N-Acetyltransferase-2 Genetic Polymorphism, Well-done Meat Intake, and Breast Cancer Risk among Postmenopausal Women[1]

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Abstract

Heterocyclic amines found in well-done meat require host-mediated metabolic activation before initiating DNA mutations and tumors in target organs. Polymorphic *N*-acetyltransferase-2 (NAT2) catalyzes the activation of heterocyclic amines via *O*-acetylation, suggesting that *NAT2* genotypes with high *O*-acetyltransferase activity (rapid/intermediate acetylator phenotype) increase the risk of breast cancer in women who consume well-done meat. To test this hypothesis, DNA samples and information on diet and other breast cancer risk factors were obtained from a nested case-control study of postmenopausal women. Twenty-seven *NAT2* genotypes were determined and assigned to rapid, intermediate, or slow acetylator groups based on published characterizations of recombinant NAT2 allozymes. *NAT2* genotype alone was not associated with breast cancer risk. A significant dose-response relationship was observed between breast cancer risk and consumption of well-done meat among women with the rapid/intermediate NAT2 genotype (trend test, $P = 0.003$) that was not evident among women with the slow acetylator genotype (trend test, $P = 0.22$). These results suggest an interaction between *NAT2* genotype and meat doneness, although a test for multiplicative interaction was not statistically significant ($P = 0.06$). Among women with the rapid/intermediate NAT2 genotype, consumption of well-done meat was associated with a nearly 8-fold (odds ratio, 7.6; 95% confidence interval, 1.1–50.4) elevated breast cancer risk compared with those consuming rare or medium-done meats. These results are consistent with a role for *O*-acetylation in the activation of heterocyclic amine carcinogens and support the hypothesis that the *NAT2* acetylation polymorphism is a breast cancer risk factor among postmenopausal women with high levels of heterocyclic amine exposure.

Introduction

Heterocyclic amines, such as PhIP,[5] 2-amino-3-methylimidazo[4,5-*f*]quinoline, and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline, which are formed when meat is cooked at high temperatures until well done, induce mammary gland tumors in the rat (1–3). The consumption of well-done meat has been associated with an elevated risk of human breast cancer in some but not all epidemiological studies (4). Heterocyclic amine carcinogens require host-mediated metabolic activation before initiating DNA mutations that progress to tumors in target organs (3). *N*-Acetyltransferases catalyze the activation (*O*-acetylation) of heterocyclic amine carcinogens (5–8) and are subject to genetic polymorphism (9). The *NAT2* polymorphism is very common in the human population, and individuals can be subdivided into rapid, intermediate, and slow acetylator phenotypes (9, 10).

Studies investigating the relationship between NAT2 acetylator polymorphism and breast cancer have yielded mixed results. NAT2 acetylator phenotype was not associated with breast cancer in three studies (11–13). However, other studies have suggested that the rapid NAT2 acetylator phenotype is associated with breast cancer risk (14–16) or advanced disease at first presentation (17). Part of the inconsistency may be due to the fact that NAT2 phenotyping assays were performed after cancer diagnosis and/or treatment, and disease status or its sequelae may alter acetylation rate. Furthermore, none of these studies took into consideration the potential modifying effects of heterocyclic amine exposure.

Recent breast cancer studies have used *NAT2* genotyping assays to assign acetylation status of study participants (18). Genotype, unlike acetylator phenotype determination, is not influenced by age, diet, disease state, environmental chemical exposures, or concurrent drug therapy. These studies assessed three (19–22), four (23, 24), or six (25) SNPs within the *NAT2* coding region.

Twenty-six *NAT2* alleles had been identified in human

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[5] The abbreviations used are: PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; NAT2, *N*-acetyltransferase-2; SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

populations when this study was initiated (9, 26). The "wild-type" allele is denoted as *NAT2*4*. The other 25 alleles possess a combination of one to four SNPs at 11 sites within the 870-bp coding region. Seven SNPs result in amino acid changes (G191A, T341C, A434C, G590A, A803G, A845C, and G857A), whereas four do not (T111C, C282T, C481T, C759T). Based on prokaryotic recombinant enzyme expression data, 5 *NAT2* alleles (*NAT2*4*, *NAT2*12A*, *NAT2*12B*, *NAT2*12C*, and *NAT2*13)* encode proteins with a high (rapid) *O*-acetylation capacity toward *N*-hydroxy amines, whereas the other *NAT2* alleles encode proteins with reduced capacity (8, 27). Allelic frequency varies with ethnicity (9), but approximately 50% of Caucasians are rapid/intermediate acetylators (10).

