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Acetylation Polymorphism and Prevalence of Colorectal Adenomas[1]

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Abstract

Polymorphic *N*-acetyltransferase (NAT2), an enzyme present in the colon, may affect incidence of colon cancer. Individuals with *NAT2* fast acetylator genotypes may have higher colon cancer risks due to faster conversion of certain carcinogens to mutagens. We determined *NAT2* genotypes in 447 subjects with distal colon adenomas and in 487 controls. No significant increase in adenoma prevalence among fast acetylators was observed. However, there was a suggestion of ethnic differences in *NAT2* effects. For example, white fast acetylators potentially had slightly increased risks for adenomas (odds ratio, 1.29; 95% confidence interval, 0.90–1.84), whereas fast acetylation was potentially protective among blacks (odds ratio, 0.64; 95% confidence interval, 0.32–1.28). The apparent difference between blacks and whites may simply reflect random variation around an overall null effect, or it could represent a real difference. There was preliminary evidence for a possible interaction between *NAT2* and the glutathione transferase M1 null genotype. Smokers' adenoma prevalence was 10-fold higher for fast acetylators with the null genotype compared to slow acetylators without the null genotype. Large, multiethnic populations and analysis of combinations of genes for carcinogen metabolism may be needed to further assess the role of *NAT2* in colorectal tumorigenesis.

Introduction

The classical acetylation polymorphism results from different forms of the NAT2[3] enzyme, leading to fast or slow acetylation of arylamine and hydrazine drugs and carcinogens. Roughly one half of all whites are slow acetylators due to single-base mutations in both copies of their *NAT2* genes (1–4). However, the prevalence and distribution of these mutations vary by ethnicity. Aromatic amines from cigarette smoke [a risk factor for colon adenomas (5)] are acetylated by NAT2, and heterocyclic amine carcinogens from cooked meat are converted to mutagens by this enzyme (6). The meat carcinogens may contribute to the hypothesized association between fast acetylation and colon cancer (7–10). A possible association between fast acetylator genotype and mutations in the K-*ras* gene in colorectal carcinomas has also been reported (11). If confirmed, a relationship between fast acetylation and cancer could lead to public health measures aimed at reducing heterocyclic amine exposures. We assessed the role of the acetylation polymorphism in the prevalence of colon adenomas (precursors to cancer) by use of a sigmoidoscopy-based, molecular, case-control study.

Materials and Methods

Subjects. Subjects were from either of two Southern California Kaiser Permanente Medical Centers (Bellflower or Sunset) and had a sigmoidoscopy during the period from January 1, 1991 through August 25, 1993. Eligible subjects were English speaking, ages 50–74 years, and residents of Los Angeles County. They had no severe gastrointestinal symptoms, invasive cancer, inflammatory bowel disease, familial polyposis, previous bowel surgery, or disability precluding an interview. Case subjects had a first time diagnosis of a histologically confirmed adenoma. Controls had no current or past polyp and were individually matched to cases by gender, age, date of sigmoidoscopy, and center. Of 628 eligible case subjects and 689 controls, 70 case subjects and 94 controls refused interviews and 29 case subjects and 32 controls could not be contacted. The response rate was 84% among case subjects and 82% among controls. Indications for sigmoidoscopy were "routine" for 45% of the case subjects and 44% of the controls, "specific minor symptoms" for 16% of the case subjects and 13% of the controls, and were not given for 39% of the case subjects and 43% of the controls. The average depth of the flexible sigmoidoscope was 55 cm for case subjects and 59 cm for controls. Fifteen case subjects also had carcinoma *in situ*. Participants provided data on smoking, therapeutic drug use, physical activity, height, weight, family history of cancer, and other factors during a 45-min in-person interview. The interviewer remained unaware of case or control status for 70% of case subjects and 87% of controls. A semiquantitative food frequency questionnaire was completed by 519 case subjects and 556 controls. *NAT2* genotyping was performed on 937 of 1033 blood samples. (The last 96 specimens were collected well after the current study was completed and will be included in future analyses.) Information on ethnicity was lacking for three genotyped participants. The analysis therefore includes data for 447 case subjects and 487 controls. All work had the approval of institutional human subjects committees and appropriate informed consent.

