leads for the prevention of colorectal adenoma and cancer.

Table 5. *NAT2* diplotypes, smoking and advanced adenoma risk.

***NAT2* diplotypes**	**Cases (n = 772)**		**Controls (n = 777)**		**All subjects**		**Recent smokers**		**Acetylator phenotype‡**
	n	**%**	**n**	**%**	**OR* (95% CI)**	**p-value**	**OR* (95% CI)**	**p-value**	
*NAT2*5B/*5B*	121	15.7	98	12.6	1.0		1.0		S
*NAT2*5B/*6A*	155	20.1	124	16.0	1.0 (0.7–1.4)	0.96	1.2 (0.4–3.3)	0.69	S
*NAT2*6A/*6A*	59	7.6	73	9.4	0.6 (0.4–1.0)	0.06	0.3 (0.1–1.0)	0.05	S
*NAT2*5A/*5B*	16	2.1	18	2.3	0.7 (0.4–1.5)	0.40	2.3 (0.2–21.1)	0.47	S
*NAT2*5B/*7B*	16	2.1	17	2.2	0.8 (0.4–1.7)	0.57	0.4 (0.08–1.5)	0.17	S
Other§	46	6.0	46	5.9	0.9 (0.5–1.5)	0.63	1.2 (0.3–5.0)	0.76	S
*NAT2*4/*5B*	109	14.1	138	17.8	0.6 (0.4–0.9)	0.02	0.3 (0.1–0.8)	0.01	I
*NAT2*4/*6A*	86	11.1	104	13.4	0.7 (0.5–1.0)	0.07	0.5 (0.2–1.3)	0.16	I
Other¶	39	5.1	29	3.7	1.1 (0.6–2.1)	0.68	0.6 (0.1–2.1)	0.39	I
*NAT2*4/*4*	37	4.8	41	5.3	0.7 (0.4–1.3)	0.28	0.2 (0.04–0.7)	0.02	R
Other#	1	0.1	5	0.6	NA	NA	NA	NA	R
Undeterminable	87	11.3	83	10.7					
Potential novel**	0	0.0	1	0.1					

**Adjusted for gender, age and race.*

‡S: Slow; I: Intermediate; R: Rapid.

*§Other diplotypes with slow acetylator phenotypes with <2% frequency each among the controls: NAT2*6A/*7B, NAT2*5A/*6A, NAT2*5A/*7B, NAT2*5B/*5C, NAT2*5C/*6A, NAT2*5A/*5C, NAT2*5C/*7B, NAT2*6A/*6B, NAT2*7B/*7B.*

*¶Other diplotypes with intermediate acetylator phenotypes with <2% frequency each among the controls: NAT2*4/*7B, NAT2*4/*5A, NAT2*5B/*12A, NAT2*6A/*12A, NAT2*5B/*13, NAT2*4/*5C, NAT2*6A/*13, NAT2*5C/*13, NAT2*7B/*12A.*

*#Other diplotypes with rapid acetylator phenotypes with <2% frequency each among the controls: NAT2*4/*12A, NAT2*12A/*12A.*

***Potential novel genotype: [NAT2_05]CC, [NAT2_06]CC, [NAT2_07]TT, [NAT2_08]GA, [NAT2_01]GG, [NAT2_02]GG.*

CI: Confidence interval; NA: Not applicable; NAT: N-acetyltransferase; OR: Odds ratio.

Table 6. *NAT1* diplotypes, recency of smoking and advanced adenoma risk.

NAT1 diplotypes	Cases (n = 772)		Controls (n = 777)		All subjects		Recent smokers	
	N	%	N	%	OR* (95% CI)	p-value	OR* (95% CI)	p-value
*NAT1*4/*4*	388	50.3	364	46.8	1.0		1.0	
*NAT1*4/*10*	190	24.6	224	28.8	0.8 (0.6–1.0)	0.09	0.5 (0.3–0.9)	0.03
*NAT1*10/*10*	33	4.3	34	4.4	0.9 (0.5–1.5)	0.66	0.5 (0.2–1.4)	0.20
*NAT1*4/*3*	23	3.0	14	1.8	1.5 (0.8–3.1)	0.20	2.3 (0.3–20.6)	0.45
Other‡	25	3.2	20	2.6	0.8 (0.5–1.2)	0.30	0.4 (0.2–1.0)	0.05
Undeterminable	112	14.5	120	15.4				
Uncommon diplotype§	1	0.1	1	0.1				

**Adjusted for gender, age and race. ‡Other diplotypes (<1.8% frequency): NAT1*3/*10, NAT1*10/*11A, NAT1*3/*11A, NAT1*11A/*11A, NAT1*3/*3. §Uncommon diplotype combination: [NAT1_03]CC, [NAT1_04]AA, [NAT1_01]TA, [NAT1_02]CC.*
CI: Confidence interval; NAT: N-*acetyltransferase; OR: Odds ratio.*

