Title: N-acetyltransferase 2 polymorphism and breast cancer risk with smoking: a case control study in Japanese women

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Abstract

Background: Recent studies have suggested that the association between smoking and breast cancer risk might be modified by polymorphisms in the N-acetyltransferase 2 gene (NAT2). Most of these studies were conducted in Western countries, with few reports from East Asia.

Methods: We conducted a case-control study of 511 breast cancer cases and 527 unmatched healthy controls from December 2010 to November 2011 in Japan. Unconditional logistic regression was used to analyze the association of smoking with breast cancer risk stratified by NAT2 phenotype.

Results: In this population, 11% of the cases and 10% of the controls were classified as a slow acetylator phenotype. Compared to never smokers, current smokers had an increased breast cancer risk in multivariate analysis (odds ratio (OR) = 2.27, 95% confidence interval (95%CI) = 1.38-3.82). Subgroup analyses of menopausal status indicated the same tendency. Subgroup analyses of NAT2 phenotype, the ORs in both of rapid and slow acetylator phenotype subgroups were comparable, and no interactions were observed between smoking status and NAT2 phenotype ($p = 0.97$). A dose-dependent effect of smoking on breast cancer risk was seen for the rapid acetylator phenotype, but not for the slow acetylator phenotype.

Conclusion: Given the high frequency of the rapid acetylator phenotype, these results show that smoking is a risk factor for breast cancer among most Japanese women. It may be of little significance to identify the NAT2 phenotype in the Japanese population.

Background

The association between smoking and breast cancer risk has been investigated in a number of epidemiological studies, with controversial outcomes. In a recent review by the International Agency for Research on Cancer [1], it was concluded that "the observed associations are weaker and less consistent for breast cancer than for other tobacco-related cancers." Recent epidemiological studies of smoking and breast cancer have also examined polymorphism in the N-acetyltransferase 2 (NAT2) gene. NAT2 is one of two human N-acetyltransferases that acetylate and detoxify aromatic amines, which are carcinogens in tobacco smoke. The hypothesis that females with a slow acetylator phenotype may be more susceptible to onset of breast cancer due to smoking compared to those with a rapid acetylator phenotype has been examined in several studies, but with inconsistent results.

In a meta-analysis [2], Ambrosone et al. showed a significantly increased breast cancer risk for ever smokers compared to never smokers among females with a slow acetylator phenotype (relative risk (RR) = 1.27, 95% confidential interval (95%CI) = 1.16-1.40), but not among those with a rapid acetylator phenotype (RR = 1.05, 95%CI = 0.95-1.17). In contrast, in a large case-control study nested in cohorts [3], Cox et al. showed that long-term smoking significantly increases breast cancer risk, regardless of the NAT2 acetylator phenotype. Most studies have been conducted in Western countries, and there are few reports from East Asia. Therefore, we conducted a case-control study in Japan to investigate the interaction between NAT2 polymorphisms and breast cancer risk caused by smoking in the Japanese population, which is genetically different from the Western population.

Materials and Methods

Subjects

A multicenter population-based case-control study was conducted between December 2010 and November 2011 in Japan. The cases were consecutive patients with noninvasive or invasive breast cancer aged over 20 years old who were treated at Okayama University Hospital, Okayama Rousai Hospital and Mizushima Kyodo Hospital in Okayama and at Kagawa Prefecture Central Hospital in Kagawa. The controls were women aged over 20 years old without a history of breast cancer who underwent breast cancer screening at Mizushima Kyodo Hospital in Okayama and at Kagawa Prefectural Cancer Detection Center in Kagawa. All subjects gave written informed consent before enrollment in the study. A blood sample (5 ml) was collected from each subject to use for single nucleotide polymorphism (SNP) analysis. Subjects were also given questionnaires that they completed at home and mailed back to Okayama University Hospital. The study was approved by the institutional ethics committee on human research at Okayama University.

The questionnaires collected information on age, height, body weight, smoking history, alcohol intake, habitual exercise, menstruation status, age at menarche, age at first full term pregnancy, number of children, breastfeeding, age at menopause, history of hormone replacement therapy, history of oral contraceptive use, history of benign breast disease, familial history of breast cancer (including mother, sisters and daughters). Smoking data included the amount of daily smoking (cigarettes/day), the duration of smoking (in years), and the time since cessation of smoking (in years). Body mass index (BMI) was calculated as body weight/square of height.

