N-Acetyltransferase 1 Genetic Polymorphism, Cigarette Smoking, Well-Done Meat Intake, and Breast Cancer Risk[1]

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Abstract

***N*-Acetyltransferase 1 (NAT1), encoded by the polymorphic *NAT1* gene, has been shown to be one of the major enzymes in human breast tissue that activates aromatic and heterocyclic amines. Humans are mainly exposed to these carcinogens through cigarette smoking and consumption of well-done meat. To test the hypothesis that variations in the *NAT1* gene are related to breast cancer risk, particularly among women who smoke or consume high levels of well-done meat, a nested case-control study was conducted in a prospective cohort study of 41,837 postmenopausal Iowa women. Information on cigarette smoking and other breast cancer risk factors was obtained at the baseline survey conducted in 1986. DNA samples and information on the consumption of well-done meat were obtained, in the case-control study, from breast cancer cases diagnosed from 1992 to 1994 and a random sample of cancer-free cohort members. Genomic DNA samples obtained from 154 cases and 330 controls were assayed for 11 *NAT1* alleles (*NAT1*3, *4, *5, *10, *11, *14, *15, *16, *17, *19,* and **22*). The *NAT1*4* allele was the predominant allele observed in this study population, accounting for 73.2% (72.4% in cases *versus* 73.8% in controls) of the total alleles analyzed. Compared to controls, breast cancer cases had a slightly higher frequency of the *NAT1*10* allele (18.8% in cases *versus* 17.3% in controls) and a substantially higher frequency of the *NAT1*11* allele (3.6% *versus* 1.2%). In multivariate analyses, we found a 30% [95% confidence interval (CI) = 0.8–1.9] elevated risk of breast cancer associated with the *NAT1*10* allele and a nearly 4-fold (95% CI = 1.5–10.5) elevated risk associated with the *NAT1*11* allele. The positive association of breast cancer with the *NAT1*11* allele was more evident among smokers [odds ratio (OR) = 13.2, 95% CI = 1.5–116.0] and those who consumed a high level of red meat (OR = 6.1, 95% CI = 1.1–33.2) or consistently consumed their red meat well done (OR = 5.6, 95% CI = 0.5–62.7). The association of the *NAT1*10* allele with breast cancer was mainly confined to former smokers (OR = 3.3, 95% CI = 1.2–9.5). These findings are consistent with a role for the *NAT1* gene in the etiology of human breast cancer.**

Introduction

Aromatic and heterocyclic amines have been shown in animal studies to induce tumors of various organs (1–3), including those of the mammary glands (2–4). Humans are mainly exposed to these carcinogens through cigarette smoking and consumption of well-done meat (5–8). We have shown recently in a case-control study that well-done meat intake may be an important risk factor for breast cancer (9). Similar findings have been reported elsewhere, although there are some inconsistencies among the studies (10–14). The results on the association between cigarette smoking and breast cancer risk have been conflicting, with some studies reporting increased risk of breast cancer among smokers and others reporting no association or even a reduced risk (15–19).

Part of the inconsistencies observed in previous studies may reflect that only a few of them have investigated potential modifying effects of relevant carcinogen-metabolizing enzymes on the associations of breast cancer with cigarette smoking and/or consumption of well-done meat (20–23). *N*-Acetyltransferases are believed to be important in the activation and detoxification of aromatic and heterocyclic amines (24–26). In humans, two *N*-acetyltransferases (NAT1[5] and NAT2) have been identified (26–28). The *NAT2* gene is known to be polymorphic in humans (26, 27). Recently, the *NAT1* gene has also been shown to be polymorphic (29). Both NAT1 and NAT2 have been shown to catalyze the metabolic activation of aromatic and heterocyclic amine carcinogens (24, 30–32). Several epidemiological studies, including our own study among postmenopausal Iowa women (23), have evaluated the association

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[5] The abbreviations used are: NAT1 and NAT2, *N*-acetyltransferase 1 and 2, respectively; IWHS, Iowa Women's Health Study; OR, odds ratio; CI, confidence interval.