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Kronbeorg O: Colon polyps and cancer. *Endoscopy* 36, 3–7 (2004).
2. Giovannucci E: An updated review of the epidemiological evidence that cigarette smoking increases the risk of colorectal cancer. *Cancer Epidemiol. Biomarkers Prev.* 10, 725–731 (2001).
3. Ji BT, Weissfeld J, Chow W-H, Huang W-Y, Schoen R, Hayes RB: Tobacco smoking and colorectal hyperplastic and adenomatous polyps. *Cancer Epidemiol. Biomarkers Prev.* 15, 897–901 (2006).
4. Lang NP, Chu DZ, Hunter CF, Kendall DC, Flammang TJ, Kadlubar FF: Role of aromatic amine acetyltransferase in human colorectal cancer. *Arch. Surg.* 121, 1259–1261 (1986).
5. Hein DW, Doll MA, Rustan TD *et al.*: Metabolic activation and deactivation of arylamine carcinogens by recombinant human NAT1 and polymorphic NAT2 acetyltransferases. *Carcinogenesis* 14, 1633–1638 (1993).
6. Hein DW, Doll MA, Rustan TD, Ferguson RJ: Metabolic activation of *N*-hydroxylamines and *N*-hydroxylamides by 16 recombinant human NAT2 allozymes: effects of 7 specific NAT2 nucleic acid substitutions. *Cancer Res.* 55, 3531–3536 (1995).
7. Hickman D, Risch A, Buckle V *et al.*: Chromosomal localization of human genes for arylamine *N*-acetyltransferase. *Biochem. J.* 297, 441–445 (1994).
8. Husain A, Barker D, States C, Doll M, Hein DW: Identification of the major promoter and noncoding exons of the human arylamine *N*-acetyltransferase 1 gene (*NAT1*). *Pharmacogenetics* 14, 397–406 (2004).
9. Blum M, Grant DM, McBride W, Heim M, Meyer UA: Human arylamine *N*-acetyltransferase genes: isolation, chromosomal localization, and functional expression. *DNA Cell Biol.* 9, 193–203 (1990).
10. Vatsis KP, Weber WW, Bell DA *et al.*: Nomenclature for *N*-acetyltransferases. *Pharmacogenetics* 5, 1–7 (1995).
11. Hein DW, Doll M, Fretland A *et al.*: Molecular genetics and epidemiology of the *NAT1* and *NAT2* acetylation polymorphism. *Cancer Epidemiol. Biomarkers Prev.* 9, 29–42 (2000).

•• Excellent review of *N*-acetyltransferase (*NAT*) polymorphisms, haplotype and phenotype designations.

12. Kirlin WG, Ogolla F, Andrews AF *et al.*: Acetylator genotype-dependent expression of arylamine *N*-acetyltransferases in human colon cytosol from noncancer and colorectal cancer patients. *Cancer Res.* 51, 549–555 (1991).
13. Smith CA, Wadelius M, Gough AC, Harrison DJ, Wolf CR, Rane A: A simplified assay for the arylamine *N*-acetyltransferase 2 polymorphism validated by phenotyping with isoniazid. *J. Med. Genet.* 34, 758–760 (1997).
14. O'Neill WM, Drobitch RK, MacArthur RD *et al.*: Acetylator phenotype and genotype in patients infected with HIV. Discordance between methods for phenotype determination and genotype. *Pharmacogenetics* 10, 171–182 (2000).
15. Hein DW: *N*-acetyltransferase 2 genetic polymorphism: effects of carcinogen and haplotype on urinary bladder cancer risk. *Oncogene* 25, 1649–1658 (2006).
16. Tiemersma E, Bunschoten A, Kok F, Glatt H, de Boer S, Kampman E: Effect of *SULT1A1* and *NAT2* genetic polymorphism on the association between cigarette smoking and colorectal adenomas. *Int. J. Cancer* 108, 97–103 (2004).
17. Potter J, Bigler J, Fosdick L *et al.*: Colorectal adenomatous and hyperplastic polyps: smoking and *N*-acetyltransferase 2 polymorphisms. *Cancer Epidemiol. Biomarkers Prev.* 8, 69–75 (1999).
18. Probst-Hensch N, Haile R, Ingles S *et al.*: Acetylation polymorphism and prevalence of colorectal adenomas. *Cancer Res.* 55, 2017–2020 (1995).
19. Lin H, Probst-Hensch N, Hughes N *et al.*: Variants of *N*-acetyltransferase *NAT1* and a case–control study of colorectal adenomas. *Pharmacogenetics* 8, 269–281 (1998).
20. Probst-Hensch N, Haile R, Li D *et al.*: Lack of association between the polyadenylation polymorphism in the *NAT1* gene and colorectal adenomas. *Carcinogenesis* 17, 2125–2129 (1996).
21. Brockton N, Little J, Sharp L, Cotton SC: *N*-acetyltransferase polymorphisms and colorectal cancer: A HuGE Review. *Am. J. Epidemiol.* 151, 846–861 (2000).

