American Journal of Epidemiology

Vol. 152, No. 3
Printed in U.S.A.

Breast Cancer and Active and Passive Smoking: The Role of the *N*-Acetyltransferase 2 Genotype

A. Morabia,[1] M. S. Bernstein,[1] I. Bouchardy,[2] J. Kurtz,[3] and M. A. Morris[2]

The association of breast cancer with passive and active smoking was investigated in slow and fast acetylators of aromatic amines in a Geneva, Switzerland, study in 1996–1997. A slow acetylator was homozygous for one, or heterozygous for two, of three *N*-acetyltransferase 2 (*NAT2*) polymorphisms determined on buccal cell DNA from 177 breast cancer cases and 170 age-matched, population controls. The reference group consisted of women never regularly exposed to active or passive smoke. Among premenopausal women, the odds ratios were homogeneous in slow and fast acetylators: 3.2 (95% confidence interval (CI): 1.2, 8.7) for passive smoking and 2.9 (95% CI: 1.1, 7.5) for active smoking. Among postmenopausal women, the odds ratios for fast acetylators were 11.6 (95% CI: 2.2, 62.2) for passive and 8.2 (95% CI: 1.4, 46.0) for active smoking; the corresponding effects were also apparent but less strong in slow acetylators. After the nonexposed and the passive smokers were grouped in a single reference category, active smoking was associated with postmenopausal breast cancer in slow acetylators (odds ratio (OR) = 2.5, 95% CI: 1.0, 6.2) but not in fast acetylators (OR = 1.3, 95% CI: 0.5, 3.3). Thus, the associations of both passive and active smoking with breast cancer appear stronger in fast than in slow *NAT2* genotypes. Separating passive smokers from the nonexposed impacts on the inference about a possible *NAT2*-smoking interaction. *Am J Epidemiol* 2000;152:226–32.

breast neoplasms; case-control studies; genes; tobacco; tobacco smoke pollution

Suspicions that breast cancer may be linked to passive smoking go back to 1985, when Sandler et al. (1) observed a twofold increased risk of breast cancer associated with smoking by spouse. They postulated that in previous studies, "… nonsmokers may have included women who were passively exposed, limiting the possibility of observing any effect that might result from exposure to sidestream smoke" (2, p. 371). They therefore suggested, "Future studies of this question should collect data that will allow for stratification into at least three smoking categories: true nonexposed, those with passive exposure only, and active smokers" (2, p. 371).

Indeed, six studies (3–8), recently reviewed by Wells (9), have been conducted or reanalyzed according to this methodological recommendation. Five of them (3–7) were based on incident cases and consistently show that women exposed to passive smoking are at increased risk of breast cancer relative to women never exposed to either active or passive smoke (10). The association of breast cancer was of similar magnitude with passive smoking (odds ratio (OR) = 2.0, 95 percent confidence interval (CI): 1.5, 2.6) and with active smoking (OR = 2.2, 95 percent CI: 1.6, 2.9).

It has also been found that women who are active smokers and slow acetylators due to their *N*-acetyltransferase 2 (*NAT2*) genotype were at increased risk of breast cancer (11), suggesting that slow acetylation of aromatic amines may lead to a longer or greater exposure to some of the potentially carcinogenic compounds than fast acetylation. However, of the three studies (12–14) that tried to reproduce the findings of Ambrosone et al. (11), none showed higher relative risks among slow acetylators as clearly.

We had postulated that the *NAT2*-smoking interaction could explain the similar magnitude of effect of passive and active smoking on breast cancer risk; that is, women who develop breast cancer as a consequence of passive smoking would be more likely to be slow acetylators (15). We tested this hypothesis by obtaining buccal scrapes and isolating the desoxyribonucleic acid (DNA) from all surviving cases and from a random sample of age-matched controls drawn from the case-control study conducted in 1992–1993.

MATERIALS AND METHODS

A population-based case-control study was performed during 1992–1993 in Geneva, Switzerland (3). Eligible cases were all women resident in Geneva aged less than 75 years diagnosed with incident breast cancer. We interviewed 71 percent (n = 244) of 344 eligible cases and 70 percent of

Received for publication May 17, 1999, and accepted for publication September 17, 1999.

Abbreviations: CI, confidence interval; *NAT2*, *N*-acetyltransferase 2 gene; OR, odds ratio; RFLP, restriction fragment length polymorphism.