between *NAT2* polymorphisms and breast cancer risk, but the results from these studies have been inconclusive (20–23, 33). In 1996, Sadrieh *et al.* (34) reported that NAT1 but not NAT2 activity was detectable in human mammary epithelial cells. They further showed that human mammary gland catalyzed the metabolic activation of heterocyclic amines, suggesting that NAT1 may be more important than NAT2 in the etiology of breast cancer. Prompted by this observation and findings from recent studies showing the potential etiological importance of *NAT1* in other human cancers (35, 36), we conducted a case-control study among participants in the IWHS to investigate whether *NAT1* polymorphisms associate with breast cancer risk and, further, whether the association is modified by cigarette smoking or consumption of well-done meat.

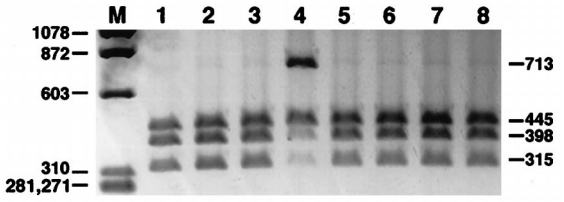

Fig. 1. Gel electrophoresis following RFLP analysis of the 1158-bp *NAT1* PCR product. *Lane M*, molecular weight marker ϕX174/*Hae*III. The restriction digest with *Ban*I and *Bg*/II indicates that the sample in *Lane 4* is heterozygous for the $A^{752}\rightarrow T$ substitution. The remaining samples do not have the $A^{752}\rightarrow T$ substitution on either allele. Additionally, no samples in *Lanes 1–8* have the $C^{97}\rightarrow T$ or $G^{350,351}\rightarrow C$ substitution on either allele.

Materials and Methods

IWHS. Detailed descriptions of this cohort study have been published elsewhere (37–39). Briefly, 41,836 Iowa women, ages 55–69 years, who completed a mailed questionnaire in January 1986, have been followed for mortality and cancer incidence. Virtually all IWHS participants were Caucasian women. The follow-up was accomplished through computer linkage of study participants with Iowa death certificate files, the National Death Index, and cancer diagnosis data collected by the Iowa State Health Registry, part of the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute. The self-administered questionnaire used in the 1986 baseline survey included information on diet and other major risk factors for cancer, such as cigarette smoking, reproductive factors, family history of cancer, prior medical conditions, and hormone use. At baseline, however, no blood sample was collected, and no information was obtained on usual intake of meat, cooking method, or usual doneness levels of meat. A case-control study with a supplementary survey to obtain information on consumption of meat and usual doneness level (see below) and DNA sample collection was conducted during 1995–1996 in a subset of cohort members.

Case-Control Study of Breast Cancer. Eligible cases for this study included all IWHS cohort members who had breast cancer diagnosed from January 1, 1992, to December 31, 1994 ($n = 453$). A control sample of 900 women was randomly selected from cohort members who were alive and free of cancer on January 1, 1992. Of these 900 women, 24 were excluded from the control group because they were either later found to have a breast cancer diagnosis during the 1992–1994 period ($n = 3$) or were selected to participate in other IWHS ancillary projects ($n = 21$). The design of the study was approved by the Institutional Review Board Human Subjects Committee at the University of Minnesota and the Iowa State Health Registry. Informed consent was obtained from all women who participated in this study.

All eligible subjects were asked to complete a self-administered semiquantitative food frequency questionnaire on meat consumption during the "reference" year. Three reference years were identified (1991, 1992, and 1993), corresponding, for cases, to the years immediately prior to breast cancer diagnosis. Women in the control group of the study were also divided randomly into three groups with approximately equal sample sizes to assess their dietary habits during the same three reference years. The food questionnaire included questions on the usual consumption and preparation methods of various meats. In addition, participants reported their usual preference for level of meat doneness using a series of color photographs that represented increasing levels of doneness of hamburger (four photos), beefsteak (four photos), and bacon (three photos). Photographs were labeled by number only and represented a range of doneness levels from extremely rare to very well done. Two hundred seventy-three (60.3%) of the selected cases and 659 (75.0%) controls participated. The major reasons for nonparticipation were refusal (29.1% of cases and 18.7% of controls), inability to locate (4.9% of cases and 3.8% of controls), or death prior to contact (5.7% of cases and 2.5% of controls).