Genotyping. PCR assays were used to test for *NAT2* mutations[4] 191A, 341C, 481T, 590A, and 857A (1–4) and the corresponding wild-type nucleotides (191G, 341T, 481C, 590G, and 857G). Buffy coat specimens from subjects were spotted onto blotter paper as templates for PCR (13). PCR primers were: ACC-L, CCATTGTGTTTTTACGTATT; ACM1W-R, CTCCTGATTTGGTCCAG; ACM1V-R, CTCCTGATTTGGTCCAA; ACM2W-L, TTTACGCTTGAACCTCG; ACM2V-L, TTTACGCTTGAACCTCA; ACM2-R4, GTTGGGTGATACATACACAA; ACM3W-L2, CCCAAACCTGGTGATGG; ACM3V-L2, CCCAAACCTGGTGATGA; ACM3-R, CACTCTGCTTCCCAAGAT; AC341T, CTCCTGCAGGTGACCAT; and AC341C, CTCCTGCAGGTGACCAC. Primer pairs (left/right and product size) for allele-specific amplification were: 341T, AC341T/ACM1W-R and AC341T/ACM1V-R (173 bp); 341C, AC341C/ACM1W-R and AC341C/ACM1V-R (173 bp); 481C, ACC-L/ACM1W-R (610 bp); 481T, ACC-L/ACM1V-R (610 bp); 590G, ACM2W-L/ACM2-R4 (341 bp); 590A, ACM2V-L/ACM2-R4

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[3] The abbreviations used are: NAT2, *N*-acetyltransferase; CI, confidence interval; CYP1A2, enzyme cytochrome P450 1A2; GSTM1, glutathione transferase M1; OR, odds ratio; bp, base pair(s); 191A, 341C, 481T, 590A, and 857A are abbreviations for *NAT2* mutations $G^{191}A$ (Arg→Glu), $T^{341}C$ (Ile→Thr), $C^{481}T$ (no amino acid change), $G^{590}A$ (Arg→Gln), and $G^{857}A$ (Gly→Glu), respectively.

[4] Formal *NAT2* nomenclature is described in the study by Vatsis *et al.* (12).

Table 1 *Characteristics of the study population*

	Case subjects (n = 447)	Controls (n = 487)
Gender (%)		
Male	63.8	67.4
Female	36.2	32.6
Age (yr)	61.7	61.7
Ethnicity (%)		
White	55.0	54.6
Black	15.7	15.2
Latino black[a]	0.5	0.4
Hispanic	16.6	17.7
Asian	10.0	11.1
Other[a]	2.2	1.0
Smoking[b] (%)		
Never smokers	37.1	42.7
Ex-smokers	42.7	44.6
Current smokers	20.1	12.7
Pack-years	22.0	16.8

[a] Excluded from further analyses due to small numbers.

[b] Never smokers smoked less than 100 cigarettes during their life. Ex-smokers stopped smoking before their sigmoidoscopy. Current smokers were smoking at the time of their sigmoidoscopy.

(341 bp); 857G, ACM3W-L2/ACM3-R (286 bp); and 857A, ACM3V-L2/ACM3-R (286 bp). Conditions for PCR and electrophoresis were as described (13, 14). Mutation 191A was detected by use of restriction enzyme (*MspI*) digestion of PCR products (14). All assays were done without knowledge of case or control status. Subjects with two slow acetylator alleles were classified as slow acetylators. The *GSTM1* null genotype was detected by using a validated PCR assay as described (15).

Data Analysis. Unmatched controls occurred, *e.g.*, when case subjects were found to be non-English speaking, or when case subjects had invasive cancer at subsequent colonoscopy. Unmatched case subjects occurred when we were unable to interview an eligible control. To make use of information on unmatched subjects, and because we had to break the matching due to stratification by ethnicity, we used unconditional logistic regression to estimate ORs. Conditional and unconditional analyses of the reduced data set gave similar results for the total study population and for whites. Data for other ethnicities were too sparse for this comparison. Using unconditional logistic regression, we controlled for matching factors [date of sigmoidoscopy (6-month intervals), age (5-year intervals), gender, and clinic attended] as indicator variables in the model.

Results

The main mutations used for *NAT2* genotyping are 191A, 341C, 481T, 590A, and 857A. Earlier work showed that all 481T mutations are linked to mutation 341C in all groups studied (13), whereas wild-type 481C is not always linked to wild-type 341T. For example, roughly 8% of African American *NAT2* genes with wild-type 481C contain mutation 341C compared to 0–3% for other ethnicities (13). Furthermore, mutation 191A is rare in all groups except African Americans (14). We therefore tested for all five mutations in blacks and for three mutations (481T, 590A, 857A) in Asians, Hispanics, and whites. Table 1 describes the study population, and Table 2 shows frequencies of *NAT2* slow acetylator mutations.