[1] Division of Clinical Epidemiology, University Hospital, Geneva, Switzerland.
[2] Division of Medical Genetics, University Hospital, Geneva, Switzerland.
[3] Division of Radiooncology, University Hospital, Geneva, Switzerland.
Reprint requests to Dr. A. Morabia, Division of Clinical Epidemiology, Geneva University Hospital, CH-1211 Geneva 14, Switzerland (e-mail: alfredo.morabia@hcuge.ch).

1,032 eligible controls, who were a random sample of women aged 30–74 years residing in Geneva during the same time period. Cases and controls were invited to come to a mobile clinic and participate in an ongoing survey on women's health. The aim of the study was not specified, and trained interviewers were blind to the case-control status. The smoking history section of the questionnaire was structured as four calendars, with one dedicated to active smoking and three separate calendars dedicated to passive exposures at home, at work, and during leisure time. Smoking exposures were recorded year by year, between age 10 and the date of interview. An episode of exposure consisted of a time period of at least 6 months during which the woman had passively or actively smoked. For each episode of active smoking, the number of cigarettes per day, filter, and cigarette brand were recorded. The number of hours per week of each passive smoking episode was recorded.

The present gene-smoking interaction study was conducted in 1996–1997. As no biologic material was collected in the original study (1992–1993) in view of a genetic study, we first identified the surviving cases from a computerized database indicating whether a person had remained a resident of Canton Geneva and, if applicable, whether the person was still living. We could identify 205 cases (84 percent) still alive and residing in Geneva. A group of frequency-matched controls were randomly selected so that the proportion of controls within each 10-year age group was similar to that of the cases. Of the 349 women selected, 287 (82.2 percent) were still residing in Geneva and eligible. A letter describing the aim of the study was sent to all eligible subjects. In case of nonresponse, we tried to contact every eligible person by telephone.

Participants were asked to come to the Division of Clinical Epidemiology of the Geneva University Hospital. All women signed an informed consent for DNA analysis. Cells were collected from the cheek mucosa using a cytologic brush. This approach minimized nonparticipation by eligible subjects.

Procedures at the laboratory

Buccal cells were sampled with two cytologic brushes per patient. After sample collection, the brushes were put immediately into 400 μl of 6 M sodium iodide. Samples were stored at 4°C before extraction. Total DNA was extracted by binding to a silica matrix (Prepagene; Bio-Rad, Hercules, California) in groups of 96 samples to allow manipulation in a microtiter plate format (QIAamp 96 DNA blood kit; Qiagen AG, Basel, Switzerland). DNA was extracted up to 6 months after sampling, with no apparent effect on efficiency. Two separate extractions were performed for each person. DNAs were stored in 10 mM Tris/0.1 mM ethylene diamine tetraacetic acid, at 4°C and at –80°C (duplicates).

Three polymorphisms of the arylamine-*N*-acetyltransferase 2 gene (*NAT2*) were analyzed by nested polymerase chain reaction and restriction digestion. A 900-base pair fragment containing the entire coding region of *NAT2* was amplified (30 cycles, standard conditions); then 1 μl of this product was subjected to nested polymerase chain reaction to amplify a 740-base pair fragment containing the sites of the three functionally important polymorphisms 481 (*Kpn*I restriction fragment length polymorphism (RFLP)), 590 (*Taq*I RFLP), and 857 (*Bam*HI RFLP). The polymerase chain reaction primers were the following: first polymerase chain reaction, NAT2CL (GCC ATG GAG TTG GGC TTA GAG), NAT2R (GTG AGT TGG GTG ATA CAT ACA CAA G); nested polymerase chain reaction, NAT2DL (ATT TTT GAT CAC ATT GTA AGA AGA), NAT2CR (CAT ACA CAA GGG TTT ATT TTG TTC C). The polymorphic sites were analyzed by serial digestion with the appropriate restriction enzymes followed by electrophoresis on horizontal 12 percent polyacrylamide gels (GenePhor; Amersham Pharmacia Biotech, Piscataway, New Jersey). Samples were coded so that the laboratory was unaware of the case-control status. They were analyzed twice, and the gels were interpreted independently, with complete concordance. A total of 347 samples were given to the laboratory, of which 330 (95 percent) were successfully amplified. The failure to amplify 5 percent of samples was a consequence of using cheek scrapes as source material and was deemed acceptable. The restriction digestion results of 94 samples could not be unequivocally interpreted, and these samples were analyzed by direct sequencing of polymerase chain reaction products using Thermosequenase and an ALF Express DNA sequencer (Pharmacia). Unequivocal results were thus obtained for 322 samples (93 percent).