Blood samples were donated by a subset of the women who participated in this supplementary mail survey. A blood collection kit, consisting of vacutainer tubes, biological specimen packaging containers and envelopes, and instructions, was mailed to all women who agreed to donate a blood sample. Study participants were instructed to contact their physicians, have their blood drawn, and return samples via express mail using preaddressed, prepaid envelopes provided by the study. Of the 930 women who completed the survey questionnaire, 488 (156 cases and 332 controls) donated a blood sample to the study, giving a response rate of 52.5% (57.1% for cases and 49.9% for controls). Blood was drawn using vacutainers containing acid citrate dextrose solution (Becton Dickinson, Franklin Lakes, NJ). Genomic DNA was extracted from peripheral blood leukocytes according to standard methods and stored at −70°C for subsequent assays.

NAT1 genotypes were determined by a PCR RFLP-based assay (40) that differentiates among eight *NAT1* alleles (*NAT1*3*, **4*, **5*, **10*, **11*, **14*, **15*, and **16*) that were identified previously in human populations. Recently, three new human *NAT1* alleles that encode proteins with reduced *N*-acetyltransferases activity have been identified: *NAT1*17*, **19*, and **22* (41, 42). *NAT1*17* contains a $C^{190}\rightarrow T$ substitution, *NAT1*19* contains a $C^{97}\rightarrow T$ substitution, and *NAT1*22* contains an $A^{752}\rightarrow T$ substitution. To distinguish among these new *NAT1* alleles, the published *NAT1* genotyping assay was modified as described below. *NAT1* genotype assignments were blind to case-control status.

To detect the $G^{350,351}\rightarrow C$, $C^{97}\rightarrow T$, and/or the $A^{752}\rightarrow T$ nucleotide substitutions, we digested 10 μl of amplified *NAT1* PCR product overnight at 37°C with *Ban*I (10 units) and *Bgl*II (5 units) in the presence of 50 mM NaCl, 10 mM Tris-HCl (pH 7.9), 10 mM $MgCl_2$, and 1 mM DTT. Samples homozygous for $C^{97}\rightarrow T$ yield bands of 398, 343, 315, and 102 bp, whereas samples homozygous for $G^{350,351}\rightarrow C$ yield 398-, 356-, 315-, and 89-bp fragments. When both alleles contain the $A^{752}\rightarrow T$ substitution, the double digest yields bands at 713 and 445 bp. When neither allele contains the $G^{350,351}\rightarrow C$, $C^{97}\rightarrow T$, and $A^{752}\rightarrow T$ nucleotide substitutions, 445-, 398-, and 315-bp bands, respectively, result (Fig. 1). To detect the $C^{190}\rightarrow T$ nucleotide substitution, we used 0.5 μl of amplified *NAT1* as

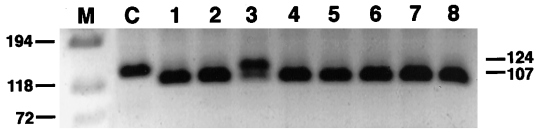

Fig. 2. Gel electrophoresis following RFLP analysis of the $C^{190}\rightarrow T$ nested PCR product. *Lane M* , molecular weight marker ϕX174/*Hae*III; *Lane C*, sample homozygous for the $C^{190}\rightarrow T$ substitution used here as a positive control. *Eco*RI digestion results indicate that the sample in *Lane 3* is heterozygous for the $C^{190}\rightarrow T$ substitution. The remaining samples do not have the $C^{190}\rightarrow T$ substitution on either allele.