Selection of SNPs

To distinguish NAT2*5, NAT2*6 and NAT2*7, which are slow acetylator alleles, from NAT2*4, a rapid acetylator allele [4], three SNPs (T341C, G590A, G857A) were analyzed, as in Cox et al [3]. NAT2*14 is also a slow acetylator allele, but its allele frequency is very low, except in the African population [5]. Therefore, we did not check for NAT2*14 in this study.

SNP genotyping

Genomic DNA was isolated from whole blood with a TaqMan® Sample-to-SNP™ kit (Applied Biosystems, Foster City, CA, USA). Samples were analyzed by a TaqMan genotyping assay using the StepOne™ real-time polymerase chain reaction (PCR) system (Applied Biosystems) in a 96-well plate that included four blank wells as negative controls. The PCR consisted of an initial denaturation step at 95°C for 20 s, followed by 40 cycles at 95°C for 3 s, and 60°C for 20 s. PCR products were analyzed by StepOne™ Software ver. 2.3 (Applied Biosystems). To assess the quality of genotyping, we conducted re-genotyping of a randomly selected 5% of samples and obtained 100% agreement.

Definition of the NAT2 acetylation phenotype

Subjects homozygous for slow acetylator alleles were classified as a slow acetylator phenotype, and those who were carriers of at least one rapid acetylator allele were classified as a rapid acetylator phenotype, consistent with the definition in previous studies [2, 3]. Accordance with the Hardy-Weinberg equilibrium in analysis of SNPs was checked using a chi-squared test.

Statistical analysis

Unconditional logistic regression analysis was used to estimate odds ratio (OR) and 95%CI for breast cancer risk with smoking status (never / former / current). Two models were used, with the first adjusted only for age (continuous), and the second adjusted for age (continuous) [3, 6-15], body mass index (continuous) [3, 6, 7, 10, 11, 15], number of children (continuous) [3, 7, 8, 10, 11, 14, 15], menopausal status (pre / postmenopausal) [10, 11, 13], family history of breast cancer (yes / no) [6-11, 14, 15], and habitual drinking (never / at some time) [8, 10, 11, 14]. Subjects were excluded from the logistic regression if any of the adjusting covariates were missing. Age at menopause and age at first full term pregnancy were excluded as covariates for the second model analysis due to a number of missing values. Durations of cigarette smoking and pack-years of smoking were calculated from data collected by questionnaire. Factors were analyzed as both categorical and continuous variables. Smoking duration was stratified into <15 and ≥15 years, and pack-years into <20 and ≥20, as in Ambrosone et al [2]. All data were also analyzed after stratification by menopausal status and NAT2 phenotype. Interactions between NAT2 phenotype and smoking were calculated by including the multiplicative terms in the models and evaluated by likelihood ratio test. All analyses were performed in Statistical Analysis System JMP ver. 11.0.0. (SAS Institute), with significance defined as $p<0.05$.

Results

A total of 515 patients with breast cancer cases and 528 healthy controls agreed to participate in the study and gave written informed consent. Among these subjects, 1 case and 1 control withdrew consent, and blood samples could not be obtained in 3 cases. Thus, 511 cases and 527 controls were included in the analyses. 51 cases and 32 controls never returned self-administered questionnaires, and some cases were complemented information by their medical records.

Baseline characteristics and potential risk factors for breast cancer are shown in Table 1. Cases were older than controls (mean age ± standard deviation (SD): 55.0 ± 12.3 vs. 52.7 ± 11.0 years, p = 0.002 by Student t test). Age, height, age at menarche, number of children, history of oral contraceptive use, and habitual exercise showed significant differences between cases and controls in an unadjusted model.

Frequencies of NAT2 genotypes are shown in Table 2. The NAT2 genotype frequencies in controls conformed to the Hardy-Weinberg equilibrium (T341C, p=1; G590A, p=0.08; G857A, p=0.5 by chi-square test). The NAT2 slow acetylator phenotype accounted for 11% of cases and 10% of controls, and was not associated with a significantly increased risk of breast cancer compared to the rapid acetylator phenotype (OR = 1.15, 95%CI = 0.77-1.71). The mean age at menarche for the slow acetylator phenotype was older than that for the rapid acetylator phenotype (13.60 ± 0.15 vs. 13.04 ± 0.05 years, p = 0.0006 by Student t test). Other potential confounders listed in Table 1 did not differ significantly between the rapid and slow acetylator phenotypes (data not shown).