Many studies examining the joint effects of heterocyclic amine exposure and enzyme polymorphisms have focused on colorectal cancer. High meat intake and consumption of fried meats have been associated with colorectal cancer among rapid NAT2 acetylators (28–31). However, many heterocyclic amines are mammary carcinogens, and, in a recent study (32), we found that another *N*-acetyltransferase polymorphism (*NAT1)* was associated with breast cancer risk in individuals who consumed consistently well-done meat. This study was undertaken to investigate whether the *NAT2* polymorphism is associated with breast cancer risk and whether consumption of well-done meat modifies this risk.

Materials and Methods

Subjects. Human DNA samples were obtained from the nested case-control study of the Iowa Women's Health Study, a prospective cohort of 41,836 women, virtually all Caucasian, aged 55–69 years at the 1986 baseline survey. Cohort members have been followed since 1986 for mortality and cancer incidence. Detailed descriptions of this cohort study and the nested case-control study have been published elsewhere (4, 32–35). Briefly, cohort members diagnosed with breast cancer between 1992 and 1994 were classified as cases ($n = 456$), and a random sample of cohort members who were cancer free in 1992 were classified as controls ($n = 876$). Each eligible subject ($n = 1332$) was asked to complete a food frequency questionnaire about meat intake habits during the year prior to breast cancer diagnosis or, for controls, during one of three randomly assigned reference years (1991, 1992, or 1993). The questionnaire assessed usual intake and preparation methods of 15 different meats. Using a series of color photographs, information on meat doneness level was obtained for hamburger, beef steak, and bacon. Of the 930 women who completed this supplementary questionnaire, 878 provided a buccal cell sample, and 488 also provided a blood sample (through the mail) for genomic DNA extraction.

***NAT2* Genotype Assay.** *NAT2* genotype was determined using a modification of our PCR-RFLP assay (36), which is designed to avoid the pitfalls reported recently for *NAT2* genotyping (37). The published assay (36) was modified to distinguish between the 26 known human *NAT2* alleles (9). All *NAT2* genotype assignments were blind to case-control status. *NAT2* was amplified by PCR using 50–250 ng of genomic DNA in a 50-μl reaction containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate, 0.5 μg of primer 5′-GGCTATAAGAACTCTAGGAAC-3′, 0.7 μg of 5′-AAGGGTTTATTTGTTCCTTATTCTAAAT-3′, and 1.25 units of Taq DNA polymerase. The mixture was subjected to a 5-min pretreatment at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, and a 5-min extension step at 72°C.

G191A, A434C, and C481T were detected by digesting 15 μl of the *NAT2* PCR product at 37°C (>3 h) in a total volume of 30 μl with restriction enzymes *Msp*I (10 units) and *Kpn*I (5 units) in NEBuffer 1 (New England Biolabs, Beverly, MA) supplemented with 100 μg/ml BSA. G191A causes loss of a *Msp*I site, yielding bands of 416, 384, and 66 bp, whereas A434C adds an additional *Msp*I site, yielding bands of 416, 244, 93, 66, and 47 bp. C481T results in loss of the *Kpn*I restriction site, yielding fragments of 707, 93, and 66 bp. When neither allele contains G191A, A434C, or C481T, 416-, 291-, 93-, and 66-bp bands result. T111C, G590A, C759T, and G857A were distinguished after digestion of the *NAT2* PCR product with *Taq*I (10 units) and *Bam*HI (10 units) at 37°C (>3 h) followed by 65°C (>3 h) in NEBuffer *Bam*HI (New England Biolabs) supplemented with 100 μg/ml BSA. T111C adds a *Taq*I restriction site, resulting in 252-, 226-, 170-, 98-, 80-, and 40-bp fragments. G590A and C759T delete *Taq*I restriction sites, yielding bands of 396, 332, 98, and 40 bp and 332, 268, 226, and 40 bp, respectively. G857A causes loss of the *Bam*HI restriction site, yielding 332-, 226-, 170-, and 138-bp bands. When neither allele contains T111C, G590A, C759T, or G857A, 332-, 226-, 170-, 98-, and 40-bp bands result. C282T and A845C were detected by digesting PCR-amplified *NAT2* with the restriction enzymes *Fok*I (2 units) and *Dra*III (1.5 units) at 37°C (>3 h) in NEBuffer 3 (New England Biolabs) supplemented with 100 μg/ml BSA. C282T causes loss of a *Fok*I site, yielding bands of 667 and 199 bp. A845C adds a *Dra*III site, resulting in 429-, 238-, 153-, and 46-bp bands. When neither allele contains C282T or A845C, 429-, 238-, and 199-bp fragments result.