Statistical methods

Information concerning exposure to tobacco smoke was obtained from the original interview. Ever smokers had smoked at least 100 cigarettes in their lifetime. Among ever smokers, current smokers were women who reported having smoked regularly within the 2 years preceding interview. Passive smokers were defined as women who had been exposed at least 1 hour per day during 1 year or more either at home, at work, or during leisure time.

A woman was defined as a slow acetylator if she was homozygous for one, or heterozygous for two, of the three following *NAT2* polymorphisms: 481C>T (*Kpn*I RFLP), 590G>A (*Taq*I), and 857G>A (*Bam*HI). This definition assumed that there could be no single allele with two or more of the tested mutations (11, 16).

The odds ratios for developing breast cancer can be interpreted as relative risks of breast cancer. They were estimated using a logistic regression model that included as potential confounders the three variables that appeared to be related to breast cancer in the full sample, that is, age, education, and positive family history of breast cancer. Analyses were also stratified by menopause, defined as having had the last menses at least 1 year before interview or a bilateral ovariectomy.

A possible interaction among *NAT2*, smoking, and breast cancer was investigated using multiple logistic regression. Multiplicative interaction was assessed by adding a product term between *NAT2* status and each smoking exposure. The interaction coefficient can be interpreted as the log of the ratio of the smoking by breast cancer odds ratios in fast relative to slow acetylators. Additive interaction was assessed by the relative excess risk due to interaction that was esti-

mated (with its 95 percent confidence limits) according to the method of Hosmer and Lemeshow (17).

As DNA could not be analyzed for 25 women (10 cases and 15 controls), we performed a sensitivity analysis with four hypothetic scenarios: 1) all 25 missing subjects were fast acetylators; 2) all 25 missing subjects were slow acetylators; 3) missing cases were slow acetylators but controls were fast acetylators; and 4) missing controls were slow acetylators but cases were fast. Results of the four scenarios were highly consistent with those obtained without the 25 women.

RESULTS

Of the 205 cases from the original study who could potentially participate in this study, 170 agreed to participate (83 percent). Differences in breast cancer risk factors and in smoking status between this subsample and the original case series can be obtained by comparing tables in the present paper with tables of the original publication (3). Deficits of participation were found in the following categories: primary education, nulliparity, and never oral contraceptive use. The proportion of ever passive smokers was 44 percent in the present sample and 34 percent in nonparticipants. Corresponding proportions of ever active smokers were 47 percent and 51 percent.

Of the randomly sampled subgroup of 287 control women, 177 (62 percent) participated. DNA was successfully analyzed in 160 cases and 162 controls (93 percent). Except for age matching and for a lower participation of women with primary education, characteristics of the present sample of controls were very similar to those of the nonparticipants.

Table 1 shows that the compared groups had the same age distribution. There were a reduction in risk of breast cancer with older age at menarche and an increased risk in women with age at first birth between 25 and 29 years and in women with a family history of breast cancer.

The allele frequencies of the *NAT2* genetic variants were 46.1 percent for 481T, 27.6 percent for 590A, and 4.7 percent for 857A. These frequencies are in close agreement with those published by Cascorbi et al. (16) for the German population. The overall frequency of slow acetylation genotypes was 56.5 percent. It was slightly higher among cases (58.8 percent) and among passive (58.7 percent) or active (57.6 percent) smokers, but these differences were not statistically significant.

When pooling premenopausal and postmenopausal women, we found the adjusted odds ratio of breast cancer to be 3.1 (95 percent CI: 1.5, 6.0) for passive smokers and 3.3 (95 percent CI: 1.7, 6.5) for active smokers (not shown in a table). After stratification for *NAT2* (table 2), both active and passive smoking increased breast cancer risk, with higher relative risks observed for the fast acetylation genotype. The one apparent exception, the current smokers of less than 20 cigarettes per day, may be an effect of the small size of this subgroup.