Breast cancer risk associated with smoking history is shown in Table 3. Compared to never smokers, current smokers had an increased breast cancer risk in multivariate analysis (OR = 2.27, 95%CI = 1.38-3.82).

Subgroup analyses of menopausal status were shown in Table 4. Regardless of menopausal status, there were roughly same tendencies seen in the analyses of total study population, except for former smoker in postmenopausal group. Compared to never smokers, former smokers were associated with lower risk of breast cancer (OR = 0.41, 95%CI = 0.16-0.94).

In the rapid acetylator phenotype subgroup analysis shown in Table 5, current smokers had a similar increased breast cancer risk (OR = 2.36, 95%CI = 1.41-4.03), but in the slow acetylator phenotype subgroup, there was a very wide confidence interval and no significant difference between current smokers and never smokers (OR = 2.60, 95%CI = 0.31-54.1). No interactions were observed between smoking status and NAT2 phenotype (p = 0.97).

In analyses of the dose-dependent effect of smoking on breast cancer risk, only the heavy smoker group differed significantly compared to never smokers. The OR for smoking for >15 years was 1.65 (95%CI = 1.03-2.67) and that for smoking for >20 pack-years was 2.48 (95%CI = 1.21-5.37). Differences between light smokers and never smokers were not significant (Table 3). The same tendencies were found for the rapid acetylator phenotype (OR = 1.65, 95%CI = 1.01-2.72; OR = 2.42, 95%CI = 1.17-5.30, respectively, shown in Table 5). For the slow acetylator phenotype, all data had very wide confidence intervals and no significance.

In analyses of continuous variables, weak associations were observed between smoking extent and breast cancer risk (Table 3). The OR for each additional year of smoking was 1.02 (95%CI = 1.01-1.04) and that for each additional pack-year was 1.03 (95%CI = 1.01-1.06). In subgroup analyses, the rapid acetylator phenotype showed almost the same results, but there were no significant associations for the slow acetylator phenotype.

Discussion

This study showed a significant association between breast cancer and smoking. Compared to never smoker, current smoker had a significantly increasing risk with breast cancer. And, dose-response relationship was observed between the risk and smoking dosage. These tendencies were consistent regardless of menopausal status and NAT2 phenotype.

Compared with previous studies [2, 3], we obtained two important findings. First, there is a difference in the phenotype distribution between the Western and Japanese populations. A review of population differences in allele frequencies by Kurose et al. showed NAT2*5: 0.425 - 0.498, NAT2*6: 0.264 - 0.295, and NAT2*7: 0.013 - 0.026 in Europeans and Caucasians, and NAT2*5: 0.014, NAT2*6: 0.205, and NAT2*7: 0.088 in Japanese [4]. These data are consistent with the phenotype distributions in the current study and previous studies [2, 3].

The second finding in this study was a significant association of breast cancer risk and smoking for the rapid acetylator phenotype. Studies in Western countries [2, 3] are inconsistent with regard to the increase in breast cancer risk attributed to smoking among subjects with a rapid acetylator phenotype, but did show a consistent risk for the slow acetylator phenotype. Based on these studies [2, 3], a larger sample size for the slow acetylator phenotype in our study may also have produced a positive association between breast cancer risk and smoking.

However, almost no association was observed for the slow acetylator phenotype. This might be because of small sample size of slow acetylator phenotype. This is one of the limitations in the study.

A second limitation is the hospital-based case-control study design, in which we cannot rule out selection bias associated with cancer screening. Breast cancer screening rates in

Japan is about 30-40% [16]. In general, health-conscious women are more likely to visit cancer screening and less likely to smoke. Therefore cancer screening visitors cannot represent an entire Japanese population.

A third limitation is the recall bias of smoking status and smoking dose that derived from self-administered questionnaire. Especially, compared to the case group that could be complemented information by their medical records, the control group might be calculated more inaccurately. The low odds ratio of former smokers in postmenopausal group might be due to this kind of biases.

In a systematic review of breast cancer risk with smoking in Japan [17], it was concluded that "tobacco smoking possibly increases the risk of breast cancer in the Japanese population." This report included three cohort and eight case-control studies, and there were significant associations only in one cohort and four case-control studies; therefore, a definitive association has not been shown.