the template in a 20-μl reaction containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 210 ng of primer (169–189) 5′-GATCAAGTTGTGAGAAGAA**TT**-3′ (boldface type indicates the nucleotide change made in the primer sequence to generate a partial *Eco*RI restriction site, which is underlined), 190 ng of primer (292–274) 5′-CTGGAGTGCTGTAAACATA-3′, and 0.5 unit of Taq DNA polymerase. The mixture was pretreated at 94°C for 5 min, followed by 15 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C and a final 5-min extension step at 72°C. The nested PCR product was digested with the restriction enzyme *Eco*RI (10 units) overnight at 37°C in the presence of 50 mM NaCl, 100 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, and 0.025% Triton X-100. Samples that were homozygous for the $C^{190}\rightarrow T$ nucleotide substitution resulted in the loss of the *Eco*RI restriction site, yielding a band of 124 bp instead of 107- and 17-bp bands (Fig. 2).

Of the 488 DNA samples included in the study, 4 did not amplify and 2 contained alleles that were different from those under study. Therefore, the data analysis was based on 154 cases and 328 controls. ORs were used to measure the strength of the association between exposures and cancer risk. Unconditional logistic regression was used to control for potential confounders assessed at the baseline survey and to derive adjusted ORs and 95% CIs. Red meat intake and doneness levels of these meats were used to estimate exposure to well-done meat. Intakes (in g) of hamburgers, cheeseburgers, beefsteaks, pork chops, bacon, breakfast sausage links, breakfast sausage patties, other sausages, bratwurst, and hot dogs or franks were summed to form the red meat variable. Women who reported eating hamburger, beefsteak, and bacon consistently at well- or very well-done levels were classified as the "exposed" group. These variables, along with variables on cigarette smoking, were analyzed in combination with *NAT1* genotype to evaluate potential gene-environment interactions in the etiology of breast cancer.

Results

Table 1 shows the case-control distribution and ORs for selected demographic variables and major risk factors identified for this cohort of women in the early years of follow-up. Information related to cigarette smoking and well-done meat intake, the main effects studied here, is also presented in Table 1. Among women who completed the supplementary survey, the risk of breast cancer was found to be positively associated with aging, education, family history of breast cancer, estrogen use, waist:hip ratios, smoking, and well-done meat intake, although not all elevated ORs were statistically significant. With only a few exceptions, similar associations were observed among women who provided a blood sample to the study.

The allele frequencies of the *NAT1* gene are presented in Table 2. There was no statistically significant difference in the overall distribution of allele frequency between cases and controls. *NAT1*4*, the wild-type allele, presented in nearly three-quarters of the chromosomes tested, and its frequency was comparable between cases and controls. Slightly more cases (18.8%) than controls (17.3%) had the *NAT1*10* allele. Other *NAT1* alleles were relatively uncommon in this study population. Of note, the *NAT1*11* frequency was three times more common in cases (3.6%) than in controls (1.2%).

The frequency distribution of specific *NAT1* genotypes by case-control status is presented in Table 3. Because of a small sample size and relatively low frequency of most *NAT1* variants, ORs were calculated for selected genotype groups (Table 4). The *NAT1*3* allele was uncommon, and this allele associated primarily with the wild-type *NAT1*4* to form the heterozygous *NAT1*3/*4* genotype. Therefore, *NAT1*3/*3*, *NAT1*3/*4*, and *NAT1*4/*4* were combined into one group and used as the reference group in the OR estimates of other genotypes. A 30% increase in breast cancer risk was associated with the *NAT1*10/any* genotype, but the OR was not statistically significant. No elevated risk (OR = 0.8, 95% CI = 0.3–2.4) was found for *NAT1*10* homozygotes (5 cases and 13 controls). Eleven cases (7.1%) and 7 controls (2.1%) were *NAT1*11* homozygotes or heterozygotes, with a nearly 4-fold significantly elevated risk of breast cancer. Only one *NAT1*11* homozygote and two *NAT1*10/*11* heterozygotes were identified in this study. Breast cancer was not associated with the presence of *NAT1*14*, *NAT1*15*, *NAT1*17*, or *NAT1*22*, all of which were rare alleles in this study population. Adjustment for a family history of breast cancer did not change the ORs presented in Table 4, suggesting that these *NAT1* alleles may not contribute substantially to the familial clustering of breast cancer among postmenopausal Iowa women.