Studies of human *NAT2* genes in monkey kidney COS-1 cells showed that mutations 341C and 481T together, but not alone, produce a slow acetylator allele (2). However, mutation 481T does not change the NAT2 amino acid sequence, and studies of human *NAT2* in *Escherichia coli* support an interpretation that 341C alone can be a slow acetylator mutation (16). Therefore, two sets of calculations were performed for the effect of *NAT2* in blacks. One set treated 341C as an independent slow acetylator mutation, while the other set treated the 341C-481T combination as a slow acetylator determinant.

Prevalence ORs for adenomas in fast acetylators were moderately increased among whites (OR, 1.29) and Hispanics (OR, 1.38), absent among Asians (OR, 1.08), but preventive among blacks (ORs, 0.64 and 0.44). Only the 0.44 OR was statistically significant. There was no substantial difference in the results when stratified by adenoma size (Table 3).

For whites, 54 cases of incident adenomas were compared to all controls. A case subject was coded as having an incident adenoma if the medical records showed that the patient had a previous negative sigmoidoscopy. The OR was 1.17 (95% CI, 0.63–2.19) compared to 1.29 for all cases among whites (Table 3). We assessed the sensitivity of the OR to different dates of the previous negative sigmoidoscopy. Analysis of 39 case subjects who had their previous negative sigmoidoscopy no earlier than 1985 gave an OR of 0.95 (95% CI, 0.46–1.96) for the same comparison as above.

White, fast-acetylating current smokers had a 2.25 OR for adenomas (95% CI, 1.00–5.08) compared to white, slow-acetylating never smokers (Table 4). The results suggest a slight modification of the smoking effect by the acetylation phenotype. We estimate that 16.5% of the adenomas among fast-acetylating smokers in our study population were attributable to exposure to both of these factors.

The power to estimate the combined smoking and *NAT2* effect, as shown for whites above, was low for other ethnic groups. ORs for black, slow-acetylating current smokers were 6.80 (95% CI, 1.44–32.2) when 341C was treated as an independent slow acetylator mutation and 3.60 (95% CI, 0.73–17.77) when the 341C-481T combination was treated as a slow acetylator determinant. Corresponding calculations for black, fast-acetylating current smokers gave ORs of 1.56 (95% CI, 0.42–5.87) and 0.83 (95% CI, 0.22–3.18), respectively. Again, there is a suggestion of a modification of the smoking effect by the acetylation phenotype. However, we observed in blacks a larger smoking effect for slow acetylators than for fast acetylators. Blacks as a group were more likely than whites to smoke (26% of controls compared to 11%).

Among white current smokers, we found an OR of 10.33 (95% CI, 1.94–55.04) for adenomas of individuals with both *NAT2* fast acetylator and *GSTM1* null genotypes, when compared to slow acetylators without the *GSTM1* null genotype. (The confidence interval represents a large sample estimate and may therefore be underestimated. An exact confidence interval could not be calculated, due to insufficient computer memory for the size of the data set and the number of

Table 2 *Frequencies of NAT2 slow acetylator mutations*[a]

Ethnic group	No. of genes analyzed	Frequencies of mutations					Combined total
		191A	341C	481T	590A	857A	
Black	148	0.04	0.37	0.30	0.23	0.01	0.65[b]
White	532			0.47	0.28	0.03	0.78[c]
Hispanic	172			0.37	0.23	0.13	0.73[c]
Asian	108			0.04	0.30	0.07	0.41[c]

[a] Data are from 480 individuals, *i.e.*, all controls except those in the Latino black and "other" groups. *NAT2* genes in all case subjects and controls, except one, contained no more than one slow acetylator determinant. (*i.e.*, 191A, 341C, the 341C-481T combination, 590A, or 857A). The one exception contained both 191A and 590A (lab No. 287).

[b] The combined total for blacks reflects 191A + 341C + 590A + 857A. Frequencies of combinations of 341C, 341T, 481C, and 481T among black controls were: 341C-481T, 0.30 (45 genes); 341C-481C, 0.07 (10 genes); 341T-481T, 0; 341T-481C, 0.63 (93 genes). The combinations were detected by using allele-specific left and right PCR primers.

[c] Mutation 191A is rare in whites, Hispanics, and Asians (14). Also, 97–100% of 481C nucleotides were linked to 341T, and 100% of 481T mutations were linked to 341C in a previous study of these groups (13). Therefore, we tested for only 481T, 590A, and 857A among whites, Hispanics, and Asians.