The limitation of the case-control study prevents establishment of strong evidence for an increased risk of breast cancer due to smoking based on the NAT2 phenotype. Nonetheless, this study is of some importance, in that it suggests that in Japan there is little significance in identifying subjects with greater susceptibility to smoking by determining the NAT2 status, as done in Western countries.

Conclusion

Smoking might be associated with a breast cancer risk in most Japanese women, and identification of the NAT2 phenotype may not be of importance in the Japanese population.

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Conflict of interest: No potential conflicts of interest were disclosed.

References

1. International Agency for Research on Cancer. Personal Habits and Indoor Combustions. IARC monographs on the evaluation of carcinogenic risks to humans, vol 100E. Lyon: International Agency for Research on Cancer, 2012. 93p.
2. Ambrosone CB, Kropp S, Yang J, Yao S, Shields PG, Chang-Claude J. Cigarette smoking, N-acetyltransferase 2 genotypes, and breast cancer risk: pooled analysis and meta-analysis. Cancer Epidemiol Biomarkers Prev. 2008;17:15–26.
3. Cox DG. Dostal L, Hunter DJ, Marchand LL, Hoover R, Ziegler RG, et al. N-acetyltransferase 2 polymorphisms, tobacco smoking and breast cancer in the breast and prostate cancer cohort consortium. Am J Epidemiol 2011;174:1316–22.
4. Kurose K, Sugiyama E, Saito Y. Population differences in major functional polymorphisms of pharmacokinetics/pharmacodynamics-related genes in Eastern Asians and Europeans: implications in the clinical trials for novel drug development. Drug Metab Pharmacokin 2012;27:9–54.
5. Sabbagh A, Langaney A, Darlu P, Gerard N, Krishnamoorthy R, Poloni ES. Worldwide distribution of NAT2 diversity: implications for NAT2 evolutionary history. BMC Genet 2008;9:21.
6. Ambrosone CB, Freudenheim JL, Graham S, Marshall JR, Vena JE, Brasure JR, et al. Cigarette smoking, N-acetyltransferase 2 genetic polymorphisms, and breast cancer risk. JAMA 1996;276:1494-501.
7. Hunter DJ, Hankinson SE, Hough H, Gertig DM, Garcia-Closas M, Spiegelman D, et al. A prospective study of NAT2 acetylation genotype, cigarette smoking, and risk of breast cancer. Carcinogenesis 1997;18:2127‑32.
8. Millikan RC, Pittman GS, Newman B, Tse CJ, Selmin O, Rockhill B,et al. Cigarette

smoking, N-acetyltransferases 1 and 2, and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1998;7:371‑8.

9. Morabia A, Bernstein MS, Bouchardy I, Kurtz J, Morris MA. Breast cancer and active and passive smoking: the role of the Nacetyltransferase 2 genotype. Am J Epidemiol 2000;152:226‑32.
10. Krajinovic M, Ghadirian P, Richer C, Sinnett H, Gandini S, Perret C, et al. Genetic susceptibility to breast cancer in french-canadians: role of carcinogen-metabolizing enzymes and gene-environment interactions. Int J Cancer 2001;92: 220‑5.
11. Chang-Claude J, Kropp S, Jager B, Bartsch H, Risch A. Differential effect of NAT2 on the association between active and passive smoke exposure and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11:698‑704.
12. van der Hel OL, Bueno-de-Mesquita HB, van Gils CH, Roest M, Slothouber B, Grobbee DE, et al. Cumulative genetic defects in carcinogen metabolism may increase breast cancer risk (the netherlands). Cancer Causes Control 2005;16: 675‑81.
13. Alberg AJ, Daudt A, Huang H, Hoffman SC, Comstock GW, Helzlsouer KJ, et al. N-acetyltransferase 2 (NAT2) genotypes, cigarette smoking, and the risk of breast cancer. Cancer Detect Prev 2004;28:187‑93.
14. Sillanpaa P, Hirvonen A, Kataja V, Eskelinen M, Kosma V, Uusitupa M, et al. NAT2 slow acetylator genotype as an important modifier of breast cancer risk. Int J Cancer 2005;114:579‑84.
15. Lissowska J, Brinton LA, Zatonski W, Blair A, Bardin-Mikolajczak A, Peplonska B, et al. Tobacco smoking, NAT2 acetylation genotype and breast cancer risk. Int J Cancer 2006;119: 1961‑9.
16. Annual report of breast cancer screening rates. Ministry of Health, Labour and Welfare

(MHLW). http://www.mhlw.go.jp/

stf/houdou/2r9852000001igt0-att/2r9852000001iguh.pdf. Accessed 19 Jan 2016.