Among premenopausal women (89 cases and 93 controls), odds ratios were homogeneous in slow and fast acetylators (figure 1): the *NAT2*-adjusted odds ratio was 3.2 (95 percent CI: 1.2, 8.7) for passive smoking and 2.9 (95 percent CI: 1.1, 7.5) for active smoking (not shown in a table).

Among postmenopausal women (81 cases and 84 controls), a strong and statistically significant association was found in fast acetylators with both passive (OR = 11.6, 95 percent CI: 2.2, 62.2) and active (OR = 8.2, 95 percent CI: 1.4, 46.0) smoking (figure 1). In slow acetylators, a smaller effect was also apparent for active smoking (OR = 2.9, 95 percent CI: 0.8, 11.2) but not for passive smoking (OR = 1.1, 95 percent CI: 0.3, 4.3). The number of unexposed cases was small in both slow ($n = 5$) and fast ($n = 2$) acetylators. In the analyses of interaction, the ratio of odds ratios for fast relative to slow acetylators was 11.6 (95 percent CI: 1.3, 111.1) for passive smoking and 3.3 (95 percent CI: 0.4, 33.3) for active smoking. The relative excess risk due to interaction was 5.1 (95 percent CI: –4.1, 14.2) for passive smoking and 2.9 (95 percent CI: –6.2, 12.0) for active smoking.

We also categorized passive smokers according to the number of hours-per-day-years (3) of exposure, but these analyses did not lead to different interpretations (not shown). The median age of first exposure was 20 years for passive and 18 years for active smoking. We used these cutoffs to determine whether the effect of age at first exposure differed according to *NAT2* status. Nonexposed women were not included in these analyses. The odds ratios for first exposure to passive smoking at age 20 or before (relative to after age 20) were 1.6 (95 percent CI: 0.7, 3.9) in slow acetylators and 0.6 (95 percent CI: 0.2, 1.7) in fast acetylators (p for interaction = 0.15). The corresponding odds ratios for first active smoking at age 18 or before (relative to after age 18) were 1.2 (95 percent CI: 0.6, 2.7) in slow and 0.4 (95 percent CI: 0.2, 1.1) in fast acetylators (p for interaction = 0.10).

In order to compare the present results with those of previous reports that lacked information on passive smoking (11–14), we repeated the analyses after grouping the nonexposed and the passive smokers in a single category. Among premenopausal women, there was no significant association for active versus never-active smokers in either slow (OR = 1.3, 95 percent CI: 0.5, 3.0) or fast (OR = 1.5, 95 percent CI: 0.6, 4.3) acetylators. In contrast, among postmenopausal women, active smoking was a risk factor for breast cancer in slow acetylators (OR = 2.5, 95 percent CI: 1.0, 6.2) but not in fast acetylators (OR = 1.3, 95 percent CI: 0.5, 3.3). The test for multiplicative interaction had $p = 0.30$.

DISCUSSION

The biologic rationale behind studying *NAT2* was that tobacco bicyclic aromatic amines, such as β-naphthylamine and 4-aminobiphenyl, are environmental carcinogens for the breast. These amines are proven animal bladder carcinogens. Human subjects with the slow *NAT2* genotype or phenotype are at higher risk of bladder cancer than are fast acetylators (18). Ambrosone et al. (11) reported an increased risk of postmenopausal breast cancer with active smoking in slow acetylators only, suggestive of a *NAT2*-smoking interaction with respect to breast cancer risk.

We expected to find a stronger association of smoking and breast cancer in slow acetylators (15), but we found

TABLE 1. Risk factors for breast cancer in 170 cases and 177 controls, Geneva, Switzerland, 1992–1994

Risk factor	Cases (no.)	Controls (no.)	OR*,†	95% CI*
Age (years)				
<40	8	8	1.0	
40–44	12	13	0.8	0.2, 3.0
45–49	38	37	0.8	0.2, 2.4
50–54	33	44	0.5	0.2, 1.7
55–59	28	29	0.7	0.2, 2.3
60–64	23	20	0.9	0.3, 3.0
65–69	14	13	0.8	0.2, 3.1
70–74	14	13	0.9	0.2, 3.5
Education				
Elementary	21	15	1.0	
Apprenticeship	97	88	0.7	0.3, 1.6
≥High school	52	74	0.4	0.2, 0.9
BMI* (kg/m^2)				
<21	40	52	1.0	
21–22	48	43	1.3	0.7, 2.4
23–25	39	45	1.0	0.5, 2.0
≥26	43	37	1.2	0.6, 2.3
Age at menarche (years)				
<12	24	21	1.0	
12	33	42	0.6	0.3, 1.3
13	52	39	1.0	0.5, 2.2
14	38	36	0.8	0.4, 1.8
≥15	23	39	0.4	0.2, 1.0
Age at first live birth (years)‡				
<25	44	54	1.0	
25–29	47	37	1.8	1.0, 3.4
≥30	33	36	1.1	0.6, 2.1
No birth	15	19	1.0	0.5, 2.4
No conception	31	30	1.3	0.6, 2.8
Oral contraception				
Never	73	72	1.0	
Ever	97	105	1.0	0.6, 1.7
Breast cancer in mother or sister				
No	153	166	1.0	
Yes	17	11	2.0	0.9, 4.7