To evaluate potential modifying effects of *NAT1* polymorphism on the associations of breast cancer with cigarette smoking or consumption of well-done meat, adjusted ORs were calculated according to the joint distribution of selected *NAT1* genotypes and exposure levels of these two lifestyle factors (Table 5). Cigarette smoking was not related to breast cancer risk among the combined *NAT1*3/*3*, *3/*4*, and *4/*4* genotype group. A 13-fold elevated risk (95% CI = 1.5–116.0), however, was observed for cigarette smoking among women with the *NAT1*11/any* genotype. Among the *NAT1*10/any* stratum, smoking was associated with a slightly elevated risk (OR = 1.4, 95% CI = 0.7–2.9) of breast cancer when subjects with the *NAT1*10/*11* genotype (two cases, no controls) were excluded. When these subjects were included in the analysis, a 60% elevated risk (95%CI = 0.8–3.3) was associated with cigarette smoking. Similarly, inclusion of subjects with the *NAT1*10/*11* genotype in the *NAT1*10/any* stratum increased the OR from 2.2 (95% CI = 0.7–6.6) to 3.3 (95% CI = 1.2–9.5) among those who had quit smoking >10 years ago.

High consumption of red meat was positively associated with the risk of breast cancer only among the *NAT1*11/any* stratum, with an OR of 6.1 (95% CI = 1.1–33.2) observed in the highest meat intake group. Women who reported consuming red meat at a consistently well-done level were at an increased risk of breast cancer across all genotype groups. The risk was particularly high (OR = 5.6, 95% CI = 0.5–62.7) for women with the *NAT1 *11/any* genotype who consumed red meat at a consistently well-done level. This OR, however, was not statistically significant, perhaps due to a small sample size.

Table 1 Comparison of cases and controls by selected demographic and risk factors among postmenopausal Iowa women

	Subjects who completed survey			Subjects who provided a blood sample		
	Cases (n = 273)	Controls (n = 657)	OR (95% CI)	Cases (n = 156)	Controls (n = 332)	OR (95% CI)
Age, ≥64 yr	92	189	1.26 (0.93–1.70)	46	83	1.26 (0.82–1.92)
High school education or higher	128	277	1.21 (0.91–1.61)	81	153	1.26 (0.86–1.85)
First-degree relatives with breast cancer	47	68	1.80 (1.21–2.69)	26	38	1.55 (0.90–2.66)
Ever used estrogen	125	251	1.37 (1.03–1.83)	66	143	0.97 (0.66–1.21)
Waist:hip ratio of ≥0.77	211	498	1.09 (0.78–1.52)	116	262	0.78 (0.50–1.42)
Menarche at ≤12 yr	106	278	0.87 (0.65–1.16)	61	149	0.79 (0.54–1.16)
Menopause at ≥52 yr	87	216	0.96 (0.71–1.29)	47	106	0.92 (0.61–1.39)
First live birth at ≥25 yr	70	176	0.94 (0.68–1.30)	45	88	1.12 (0.74–1.72)
Consumed ≥2.6 g of alcohol per day	70	166	1.02 (0.74–1.41)	40	84	1.02 (0.66–1.58)
Ever smoker	90	198	1.14 (0.84–1.54)	51	98	1.17 (0.78–1.76)
Quit >10 yr ago	40	72	1.40 (0.92–2.13)	24	32	1.67 (0.94–2.98)
1–14 cigarettes/day or smoked <25 yr	14	40	0.88 (0.47–1.66)	9	15	1.34 (0.57–3.15)
15 cigarettes/day and smoked >25 yr	36	85	1.07 (0.70–1.63)	18	51	0.79 (0.44–1.41)
Red meat intake						
2nd highest *vs.* lowest tertile	83	217	1.01 (0.70–1.44)	50	111	0.97 (0.60–1.59)
Highest *vs.* lowest tertile	108	224	1.27 (0.90–1.79)	62	126	1.06 (0.67–1.70)
Consistently ate meats well done	80	143	1.53 (1.10–2.12)	44	75	1.36 (0.88–2.12)