17. Nagata C, Mizoue T, Tanaka K, Tsuji I, Wakai K, Inoue M, et al. Tobacco smoking and breast cancer risk: an evaluation based on a systematic review of epidemiological evidence in the Japanese population. Jpn J Clin Oncol 2006;36:387–94.

Table 1 Baseline characteristic of the study population

	Case (N=511)	Control (N=527)
	Mean (SD) or n (%)	Mean (SD) or n (%)
Age (years)	55.0 (12.3)	52.7 (11.0)
<40	54 (11%)	73 (14%)
40-59	267 (52%)	314 (60%)
60-79	183 (36%)	136 (26%)
≥80	6 (1%)	1 (0%)
unknown	1 (0%)	3 (1%)
Height (cm)	155.2 (5.8)	156.0 (5.6)
Weight (kg)	54.7 (8.9)	54.3 (8.4)
Body mass index (kg/m^2)	22.7 (3.6)	22.3 (3.2)
<18.5	39 (8%)	32 (6%)
18.5-24.9	348 (68%)	372 (71%)
25-29.9	85 (17%)	73 (14%)
30-34.9	18 (4%)	13 (2%)
≥35	3 (1%)	1 (0%)
unknown	18 (4%)	36 (7%)
Age at menarche (years)	13.3 (1.6)	12.9 (1.4)
Age at menopause (years)	49.9 (4.4)	49.9 (4.7)
Age at first full term pregnancy (years)	26.1 (4.2)	26.0 (3.4)
Number of children	1.68 (1.10)	1.87 (1.05)
Menopausal status		
postmenopausal	303 (59%)	294 (56%)
premenopausal	207 (41%)	231 (44%)
unknown	1 (0%)	2 (0%)
Breast feeding		
no	134 (26%)	113 (21%)
yes	352 (69%)	377 (72%)
unknown	25 (5%)	37 (7%)
History of benign breast disease		
no	372 (73%)	375 (71%)
yes	97 (19%)	101 (19%)
unknown	42 (8%)	51 (10%)
Family history of breast cancer		
no	411 (80%)	399 (76%)
yes	59 (12%)	57 (11%)
unknown	41 (8%)	71 (13%)
History of hormone replacement therapy		
no	441 (86%)	441 (84%)
yes	38 (7%)	47 (9%)
unknown	32 (6%)	39 (7%)
History of oral contraceptive use		
no	453 (89%)	444 (84%)
yes	22 (4%)	41 (8%)
unknown	36 (7%)	42 (8%)
Habitual drinking		
no	247 (48%)	234 (44%)
yes	237 (46%)	258 (49%)
unknown	27 (5%)	35 (7%)
Habitual exercise		
no	255 (50%)	231 (44%)
yes	214 (42%)	262 (50%)
unknown	42 (8%)	34 (6%)

SD standard deviation

Table 2 Frequencies of the NAT2 genotype and association with breast cancer risk

	Case (N=511) n (%)	Control (N=527) n (%)	OR	95%CI
Rapid acetylator phenotype				
WT/WT	237 (46%)	279 (53%)		
WT/*T341C*	13 (3%)	7 (1%)		
WT/*G590A*	138 (27%)	118 (22%)		
WT/*G857A*	67 (13%)	72 (14%)		
Total	**455 (89%)**	**476 (90%)**	Reference	
Slow acetylator phenotype				
T341C/T341C	0 (0%)	0 (0%)		
T341C/G590A	1 (0%)	6 (1%)		
T341C/G857A	0 (0%)	1 (0%)		
G590A/G590A	26 (5%)	25 (5%)		
G590A/G857A	25 (5%)	17 (3%)		
G857A/G857A	4 (1%)	2 (0%)		
Total	**56 (11%)**	**51 (10%)**	1.15	(0.77-1.71)

NAT2 N-acetyltransferase 2, *OR* odds ratio, *CI* confidence interval, *WT* wild-type allele