* OR, odds ratio; CI, confidence interval; BMI, body mass index.
† The odds ratio for each variable was controlled for all of the other factors in the table.
‡ Age of the first live birth missing for one control.

instead more elevated relative risks for both passive and active smoking in fast acetylators, especially among postmenopausal women. If true, these results suggest that the carcinogenic substrate is not aromatic amines (which are found only as traces in tobacco smoke) but heterocyclic amines that are more concentrated in cigarette smoke than bicyclic aromatic amines and are at least 10 times more present in the sidestream than in the mainstream smoke. The ratio of sidestream to mainstream smoke is probably even greater in cigarettes with a perforated filter tip that is usually smoked by women. Heterocyclic amines, such as pyridine, are *N*-hydroxylated by cytochrome P450 1A2 and subsequently activated by *O*-acetylation by NAT2 to form electrophilic intermediates and DNA adducts that can initiate cancer (19, 20). If heterocyclic amines were the culprit, then fast acetylators would be most at risk. An analogy can be made here with colon cancer, which seems to be caused by dietary heterocyclic amines and for which a higher risk in fast acetylators has been consistently reported (21). A relation of breast cancer to dietary heterocyclic amines is not well established (22) but is strongly supported by the results of the Iowa Women's Health Study (23).

Independently of the environmental agent involved, the equivalence of relative risk of breast cancer for passive or

TABLE 2. Odds ratio of breast cancer with passive and active smoking, by *NAT2* acetylation genotype, Geneva, Switzerland, 1992–1994

Acetylation genotype	Smoking status	Breast cancer cases (no.)	Controls (no.)	OR*,†	95% CI*
Slow‡	Never exposed	10	18	1.0	
	Ever passive smokers	38	36	1.9	0.7, 4.6
	Ever active smokers	46	34	2.7	1.1, 6.6
	Exsmokers	25	18	2.8	1.05, 7.8
	Current <20 cpd*	12	7	3.7	1.05, 12.9
	Current ≥20 cpd	9	9	1.6	0.7, 6.9
Fast‡	Never exposed	6	23	1.0	
	Ever passive	30	22	5.9	2.0, 17.4
	Ever active	30	29	4.2	1.5, 12.0
	Exsmokers	14	11	5.2	1.5, 18.0
	Current <20 cpd	6	10	3.1	0.7, 12.7
	Current ≥20 cpd	10	8	5.3	1.4, 20.5

* OR, odds ratio; CI, confidence interval; cpd, cigarettes per day.
† Test of interaction among acetylation genotype, ever smoking status, and breast cancer, $p = 0.54$.
‡ Simultaneously adjusted for age, education, and family history of breast cancer.

active smoking consistently reported (3–8) remains counterintuitive. A priori, passive smokers are less exposed than active smokers who inhale their own and other people's cigarettes' sidestream smoke in addition to drawing puffs of concentrated smoke aerosols. Many compounds of intermediate tobacco combustion are more concentrated in the sidestream than in the mainstream smoke (when titrated by smoke collector devices), but the inhaled concentration is much diluted in the ambient air. It is, however, also important to stress that the magnitude of the exposure of the passive smoker is often underestimated. We have reported that the average lifetime exposure to passive smoke of the present study control population was equivalent to 30.8 hours/week during 10 years or 20.5 hours/week during 15 years (24).