Table 2 Distribution of *NAT1* alleles by cases and controls among postmenopausal Iowa women

NAT1 allele	Cases	Controls
**3*	5 (1.6)[a]	20 (3.0)
**4*	223 (72.4)	487 (74.2)
**10*	58 (18.8)	114 (17.4)
**11*	11 (3.6)	8 (1.2)
**14*	4 (1.3)	13 (2.0)
**15*	1 (0.3)	2 (0.3)
**17*	3 (1.0)	7 (1.1)
**22*	3 (1.0)	5 (0.8)
Total[b]	308 (100)	656 (100)

[a] Number of *NAT1* alleles identified; values in parentheses represent percentages.
[b] χ^2 = 8.56; degrees of freedom = 7; P = 0.29.

Table 3 Distribution of *NAT1* genotype by cases and controls among postmenopausal Iowa women

NAT1 genotype	Cases (n = 154)		Controls (n = 328)	
	No.	%	No.	%
**3/*3*	0	0	1	0.3
**3/*4*	3	2.0	12	3.6
**3/*10*	2	1.3	3	0.9
**3/*17*	0	0	2	0.6
**3/*22*	0	0	1	0.3
**4/*4*	80	52.0	186	56.4
**4/*10*	42	27.3	79	23.9
**4/*11*	9	5.8	6	1.8
**4/*14*	4	2.6	10	3.0
**4/*15*	0	0	2	0.6
**4/*17*	2	1.3	4	1.2
**4/*22*	3	2.0	2	0.6
**10/*10*	5	3.3	13	3.9
**10/*11*	2	1.3	0	0
**10/*14*	0	0	3	0.9
**10/*15*	1	0.7	0	0
**10/*17*	1	0.7	1	0.3
**10/*22*	0	0	2	0.6
**11/*11*	0	0	1	0.3

Discussion

We found in this case-control study that the *NAT1*11* allele was associated with an ~4-fold elevated risk of breast cancer. The risk was increased particularly among women who smoked cigarettes, consumed a high level of red meat, or had a preference for consistently well-done meat. The *NAT1*10* allele was related to a slightly elevated risk of breast cancer, and this association was primarily confined to former or light smokers. These findings suggest that the *NAT1* gene may play a role in the etiology of breast cancer.

Although the association between the *NAT1*11* allele and breast or other human cancers has not been reported previously, the *NAT1*10* allele has been linked to increased risk of bladder, colon, gastric, lung, and head and neck cancers in previous case-control studies (35, 36, 43–45). Recently, Millikan *et al.* (33) reported no overall association of *NAT1* polymorphism with breast cancer risk among African-American and white women. The risk, however, was substantially elevated among postmenopausal women with the *NAT1*10* allele who had quit smoking within the past 10 years. Intriguingly, no modifying effect of the *NAT1*10* allele was observed among current smokers. That study differed from ours because their *NAT1* genotype method distinguishes only four alleles (*NAT1*4*, *NAT1*3*, *NAT1*10*, and *NAT1*11*) and the *NAT1*11* allele was identified in only 1% of cases and 2% of controls among white women and was absent in African-American women.