Table 3 Smoking history and breast cancer risk

	Case (N=511) Mean (SD) or n (%)	Control (N=527) Mean (SD) or n (%)	Age-adjusted OR (95%CI)	Multivariate OR (95%CI)*
Never smoker	411 (80%)	414 (81%)	Reference	Reference
Former smoker[b]	30 (6%)	49 (10%)	0.67 (0.41-1.07)	0.63 (0.35-1.09)
Current smoker[b]	59 (12%)	28 (5%)	2.32 (1.45-3.78)	2.27 (1.38-3.82)
Unknown	11 (2%)	36 (7%)		
Ever smoker (Current or Former)[b]	89 (17%)	77 (15%)	1.26 (0.90-1.78)	1.28 (0.87-1.88)
Duration of smoking (categorical)[b]				
<15years	31 (6%)	35 (7%)	0.99 (0.59-1.65)	0.86 (0.47-1.54)
≥15years	57 (11%)	41 (8%)	1.50 (0.98-2.32)	1.65 (1.03-2.67)
unknown	1 (0%)	1 (0%)		
Duration of smoking (continuous), years[c]	4.1 (10.8)	2.6 (7.7)	1.02 (1.00-1.03)	1.02 (1.01-1.04)
Pack-years (categorical)[b]				
<20	50 (10%)	51 (10%)	1.08 (0.71-1.66)	1.07 (0.67-1.71)
≥20	29 (6%)	14 (3%)	2.10 (1.11-4.15)	2.48 (1.21-5.37)
unknown	10 (2%)	12 (2%)		
Pack-years (continuous)[c]	2.8 (8.4)	1.6 (5.5)	1.03 (1.01-1.05)	1.03 (1.01-1.06)

SD standard deviation, *OR* odds ratio, *CI* confidence interval

*Adjusted for age, body mass index, number of children, menopausal status, family history of breast cancer, and habitual drinking.

[b]In analyses of categorical variables, the never smoker group was used as a reference group.

[c]In analyses of continuous variables, ORs and 95%CIs were calculated for each additional year of smoking or for each additional pack-year.

Table 4 Smoking history and breast cancer risk stratified by menopausal status

	Premenopausal group				Postmenopausal group			
	Case (*N*=207)	Control (*N*=231)	Age-adjusted	Multivariate	Case (N=303)	Control (N=294)	Age-adjusted	Multivariate
	Mean (SD) or n (%)	Mean (SD) or n (%)	OR (95%CI)	OR (95%CI)[a]	Mean (SD) or n (%)	Mean (SD) or n (%)	OR (95%CI)	OR (95%CI)[a]
Never smoker	157 (76%)	167 (72%)	Reference	Reference	254 (84%)	247 (84%)	Reference	Reference
Former smoker[b]	19 (9%)	23 (10%)	0.92 (0.47-1.76)	0.85 (0.39-1.81)	11 (4%)	26 (9%)	0.46 (0.21-0.94)	0.41 (0.16-0.94)
Current smoker[b]	30 (14%)	18 (8%)	1.76 (0.95-3.36)	1.80 (0.93-3.57)	29 (10%)	10 (3%)	3.46 (1.68-7.75)	2.89 (1.33-6.71)
Unknown	1 (0.5%)	23 (10%)			9 (3%)	11 (4%)		
Ever smoker (Current or Former)[b]	49 (24%)	41 (18%)	1.29 (0.81-2.09)	1.30 (0.77-2.22)	40 (13%)	36 (12%)	1.26 (0.77-1.31)	1.17 (0.67-2.06)
Duration of smoking (categorical)[b]								
<15years	19 (9%)	21 (9%)	0.94 (0.48-1.84)	0.79 (0.36-1.66)	12 (4%)	14 (5%)	1.05 (0.46-2.38)	0.83 (0.31-2.12)
≥15years	29 (14%)	20 (9%)	1.61 (0.87-3.04)	1.83 (0.93-3.69)	28 (9%)	21 (7%)	1.44 (0.79-2.66)	1.45 (0.75-2.86)
unknown	1 (0%)	(0%)			(0%)	1 (0%)		
Duration of smoking (continuous), years[c]	4.2 (9.1)	2.8 (7.1)	1.02 (1.00-1.05)	1.03 (1.00-1.06)	4.1 (11.8)	2.4 (8.2)	1.02 (1.00-1.04)	1.02 (1.00-1.04)
Pack-years (categorical)[b]								
<20	33 (16%)	29 (13%)	1.24 (0.71-2.16)	1.26 (0.68-2.35)	16 (5%)	22 (7%)	0.87 (0.43-1.72)	0.78 (0.36-1.64)
≥20	9 (4%)	6 (3%)	1.60 (0.56-4.87)	1.98 (0.63-6.81)	20 (7%)	8 (3%)	2.68 (1.19-6.64)	2.85 (1.12-8.25)
unknown	7 (3%)	6 (3%)			4 (1%)	6 (2%)		
Pack-years (continuous)[c]	2.9 (7.8)	1.7 (4.9)	1.03 (1.00-1.07)	1.04 (1.00-1.08)	2.8 (8.9)	1.5 (5.9)	1.03 (1.00-1.06)	1.03 (1.00-1.06)