T341C and A803G were detected with nested PCR reactions. One μl of amplified *NAT2* was used as 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 of each deoxynucleotide triphosphate, 220 ng of primer 5′-CACCTTCTCCTGCAGGTGACC**G**-3′ and primer 5′-TGTCAAGCAGAAAATGCAAGGC-3′ or 240 ng of primer 5′-TGAGGAGAGGTTGAAGAAGTGCT-3′ and 290 ng of 5′-AAGGGTTTATTTTGTTCCTTATTCTAAAT-3′, respectively, and 0.5 unit of Taq DNA polymerase (*bold* indicates the nucleotide change made in the primer sequence to generate a partial *Aci*I restriction site, which is *underlined*.) The mixture was pretreated at 94°C for 5 min, followed by 30 cycles of 30 s at 94°C, 30 s at 58°C, and 30 s at 72°C, and a 5-min extension step at 72°C.

To distinguish between C341 and T341, 20 μl of the nested PCR product were digested at 37°C (>3 h) in a total volume of 35 μl with 5 units of *Aci*I in NEBuffer 3 (New England Biolabs). Samples homozygous for T341C yield bands of 121 and 20 bp instead of 141 bp. A803G was detected after digestion of the nested PCR product with the restriction enzyme *Dde*I (10 units) as described above for *Aci*I. When both alleles contain A803G, the 120-bp band was cut into 97- and 23-bp fragments.

Statistical Analysis. Individuals possessing two *NAT2* alleles associated with high acetylation activity (*NAT2*4*, *NAT2*12A*, *NAT2*12B*, *NAT2*12C*, and *NAT2*13*) were classified as rapid acetylators; individuals with one of these alleles were classified as intermediate acetylators, and individuals possessing none of these alleles were identified as slow acetylators. 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 1986 baseline survey and to derive adjusted OR and 95% CIs. Because none

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

	All eligible subjects			Study subjects		
	Cases (n = 456)	Controls (n = 876)	OR (95% CI)	Cases (n = 176)	Controls (n = 391)	OR (95% CI)
Demographic and major risk factors						
Age ≥ 64 yrs	171	281	1.3 (1.0–1.6)	54	105	1.2 (0.8–1.8)
Education ≥ high school	192	345	1.1 (0.9–1.4)	91	180	1.3 (0.9–1.8)
First-degree relatives with breast cancer	75	81	1.9 (1.4–2.7)	29	42	1.6 (1.0–2.7)
Waist to hip ratio ≥ 0.85	216	332	1.5 (1.2–1.9)	78	131	1.6 (1.1–2.3)
Body mass index ≥ 26	269	438	1.4 (1.1–1.8)	94	196	1.1 (0.8–1.6)
Menarche at ≤ 15 yrs	433	828	1.1 (0.7–1.8)	170	375	1.2 (0.5–3.1)
Menopause at ≥ 54 yrs	71	125	1.1 (0.8–1.5)	28	49	1.3 (0.8–2.2)
Live births ≤ 4	309	533	1.4 (1.1–1.7)	115	214	1.6 (1.1–2.3)
First live birth ≥ 25 yrs	120	228	1.0 (0.8–1.3)	49	105	1.1 (0.7–1.6)
Alcohol intake ≥ 2 g/day	123	250	0.9 (0.7–1.2)	56	127	1.0 (0.7–1.4)
Meat doneness score						
3	6	24	1.0 (reference)[a]	4	15	1.0 (reference)
4	52	200	1.1 (0.4–2.8)	34	123	1.0 (0.3–3.3)
5	71	155	1.8 (0.7–4.7)	46	90	1.9 (0.6–6.1)
6	57	103	2.2 (0.9–5.7)	43	55	2.9 (0.9–9.5)
7	31	80	1.6 (0.6–4.2)	17	52	1.2 (0.4–4.2)
8	13	18	2.9 (0.9–9.1)	7	9	2.9 (0.7–12.8)
9	12	11	4.4 (1.3–14.7)	7	5	5.3 (1.1–25.8)
Trend test			P = 0.0003			P = 0.0033