In humans, two *N*-acetyltransferases (NAT1 and NAT2) catalyze *N*- and *O*-acetylation of various procarcinogens, notably, aromatic and heterocyclic amines (24, 30–32). It is believed that these procarcinogens are activated via *O*-acetylation and detoxified by *N*-acetylation (26). The specificity of NAT1 and NAT2 in these two acetylation pathways, however, remains unclear. NAT2 is expressed primarily in the liver (46). If this enzyme is involved in *O*-acetylation of aromatic and heterocyclic amines, it may be less likely that the reactive form of these compounds could reach a high level in the breast or other target organs following activation in the liver. Given the extrahepatic distribution of the NAT1 enzyme (34, 47–50), this enzyme may be more important than the NAT2 enzyme in the activation of aromatic and heterocyclic amines in the target organs. Indeed, it has been shown that the *O*-acetylation of 2-amino-1-methyl-6-phenylimidazo[4,5*b*]pyridine and 2-amino-3-methylimidazo-(4,5-f)quinoline, two of the most abundant heterocyclic amines

Table 4 Adjusted ORs for the associations of breast cancer with *NAT1* genotype among postmenopausal Iowa women

NAT1 genotype	No. of cases	No. of controls	Age-adjusted OR (95% CI)	Multivariate-adjusted OR (95% CI)[a]
*NAT1*4/*4, *3/*4, *3/*3*	83	199	1.0 (reference)	1.0 (reference)
*NAT1*10/any*	53	101	1.3 (0.8–2.0)	1.3 (0.8–1.9)
*NAT1*11/any*	11	7	3.8 (1.4–10.2)	3.9 (1.5–10.5)
*NAT1*14/any*	4	13	0.7 (0.2–2.4)	0.7 (0.2–2.3)
*NAT1*15/any*	1	2	1.2 (0.1–13.4)	1.1 (0.1–12.3)
*NAT1*17/any*	3	7	1.0 (0.3–4.0)	1.1 (0.3–4.4)
*NAT1*22/any*	3	5	1.5 (0.3–6.2)	1.6 (0.4–6.7)

[a] Adjusted for age and family history of breast cancer.

Table 5 Adjusted ORs for the association of breast cancer with selected *NAT1* genotypes, cigarette smoking, and well-done meat intake among postmenopausal Iowa women

	*NAT1*4/*4, *3/*4, or *3/*3*		*NAT1*10/any*		*NAT1*11/any*	
	No. of cases/ no. of controls	OR (95% CI)[a]	No. of cases/ no. of controls	OR (95% CI)[a]	No. of cases/ no. of controls	OR (95% CI)[a]
Smoking characteristics						
Nonsmokers	54/134	1.0 (reference)	36/73	1.2 (0.7–2.0)	5/6	2.1 (0.6–7.1)
Smokers	28/63	1.1 (0.7–2.0)	14/25	1.4 (0.7–2.9)	5/1	13.2 (1.5–116.0)
Quit >10 yr ago	12/21	1.3 (0.6–2.8)	7/7	2.2 (0.7–6.6)	NA[b]	NA
1–14 cigarettes/day or smoked <25 yr	6/12	1.1 (0.4–3.1)	2/1	5.0 (0.4–56.2)	NA	NA
≥15 cigarettes/day and smoked ≥25 yr	10/30	0.8 (0.3–1.6)	5/17	0.6 (0.2–1.8)	NA	NA
Red meat intake (by tertile)						
T_1	30/73	1.0 (reference)	14/27	1.2 (0.6–2.6)	2/2	2.7 (0.4–20.6)
T_2	30/63	1.2 (0.6–2.1)	13/36	0.9 (0.4–1.9)	4/3	3.4 (0.7–16.2)
T_3 (high)	23/63	1.9 (0.5–1.7)	24/38	1.6 (0.8–3.1)	5/2	6.1 (1.1–33.2)
Doneness level[c]						
Not consistently well done	54/136	1.0 (reference)	29/65	1.0 (0.7–1.9)	6/4	3.6 (1.0–13.4)
Consistently well done	23/40	1.4 (0.8–2.5)	16/28	1.6 (0.7–2.7)	2/1	5.6 (0.5–62.7)

[a] Adjusted for age and family history of breast cancer. To obtain ORs from the same logistic regression model, subjects with *NAT1*10/*11* genotype (two cases, no controls) were included only in the group "*NAT1*11/any*" in the analysis. Inclusion of these two cases in the *NAT1*10/any* group increased the OR from 1.4 (95% CI = 0.7–2.9) to 1.6 (95% CI = 0.8–3.3) for smokers and from 2.2 (95% CI = 0.7–6.6) to 3.3 (95% CI = 1.2–9.5) for those who had quit smoking >10 years ago.
[b] OR was not estimated due to the small sample size; NA, not applicable.
[c] Included hamburgers, beefsteak, and bacon for which information on doneness levels of meat preparation was collected.

in well-done meat (5, 6), is largely carried out by NAT1 in human mammary glands (34).