Table 3 *Adjusted[a] ORs for adenomas in fast compared to slow acetylators,[b] by ethnicity and adenoma size[c]*

	OR (95% CI)
All adenomas	
Overall	1.08 (0.83–1.40)
White	1.29 (0.90–1.84)
Black	0.64 (0.32–1.28)[d]
	0.44 (0.21–0.91)[e]
Hispanic	1.38 (0.72–2.67)
Asian	1.08 (0.40–2.93)
Adenomas < 1 cm	
White	1.42 (0.96–2.12)
Black	0.75 (0.33–1.68)[d]
	0.47 (0.20–1.09)[e]
Hispanic	1.44 (0.71–2.93)
Asian	0.74 (0.25–2.18)
Adenomas ≥ 1 cm	
White	1.05 (0.62–1.77)
Black	0.48 (0.16–1.43)[d]
	0.40 (0.13–1.21)[e]
Hispanic	1.21 (0.37–3.94)
Asian	3.54 (0.39–32.6)

[a] Based on unconditional logistic regression with adjustment for age (5-year intervals), gender, date of sigmoidoscopy (6-month intervals), and clinic attended.
[b] Classification of slow acetylators was based on mutations 481T, 590A, and 857A for Asians, Hispanics, and whites. Classification of slow acetylators for blacks was based on 191A, 341C, 481T, 590A, and 857A, as described in footnotes *d* and *e*.
[c] Based on the size of the largest adenoma.
[d] Based on treating 341C as an independent slow acetylator mutation.
[e] Based on treating the 341C-481T combination as a slow acetylator determinant.

covariates.) Similar comparisons for all subjects and for never smokers gave ORs of 1.33 (95% CI, 0.80–2.22) and 1.02 (95% CI, 0.47–2.21), respectively.

Discussion

Overall no effect of fast acetylator genotype on the prevalence of colorectal adenomas was observed in our multiethnic study. Stratification by ethnicity based on biological considerations revealed that the direction of the fast acetylator effect among whites (OR, 1.29) and Hispanics (OR, 1.38) agreed with published urine phenotyping studies (7–10), and was thus compatible with a role for heterocyclic amines in the occurrence of colorectal adenomas in these groups, but further studies are needed. Currently available data on meat intake did not allow us to analyze heterocyclic amine effects. Meat intake without information on cooking may be inadequate for estimating heterocyclic amine exposures.

There was no detectable *NAT2* effect among Asians (OR, 1.08), which is consistent with a Japanese study of 36 colon cancer patients and 36 autopsy controls (11). If heterocyclic amines are important in colorectal tumorigenesis, there may be lower levels in Asian diets. Red meat intake among Asian and white controls did not differ in our study (72 g/day *versus* 66 g/day; $P = 0.55$). Smoking prevalence was lowest among Asians (9% of controls).

An effect of fast acetylation among blacks was in the direction of a possible protective effect. The *P* values for the difference between blacks and whites were 0.075 (when 341C was treated as an independent slow acetylator mutation) and 0.011 (when the 341C-481T combination was treated as a slow acetylator determinant). The apparent difference between blacks and whites may simply reflect random variation around an overall null effect, or it could represent a real difference. (Additional data will be needed to discriminate between these explanations.) Diet exposures are unlikely to provide an explanation for our observations, within the context of the heterocyclic amine hypothesis. Among control subjects, blacks and whites had fairly similar average intakes of red meat (73 g/day *versus* 66 g/day; $P = 0.42$).

Although a protective effect, if any, for blacks remains to be established, several interpretations are possible. First, blacks had a higher percentage of current smokers (26% *versus* 11%) than did whites. Arylamine carcinogens from cigarette smoke or other sources may have contributed to a protective effect for fast acetylation, as in bladder cancer (17). Second, frequencies of the major *NAT2* mutations vary by ethnicity (14), and mutations may have different effects on carcinogen acetylation. However, sample sizes were too small to estimate the effects of individual mutations.

Another possibility is that the related NAT1 enzyme is involved, since NAT1 and NAT2 metabolize some of the same procarcinogens. *NAT1* is polymorphic, and ethnic differences are likely. The *NAT1* and *NAT2* genes appear to lie physically close to one another (18), allowing for possible linkage disequilibrium. For example, *NAT2* fast acetylator alleles among blacks could be preferentially linked to *NAT1* slow acetylator alleles. Colon activity of NAT1 is higher than that of NAT2 (19), so an *NAT1* effect on adenoma risk is possible. We are currently screening for *NAT1* mutations in our subjects to investigate linkage disequilibrium and *NAT1* effects.