NAT2 N-acetyltransferase 2, *SD* standard deviation, *OR* odds ratio, *CI* confidence interval

[a]Adjusted for age, body mass index, number of children, family history of breast cancer, and habitual drinking.

[b]In analyses of categorical variables, the never smoker group was used as a reference group.

[c]In analyses of continuous variables, ORs and 95%CIs were calculated for each additional year of smoking or for each additional pack-year.

Table 5 Smoking history and breast cancer risk stratified by NAT2 status

	Rapid acetylator phenotype				Slow acetylator phenotype			
	Case (N=455) Mean (SD) or n (%)	Control (N=476) Mean (SD) or n (%)	Age-adjusted OR (95%CI)	Multivariate OR (95%CI)[a]	Case (N=56) Mean (SD) or n (%)	Control (N=51) Mean (SD) or n (%)	Age-adjusted OR (95%CI)	P for interaction[d]
Never smoker	365 (80%)	375 (79%)	Reference	Reference	46 (82%)	39 (76%)	Reference	0.97
Former smoker[b]	26 (6%)	43 (9%)	0.67 (0.39-1.11)	0.60 (0.32-1.10)	4 (7%)	6 (12%)	0.66 (0.15-2.58)	
Current smoker[b]	56 (12%)	27 (6%)	2.33 (1.44-3.85)	2.36 (1.41-4.03)	3 (5%)	1 (2%)	2.60 (0.31-54.1)	
Unknown	8 (2%)	31 (7%)			3 (5%)	5 (10%)		
Ever smoker (Current or Former)[b]	82 (18%)	70 (15%)	1.30 (0.91-1.86)	1.33 (0.90-1.99)	7 (13%)	7 (14%)	0.96 (0.30-3.15)	0.63
Duration of smoking (categorical)[b]								0.31
<15years	30 (7%)	31 (7%)	1.09 (0.64-1.86)	1.00 (0.54-1.83)	1 (2%)	4 (8%)	0.25 (0.01-1.82)	
≥15years	52 (11%)	38 (8%)	1.51 (0.96-2.37)	1.65 (1.01-2.72)	5 (9%)	3 (6%)	1.56 (0.35-8.18)	
unknown	(0%)	1 (0%)			1 (2%)	(0%)		
Duration of smoking (continuous), years[c]	4.3 (10.9)	2.6 (7.8)	1.02 (1.01-1.04)	1.02 (1.01-1.04)	3.0 (9.6)	2.4 (7.2)	1.01 (0.96-1.07)	0.82
Pack-years (categorical)[b]								0.49
<20	46 (10%)	46 (10%)	1.12 (0.72-1.75)	1.14 (0.69-1.88)	4 (7%)	5 (10%)	0.79 (0.18-3.36)	
≥20	27 (6%)	13 (3%)	2.16 (1.11-4.38)	2.42 (1.17-5.30)	2 (4%)	1 (2%)	1.56 (0.14-34.5)	
unknown	9 (2%)	11 (2%)			1 (2%)	1 (2%)		
Pack-years (continuous)[c]	2.9 (8.5)	1.6 (5.5)	1.03 (1.01-1.05)	1.03 (1.01-1.06)	2.1 (7.9)	1.6 (5.0)	1.01 (0.95-1.09)	0.97

NAT2 N-acetyltransferase 2, *SD* standard deviation, *OR* odds ratio, *CI* confidence interval

[a]Adjusted for age, body mass index, number of children, menopausal status, family history of breast cancer, and habitual drinking.

[b]In analyses of categorical variables, the never smoker group was used as a reference group.

[c]In analyses of continuous variables, ORs and 95%CIs were calculated for each additional year of smoking or for each additional pack-year.

[d]Interaction P value were calculated by using multiplicative interaction term in the multivariate models.