Although the link between *NAT1* alleles and isozyme activity has not been directly established (46), two studies have suggested that the *NAT1*10* allele may be associated with increased NAT1 activity in the bladder and colon (47, 48). However, in more recent studies, individuals with the *NAT1*10* allele did not reflect a more rapid phenotype *in vivo* (51) or *in vitro* in the colon (52). The association of breast cancer with the *NAT1*10* allele was weak in our study (OR = 1.3, 95% CI = 0.8–1.9), and the elevated risk was mainly confined to former (OR = 3.3, 95% CI = 1.2–9.5) or light (OR = 5.0, 95% CI = 0.4–56.2) smokers. This observation is consistent with the results from the recent study by Millikan *et al.* (33). The link between the *NAT1*11* allele and enzyme expression and activity remains unclear. One study reported that recombinant NAT1 11 catalyzed *N*-acetylation at rates ~2-fold higher than NAT1 4 (53), whereas a more recent study reported that recombinant NAT1 11 catalyzed *N*-acetylation at rates comparable to NAT1 4 (51). Recently, the coding region of the *NAT1*11* allele was sequenced and found to contain a G $^{445}\rightarrow$A substitution that had not been described previously (54). The G $^{445}\rightarrow$A substitution in the *NAT1*11* allele causes an amino acid change from valine to isoleucine. Recent studies show that this substitution resulted in a recombinant NAT1 protein that catalyzed the metabolic activation of *N*-hydroxy aromatic amines by *O*- and *N*,*O*-acetylation at rates up to 2-fold higher than wild-type recombinant human NAT1 (54). The results of this study showing elevated risk of breast cancer with the *NAT1*11* allele, particularly among women with high exposure to aromatic and heterocyclic amines, are consistent with increased activation of aromatic and heterocyclic amines in individuals carrying the *NAT1*11* allele.

The primary concern of this study may be the low response rate to blood sample collection. There is no reason, however, to speculate that the response rate would be associated with both case-control status and certain genotypes. Nevertheless, we have evaluated the possibility of this selection bias by comparing women who provided blood with those who completed the supplementary questionnaire regarding well-done meat intake and found that these two groups were generally comparable in breast risk factors, including cigarette smoking and well-done meat intake. We have also shown previously that women who completed the supplementary survey were similar to all eligible women who where selected for this ancillary study in virtually all major breast cancer risk factors (9). Therefore, this potential bias seems minimal. Passive smoking has been linked recently to an elevated risk of breast cancer in some studies (55). Information related to passive smoking, however, was not collected in this study.

In summary, this case-control study found that postmenopausal women with the *NAT1*11* allele may be at increased risk of breast cancer, particularly if they smoked cigarettes or consumed a high level of well-done meat. The *NAT1*10* allele was

also associated with an elevated risk among former or light smokers. These findings are consistent with the observations from recent laboratory studies, indicating the importance of the NAT1 enzyme in the *in situ* activation of heterocyclic amines in human breast epithelial cells, and point to the need in future studies to investigate potential interactions of genetic factors with intake of foods, food constituents, and nutrients in the etiology of cancer (56). Our findings, particularly those related to potential gene-environment interaction, are preliminary, however, given the small sample size and relatively low frequency of the *NAT1*11* allele in the study population. Therefore, in addition to laboratory studies to link the *NAT1*11* allele with its enzyme function, future epidemiological studies are needed to investigate the role of the *NAT1* genes in the etiology of breast cancer.

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N-Acetyltransferase 1 Genetic Polymorphism, Cigarette Smoking, Well-Done Meat Intake, and Breast Cancer Risk

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