[a] Restricted to the 273 cases and 637 controls who completed the supplementary survey.

of the previously identified breast cancer risk factors confounded the association between *NAT2* genotype and breast cancer, only age was adjusted in the model. Trend tests for dose-response relationships were performed by treating ordinal-score variables as continuous variables in the logistic regression model. Tests for interaction were based on the difference in the likelihood ratios from models with and without interaction terms of genotype and exposure. Intake levels of red meat were estimated by summing the grams of hamburgers, cheeseburgers, beef steaks, pork chops, bacon, breakfast sausage links and patties, other sausages, bratwurst, and hot dogs consumed. Meat doneness levels were classified as 1, 2, or 3 for rare/medium, well-done, and very well-done meat, respectively. A doneness score was calculated by summing the doneness levels of hamburger, beef steak, and bacon, the three meats for which information on doneness was obtained. Thus, a person who reported consuming very well-done hamburger, beef steak, and bacon received a doneness score of 9.

Results

NAT2 amplification was successful in 99% of DNA samples obtained from all 488 blood samples and the 79 buccal samples in which other genes had been successfully amplified. As shown in Table 1, our study subjects (176 cases and 391 controls) were similar to all eligible subjects for most demographic and breast cancer risk factors. Ten of 26 known *NAT2* alleles were identified in our Caucasian study population (Table 2). The relative *NAT2* allelic frequency was *NAT2*5B* > *NAT2*6A* > *NAT2*4* > *NAT2*5A* > *NAT2*5C* and *NAT2*7B* in both cases and controls. In contrast, *NAT2*13* was present in seven controls but was absent in cases. *NAT2*14* alleles were rare in both cases and controls, as was expected in the Caucasian population. Twenty-seven different *NAT2* genotypes were determined (Table 3) and assigned to slow, intermediate, and rapid acetylator groups based on recombinant expression of NAT2 allozymes (8). *NAT2* genotype frequencies were similar between cases and controls, although the most frequent *NAT2* genotype in cases was *NAT2*4/*5B* (an intermediate acetylator genotype), whereas it was *NAT2*5B/*6A* (a slow acetylator genotype) in the controls. The relative frequencies of slow, intermediate, and rapid genotypes were similar between cases (52.9%, 41.4%, and 5.7%) and controls (55.0%, 39.0%, and 5.9%).

Table 2 Distribution of *NAT2* alleles among incident breast cancer cases and controls

NAT2 Allele	Cases (n = 174)		Controls (n = 387)	
	No.	Percentage[a]	No.	Percentage[a]
*NAT2*4*	90	25.9	187	24.2
*NAT2*5A*	18	5.2	20	2.6
*NAT2*5B*	132	37.9	318	41.1
*NAT2*5C*	4	1.1	17	2.2
*NAT2*5D*	1	0.3	0	0
*NAT2*6A*	97	27.9	206	26.6
*NAT2*7B*	4	1.1	15	1.9
*NAT2*12A*	2	0.6	3	0.4
*NAT2*13*	0	0	7	0.9
*NAT2*14B*	0	0	1	0.1

[a] (Number of alleles/case or control chromosomes) × 100.

In stratified analyses (Table 4), meat doneness score was associated with an elevated risk of breast cancer in a dose-dependent manner among women with rapid/intermediate *NAT2* genotype (P = 0.003) but not among women with the slow *NAT2* genotype (P = 0.22). These results suggest an interaction between *NAT2* genotype and meat doneness, although a test for interaction based on a multiplicative model was not statistically significant (P = 0.06). Breast cancer risk appeared to increase with level of meat intake in women with rapid/intermediate acetylator genotype, but the trend was not significant (P = 0.11). Meat intake level was not associated with breast cancer risk in the slow *NAT2* acetylator genotype group (trend test, P = 0.30).