Analogic thinking with the tobacco-lung cancer association also leads to expecting a huge relative risk differential between active and passive smokers. The lung cancer

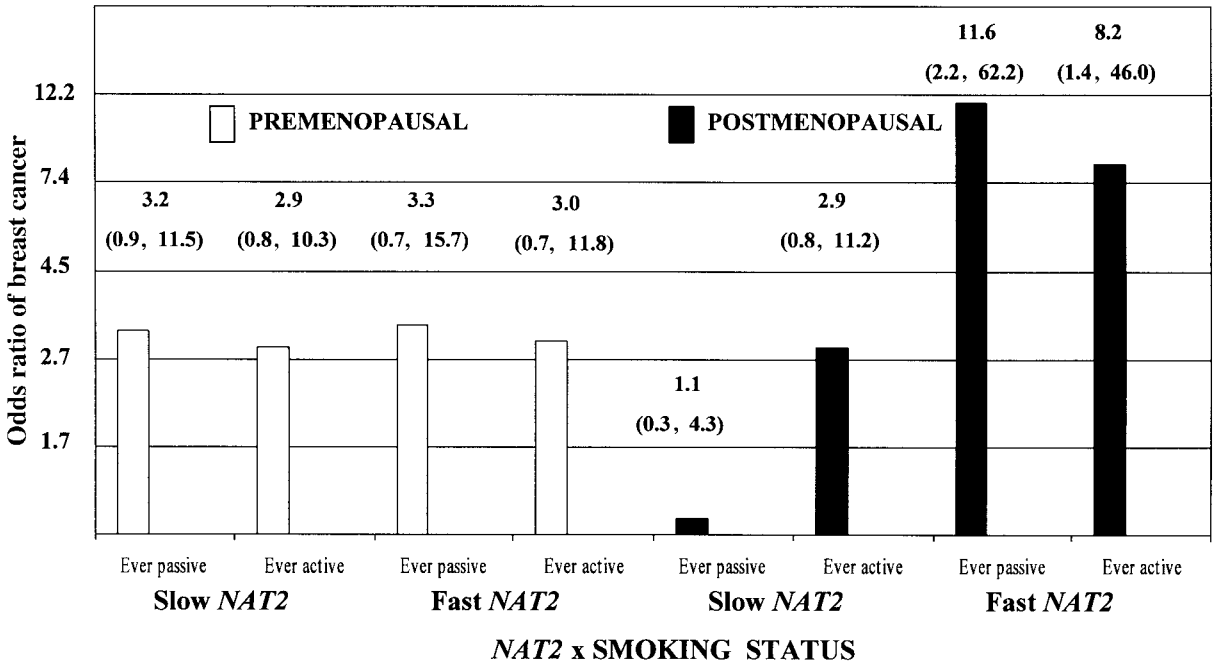

FIGURE 1. Odds ratio of breast cancer with ever passive or ever active smoking by *N*-acetyltransferase 2 (*NAT2*) genotype in pre- and postmenopausal cases and controls, Geneva, Switzerland, 1992–1994. The reference group is women never regularly exposed to active or passive smoking. Odds ratios are simultaneously adjusted for age, education, and family history of breast cancer. Numbers in parentheses, 95% confidence interval.

model may, however, not be relevant for the mammary gland that is not directly exposed to tobacco smoke. Carcinogens need to circulate in the blood before reaching the gland and seem to be able to remain for a very long time in the mammary epithelial cells (25, 26). Environmental tobacco smoke particles are small and therefore poorly filtrated by the lung. The risk differential between passive and active smokers may therefore be smaller for breast than for lung cancer.

In addition, the relative risk observed for passive smokers may also be magnified by the low dose effect hypothesis formulated by Vineis and McMichael (21) for colon cancer: the modifying effect of a genotype or phenotype can be more evident at low dose. In the present study, it is reasonable to conceive that high levels of exposure to tobacco smoke saturate the NAT2 enzyme activity, resulting in formation of abundant DNA adducts from *O*-acetylation of hydroxylated human carbonic anhydrase in both slow and fast acetylators. In contrast, at low doses of exposure to tobacco, DNA adducts may accumulate only among fast acetylators, because, among slow acetylators, the *N*-acetoxy derivatives are either produced in too small a quantity or detoxified at the same rate they are produced.

Whether the first exposure to tobacco as a teenager determines the risk of breast cancer remains elusive (27–30). The present study shows a reduced risk of young age at first exposure to active and passive smoking among fast acetylators. Larger studies of gene-smoking interactions are needed in populations where it is not uncommon that women start to smoke before age 16.