A fourth interpretation is that other carcinogen-metabolizing enzymes also differ by ethnicity, such as CYP1A2. CYP1A2 activity is polymorphic and inducible by aromatic hydrocarbons. Liver CYP1A2 is believed to *N*-hydroxylate heterocyclic amines before these metabolites are sent to the colon (6). *N*-hydroxylation followed by *O*-acetylation by NAT2 can lead to DNA adducts. Blacks have lower CYP1A2 activities compared to whites, due to unknown mechanisms (20). Lower CYP1A2 activities could theoretically shift metabolism of heterocyclic amines toward direct *N*-acetylation in the liver, which is felt to be a detoxifying and protective reaction.

We made two tentative assessments of potential interactions between NAT2 and other carcinogen-metabolizing enzymes. First, current smokers in our study probably had high CYP1A2 activities due to inducibility by tobacco smoke. We observed among whites a slight interaction between fast acetylation and smoking (Table 4), indicating either (*a*) an effect of CYP1A2 on the bioactivation of heterocyclic amines or (*b*) an effect of other carcinogens in smoke. The results agree with preliminary data supporting an interaction between CYP1A2 and NAT2 in colon cancer development (6).

Second, recent epidemiological studies have focused on the *GSTM1* null genotype, a polymorphism that occurs in 50% of whites and 30% of blacks (15). The null genotype causes lack of the GSTM1 enzyme, which detoxifies polycyclic aromatic hydrocarbons. Higher risks for lung and bladder cancer have been found among smokers with the null genotype. Although the *GSTM1* null genotype alone has little or no effect on the prevalence of colon adenomas among smokers (21), we obtained preliminary data for a possible interaction between *NAT2* and *GSTM1*. Specifically, adenoma prevalence was 10-fold higher among fast acetylating smokers with the null genotype. The confidence interval was extremely wide, however, and expanded studies are needed to confirm it.

Several strengths of our study design appear to support the validity of our results. For example, the participation rate of greater than 80%

Table 4 *Adjusted[a] ORs (and 95% CI) for adenomas according to smoking status and NAT2 genotype, for whites only*

	NAT2 genotype[b]	
	Slow acetylator	Fast acetylator
Never smoker	1.0[c]	1.20 (0.67–2.14)
Ex-smoker	0.99 (0.60–1.67)	1.34 (0.77–2.35)
Current smoker	1.68 (0.81–3.48)	2.25 (1.00–5.08)

[a] Based on unconditional logistic regression, adjusted for age, gender, date of sigmoidoscopy, and clinic attended. Adjustment for physical activity, body mass index, and intake of energy, saturated fat, fruits/vegetables, and alcohol did not substantially alter the ORs.
[b] Classification of slow acetylators was based on mutations 481T, 590A, and 857A.
[c] Reference category.

was high, and differential recall of smoking histories among asymptomatic subjects should be low. Case subjects and controls were screened with a 60-cm flexible sigmoidoscope, therefore clinical outcomes are unlikely to have been misclassified with respect to left-sided adenomas. (Our results apply only to left-sided lesions.) We determined NAT2 phenotypes on the basis of as many as five slow acetylator mutations. Total slow acetylator allele frequencies among controls agreed fairly well with phenotyping studies, indicating that these mutations accurately classified slow acetylators in our study. Our results should pertain to occurrence, not recurrence, of adenomas because we excluded individuals with a previous history of any polyp. We also identified incident cases to try to separate the effects on incidence *versus* progression. However, the number of incident cases was fairly small. Stratification by adenoma size as a proxy measure for incident adenomas did not show substantial differences in fast acetylator effects.

This is the first large, multiethnic, molecular epidemiological study of colorectal adenomas and *NAT2* genotypes. Overall our results do not support the hypothesis that fast acetylator genotype is an independent risk factor for colorectal adenoma. However, if stratified by ethnicity, there was a suggestion of increased prevalence of colon adenomas among white and Hispanic fast acetylators. Further work will require analysis of the interplay among carcinogen exposures and combinations of genes that influence carcinogen metabolism. Specifically, because NAT1 and CYP1A2 seem to be important in heterocyclic amine metabolism, we are currently performing genotyping (*NAT1*) and phenotyping (NAT1 and CYP1A2) for these enzymes.

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