To enhance the stability of risk estimates for the association between breast cancer risk and meat doneness score strat-

Table 3 Distribution of *NAT2* genotype among incident breast cancer cases and controls

NAT2 genotype	Cases (n = 174)		Controls (n = 387)	
	No.	Percentage[a]	No.	Percentage[a]
Slow acetylator				
*NAT2*5A/*5A*	1	0.6	0	0
*NAT2*5A/*5B*	7	4.0	8	2.1
*NAT2*5B/*5B*	28	16.1	67	17.3
*NAT2*5B/*5C*	2	1.2	5	1.3
*NAT2*5B/*5D*	1	0.6	0	0
*NAT2*5A/*6A*	4	2.3	6	1.6
*NAT2*5B/*6A*	32	18.4	85	21.7
*NAT2*5C/*6A*	0	0	3	0.8
*NAT2*5A/*7B*	0	0	1	0.3
*NAT2*5B/*7B*	0	0	4	1.0
*NAT2*6A/*6A*	16	9.2	31	8.0
*NAT2*6A/*7B*	1	0.6	2	0.5
*NAT2*7B/*14B*	0	0	1	0.3
Total slow acetylator	**92**	**52.9**	**213**	**55.0**
Intermediate acetylator				
*NAT2*4/*5A*	5	2.9	5	1.3
*NAT2*4/*5B*	34	19.5	80	20.7
*NAT2*4/*5C*	2	1.2	7	1.8
*NAT2*4/*6A*	27	15.5	45	11.6
*NAT2*4/*7B*	3	1.7	6	1.6
*NAT2*5B/*12A*	0	0	1	0.3
*NAT2*6A/*12A*	1	0.6	1	0.3
*NAT2*5B/*13*	0	0	1	0.3
*NAT2*5C/*13*	0	0	2	0.5
*NAT2*6A/*13*	0	0	2	0.5
*NAT2*7B/*13*	0	0	1	0.3
Total intermediate acetylator	**72**	**41.4**	**151**	**39.0**
Rapid acetylator				
*NAT2*4/*4*	9	5.2	21	5.4
*NAT2*4/*12A*	1	0.6	1	0.3
*NAT2*4/*13*	0	0	1	0.3
Total rapid acetylator	**10**	**5.7**	**23**	**5.9**

[a] (Participants with genotype/total cases or controls) × 100.

Table 4 Adjusted ORs for the association of breast cancer risk with well-done meat intake stratified by *NAT2* genotype

	NAT2 (slow)		*NAT2* (rapid/intermediate)	
	Cases/controls	OR (95% CI)[a]	Cases/controls	OR (95% CI)[a]
Meat doneness score				
3 and 4	24/71	1.0 (reference)	13/65	1.0 (reference)
5	20/51	1.2 (0.6–2.3)	25/39	3.2 (1.5–7.0)
6	26/26	3.0 (1.4–6.1)	17/28	3.0 (1.3–7.0)
7	5/33	0.4 (0.2–1.3)	12/19	3.0 (1.2–7.7)
8	3/4	2.2 (0.5–10.8)	4/5	4.0 (0.9–17.0)
9	4/3	3.9 (0.8–18.9)	3/2	7.6 (1.1–50.4)
Trend test		P = 0.22		P = 0.003
Test for interaction		P = 0.06		
Red meat intake[b]				
T1 (low)	27/64	1.0 (reference)	19/52	1.0 (reference)
T2	36/71	1.2 (0.7–2.2)	28/61	1.3 (0.6–2.5)
T3	29/76	0.9 (0.5–1.7)	35/60	1.7 (0.9–3.4)
Trend test		P = 0.30		P = 0.11
Test for interaction		P = 0.91		

[a] Adjusted for age.
[b] By tertile.

Table 5 Association of well-done meat intake and breast cancer risk stratified by *NAT2* genotype and cigarette smoking

Meat doneness score	*NAT2* (slow)		*NAT2* (rapid/intermediate)	
	Cases/controls	OR (95% CI)[a]	Cases/controls	OR (95% CI)[a]
Study participants				
3–4	24/71	1.0 (reference)	13/65	1.0 (reference)
5	20/51	1.2 (0.6–2.3)	25/39	3.2 (1.5–7.0)
6–9	38/66	1.7 (0.9–3.1)	36/54	3.3 (1.6–6.8)
Test for interaction		P = 0.154		
Never smokers				
3–4	20/51	1.0 (reference)	8/40	1.0 (reference)
5–9	39/77	1.3 (0.7–2.5)	38/66	2.9 (1.2–6.7)
Test for interaction		P = 0.148		
Ever smokers				
3–4	4/20	1.0 (reference)	4/23	1.0 (reference)
5–9	18/39	2.2 (0.7–7.5)	22/25	5.0 (1.5–16.8)
Test for interaction		P = 0.340		

[a] Adjusted for age.

ified by *NAT2* genotypes, the doneness level was collapsed into three groups (Table 5). Compared with women who consumed consistently rare or medium-done meat, breast cancer risks were elevated with increasing meat doneness, particularly among women with the rapid/intermediate *NAT2* genotype ($P < 0.01$). A similar pattern was found in analyses stratified by cigarette smoking, indicating that smoking is unlikely to explain the observed association.