An important observation from the present study for future research on the relation of smoking to breast cancer is that taking passive smoking into account had dramatic consequences on inference. It was intriguing to observe that, when active smokers were compared with never-active smokers, a reference category that includes both unexposed women and passive smokers, results suggested that postmenopausal *slow* acetylators were at higher risk of breast cancer if they smoked. This conclusion is consistent with the study of Ambrosone et al. (11). On the other hand, when nonactive, nonpassive smokers were used as the reference category, analyses led to the opposite conclusion; that is, postmenopausal *fast* acetylators were at higher risk of breast cancer if they smoked. We consider the latter results as being more valid, because it is logical to use the lowest level of exposure to tobacco as the reference category, especially in view of the epidemiologic evidence of a specific effect of passive smoking (3–8).

Given the small number of cases, confidence intervals were wide, and only very large effects could be detected with sufficient statistical power. This limitation was particularly problematic for the analyses of interaction. Even in the multiplicative model for passive smoking, the low statistical power did not allow us to establish whether the interaction was quantitative (that is, association present in both fast and slow acetylators but with a difference of magnitude) or qualitative (that is, association only among fast acetylators). Thus, larger studies are needed to elucidate gene-smoking interactions with statistical confidence.

Other limitations include the unexpected reduction in risk of breast cancer observed in the more educated women (OR = 0.4, 95 percent CI: 0.2, 0.9) that is inconsistent with what is usually known about the etiology of this disease (31) and that can be attributed to the differential participation of educated women between cases and controls. This imbalance was taken into consideration in the analysis by adjusting the odds ratio for education and does not seem to have generated substantial bias, since the observed effects for smoking or other risk factors of breast cancer were consistent with our previous findings (3) and with what is usually found by others (31).

There could have been concerns about survival bias, since biologic material was collected among survivors of the original study. However, given the high survival rate (84 percent), it is unlikely that results would have been markedly different had the DNA specimens been collected at the time of the original study.

It has been reported that smoking may reduce the risk of breast cancer in women with mutant *BRCA1* or *BRCA2* (32). This finding is only marginally relevant for epidemiologic investigations in the general population because less than one in every 500 women in the general population carries these mutant genes (33).

In conclusion, the risk of breast cancer related to smoking appears to be stronger in fast acetylators of aromatic amines. Separating passive smokers from the nonexposed has major implications on the inference about a possible *NAT2*-smoking interaction.

ACKNOWLEDGMENTS

This study was funded by the Swiss National Fund for Scientific Research (grants 32.31.326.91 and 32-47219.96) and the Dumont et Moerlen Foundation, Geneva, Switzerland.

REFERENCES

1. Sandler DP, Everson RB, Wilcox AJ. Passive smoking in adulthood and cancer risk. Am J Epidemiol 1985;121:37–48.
2. Sandler DP, Everson RB, Wilcox AJ. Cigarette smoking and breast cancer. (Letter). Am J Epidemiol 1986;123:370–1.
3. Morabia A, Bernstein M, Heritier S, et al. Relation of breast cancer with passive and active exposure to tobacco smoke. Am J Epidemiol 1996;143:918–28.
4. Smith SJ, Deacon JM, Chilvers CE. Alcohol, smoking, passive smoking and caffeine in relation to breast cancer risk in young women. UK National Case-Control Study Group. Br J Cancer 1994;70:112–19.
5. Johnson KC, Semenciw R, Hu J, et al. Active and passive smoking and premenopausal breast cancer. (Abstract). Am J Epidemiol 1998;147:S68.
6. Lash TL, Aschengrau A. Active and passive cigarette smoking and the occurrence of breast cancer. Am J Epidemiol 1999; 149:5–12.
7. Wells AJ. Breast cancer, cigarette smoking, and passive smoking. Am J Epidemiol 1991;133:208–10.
8. Hirayama T. Epidemiología y factores de riesgo del cáncer de mama. In: Diaz-Faes J, ed. Cáncer de mama. Avances en diag-