•• Excellent review of the data on associations between *NAT* genotypes and phenotypes and risks of colorectal adenoma and cancer.

22. Hein DW: Molecular genetics and function of *NAT1* and *NAT2*: role in aromatic amine metabolism and carcinogenesis. *Mutat. Res.* 506–507, 65–77 (2002).

•• Excellent review of *NAT* polymorphisms and their roles in aromatic amine metabolism.

23. Van der Hel O, Bueno de Mesquita H, Sandkuijl L *et al*.: Rapid *N*-acetyltransferase 2 imputed phenotype and smoking may increase risk of colorectal cancer in women (Netherlands). *Cancer Causes Control* 14, 293–298 (2003).
24. Lilla C, Verla-Tebit E, Risch A *et al*.: Effect of *NAT1* and *NAT2* genetic polymorphisms on colorectal cancer risk associated with exposure to tobacco smoke and meat consumption. *Cancer Epidemiol. Biomarkers Prev.* 15, 99–107 (2006).
25. Ilett KF, David BM, Detchon P, Castleden WM, Kwa R: Acetylation phenotype in colorectal carcinoma. *Cancer Res.* 47, 1466–1469 (1987).
26. Gil J, Lechner M: Increased frequency of wild-type arylamine-*N*-acetyltransferase allele *NAT2*4* homozygotes in Portuguese patients with colorectal cancer. *Carcinogenesis* 19, 37–41 (1998).
27. Slattery ML, Edwards S, Curtin K, Schaffer D, Neuhausen S: Associations between smoking, passive smoking, *GSTM-1, NAT2*, and rectal cancer. *Cancer Epidemiol. Biomarkers Prev.* 12, 882–889 (2003).
28. Barrett JC, Fry B, Maller J, Daly MJ: Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21, 263–265 (2004).
29. Martini BD, Doll MA, Hein DW: Development of a computer program for translation of human *N*-acetyltransferase-1 and -2 SNP data into genotype and phenotype: Application to tobacco use risk assessment. *Tobacco Induced Diseases* 2, 51 (2004).
30. Wallenstein S, Hodge SE, and Weston A: Logistic regression model for analyzing extended haplotype data. *Genet. Epidemiol.* 15, 173–183 (1998).
31. Morton L, Schenk M, Hein DW *et al*.: Genetic variation in *N*-acetyltransferase 1 (*NAT1*) and 2 (*NAT2*) and risk of non-Hodgkin lymphoma. *Pharmacogenet. Genomics* 16, 537–545 (2006).
32. Doll M, Hein DW: Rapid genotype method to distinguish frequent and/or functional polymorphisms in human *N*-acetyltransferase-1. *Anal. Biochem.* 301, 328–332 (2002).
33. Hein DW, Ferguson RJ, Doll MA, Rustan TD, Gray K: Molecular genetics of human polymorphic *N*-acetyltransferase: enzymatic analysis of 15 recombinant human wild-type, mutant, and chimeric NAT2 allozymes. *Hum. Mol. Genet.* 3, 729–734 (1994).
34. Vineis P, Alavanja M, Buffler P *et al*.: Tobacco and cancer: recent epidemiological evidence. *J. Natl Cancer Inst.* 96, 99–106 (2004).
- Good review of epidemiologic evidence regarding association between tobacco and cancer risk.
35. Hoffman D, Hoffman I, El-Bayoumy K: The less harmful cigarette: a controversial issue. *Chem. Res. Toxicol.* 14, 767–790 (2001).
36. Schoen RE: Surveillance after positive and negative colonoscopy examinations: issues, yields, and use. *Am. J. Gasteroenterology* 98, 1237–1246 (2003).
37. Sinha R, Peters U, Cross AJ *et al*.: Meat, meat cooking methods and preservation, and risk for colorectal adenoma. *Cancer Res.* 65, 8034–8041 (2005).
38. Bell DA, Stephens EA, Castranio T *et al*.: Polyadenylation polymorphism in the acetyltransferase 1 gene (*NAT1*) increases risk of colorectal cancer. *Cancer Res.* 55, 3537–3542 (1995).

Websites

101. Qiagen Ltd. www1.qiagen.com/default.aspx
102. National Cancer Institute: Cancer Genome Anatomy Project, SNP500Cancer Database. http://snp500cancer.nci.nih.gov
103. Haploview, Broad Institute of MIT and Harvard. www.broad.mit.edu/mpg/haploview