nóstico y tratamiento. (In Spanish). León, Spain: Santiago García, 1990:21–38.
9. Wells AJ. Re: "Breast cancer, cigarette smoking, and passive smoking." (Letter). Am J Epidemiol 1998;147:991–2.
10. Morabia A, Bernstein MS. A review of the relation of smoking (active and passive) to breast cancer. J Women Cancer 2000; 2:1–9.
11. Ambrosone CB, Freudenheim JL, Graham S, et al. Cigarette smoking, *N*-acetyltransferase 2 genetic polymorphisms, and breast cancer risk. JAMA 1996;276:1494–501.
12. Hunter DJ, Hankinson SE, Hough H, et al. A prospective study of *NAT2* acetylation genotype, cigarette smoking, and risk of breast cancer. Carcinogenesis 1997;18:2127–32.
13. Hunter DJ, Gertig DM, Spiegelman D. Re: "A prospective study of *NAT2* acetylation genotype, cigarette smoking, and risk of breast cancer." (Letter). Carcinogenesis 1998;19:1705.
14. Millikan RC, Pittman GS, Newman B, et al. Cigarette smoking, *N*-acetyltransferases 1 and 2, and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1998;7:371–8.
15. Morabia A, Bernstein M, Heritier S. Re: "Smoking and breast cancer: reconciling the epidemiologic evidence by accounting for passive smoking and/or genetic susceptibility." (Letter). Am J Epidemiol 1998;147:992–3.
16. Cascorbi I, Drakoulis N, Brockmoller J, et al. Arylamine *N*-acetyltransferase (*NAT2*) mutations and their allelic linkage in unrelated Caucasian individuals: correlation with phenotypic activity. Am J Hum Genet 1995;57:581–92.
17. Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. Epidemiology 1992;3:452–6.
18. Vineis P, Bartsch H, Caporaso N, et al. Genetically based *N*-acetyltransferase metabolic polymorphism and low-level environmental exposure to carcinogens. Nature 1994;369:154–6.
19. Hein DW, Doll MA, Rustan TD, et al. Metabolic activation and deactivation of arylamine carcinogens by recombinant human NAT1 and polymorphic NAT2 acetyltransferases. Carcinogenesis 1993;14:1633–8.
20. Hein DW, Doll MA, Fretland AJ, et al. Rodent models of the human acetylation polymorphism: comparisons of recombinant acetyltransferases. Mutat Res 1997;376:101–6.
21. Vineis P, McMichael A. Interplay between heterocyclic amines in cooked meat and metabolic phenotype in the etiology of colon cancer. Cancer Causes Control 1996;7:479–86.
22. Snyderwine EG. Dietary heterocyclic amines and breast cancer. Women Cancer 1999;1:21–9.
23. Zheng W, Gustafson DR, Sinha R, et al. Well-done meat intake and the risk of breast cancer. J Natl Cancer Inst 1998;90:1724–9.
24. Curtin F, Morabia A, Bernstein M. Lifetime exposure to environmental tobacco smoke among urban women: differences by socioeconomic class. Am J Epidemiol 1998;148:1040–7.
25. Petrakis NL, Gruenke LD, Beelen TC, et al. Nicotine in breast fluid of nonlactating women. Science 1978;199:303–5.
26. Petrakis NL. Nipple aspirate fluid in epidemiologic studies of breast disease. Epidemiol Rev 1993;15:188–95.
27. Palmer JR, Rosenberg L, Clarke EA, et al. Breast cancer and cigarette smoking: a hypothesis. Am J Epidemiol 1991;134: 1–13.
28. Lash TL, Aschengrau A. Active and passive cigarette smoking and the occurrence of breast cancer. Am J Epidemiol 1999; 149:5–12.
29. Ishibe N, Hankinson SE, Colditz GA, et al. Cigarette smoking, cytochrome P450 1A1 polymorphisms, and breast cancer risk in the Nurses' Health Study. Cancer Res 1998;58:667–71.
30. Gammon MD, Hibshoosh H, Terry MB, et al. Cigarette smoking and other risk factors in relation to p53 expression in breast cancer among young women. Cancer Epidemiol Biomarkers Prev 1999;8:255–63.
31. Kelsey JL. Breast cancer epidemiology: summary and future directions. Epidemiol Rev 1993;15:256–63.
32. Brunet JS, Ghadirian P, Rebbeck TR, et al. Effect of smoking on breast cancer in carriers of mutant *BRCA1* or *BRCA2* genes. J Natl Cancer Inst 1998;90:761–6.
33. Ford D, Easton DF, Peto J. Estimates of the gene frequency of *BRCA1* and its contribution to breast and ovarian cancer incidence. Am J Hum Genet 1995;57:1457–62.