Discussion

Metabolic activation of carcinogenic heterocyclic amines is a multistep process catalyzed by both phase I and phase II enzymes. One working hypothesis suggests initial *N*-oxidation by hepatic CYP1A2 (38, 39), followed by transport of the *N*-hydroxy-heterocyclic amine to tumor target organs, where it undergoes *O*-acetylation catalyzed by *N*-acetyltransferase(s) that ultimately leads to DNA adducts and mutations (9, 40, 41). This hypothesis suggests that, compared with women with the slow *NAT2* genotype, women with the rapid/intermediate *NAT2* genotype may more readily activate heterocyclic amines present in well-done meat to reactive metabolites that initiate DNA adducts and tumors. An alternative hypothesis suggests that activation occurs *in situ* because CYP1B1 and CYP1A1 are expressed in human mammary cells and can metabolize PhIP to *N*-hydroxy-PhIP (42–44). Regardless of whether *N*-hydroxy-PhIP is formed in the liver, mammary gland, or both, it undergoes further activation (via *O*-acetylation) by all known human NAT2 allozymes (5, 7, 27). In addition to acetyltransferases, the human mammary gland may activate dietary mutagens by several other enzyme systems, such as sulfotransferase(s) (45, 46) and prostaglandin hydroperoxidase(s) (41). Additional studies investigating the relative contribution of each of these and other enzyme systems are needed.

NAT2 genotype was not associated with breast cancer risk in our nested case-control study. However, we observed a dose-dependent elevated risk among rapid/intermediate acetylators who consumed well-done meat. Three studies reported that red meat consumption and *NAT2* genotype were not associated with breast cancer risk (21, 22, 47). However, only one study (47) collected information on degree of meat doneness to estimate the levels of heterocyclic amine exposure. The previous studies used methods to detect only three SNPs in *NAT2*, whereas our assay distinguished among 11 SNPs. Twenty-three

of the samples in our study would have been assigned an incorrect *NAT2* genotype using an assay that detects only three SNPs. The frequency of the major *NAT2* alleles in our controls was very similar to that observed in a large Caucasian (*i.e.*, German) population (48). However, *NAT2* allelic frequency varies with ethnicity (9), and this may also account for the disparity between studies.

A study reporting that NAT2 activity was below the limit of detection in human mammary cytosols (40) suggests that NAT2 may not be important for *in situ* activation of *N*-hydroxy-heterocyclic amines. However, a role for NAT2 is supported by studies reporting detection of *NAT2* mRNA in human mammary cells (40, 49), as well as by studies that found higher levels of NAT2 activity (50) and *N*-hydroxy-PhIP metabolic activation (51) in mammary cytosols from rapid *NAT2* acetylator rather than slow *NAT2* acetylator congenic Syrian hamsters. In addition, recombinant expression studies have shown that human NAT2 has a higher selectivity for some *N*-hydroxy-heterocyclic amines, including PhIP, than does NAT1 (5, 7). Finally, human mammary cells from rapid NAT2 acetylators exhibited higher levels of heterocyclic amine DNA adducts than cells derived from slow acetylators (52). Each of these findings is consistent with the results from our study suggesting that women with the rapid/intermediate *NAT2* acetylator genotype activate heterocyclic amines to DNA-adducting metabolites to a greater extent than do women with the slow *NAT2* acetylator genotype.

In summary, our results suggest that the *NAT2* polymorphism may be a breast cancer risk factor among women exposed to heterocyclic amines through the consumption of well-done meat. The findings should be interpreted with caution, due to the limitations of our study. Although our results suggest an interaction between rapid/intermediate *NAT2* genotype and meat doneness, a test for interaction based on a multiplicative model was not statistically significant. Another consideration may be the low response rate for blood sample collection and the low amplification rate of buccal DNA. There is little reason, however, to suspect that these rates would be associated with both case-control status and *NAT2* genotype. As shown in Table 1, our study subjects were similar to all eligible subjects in the associations of breast cancer risk with well-done meat intake and in virtually all other breast cancer risk factors. The high comparability between study participants and nonparticipants strongly suggests that potential selection bias in our study, if any, is unlikely to be substantial. The sample size of 174 cases and 387 controls is small, limited to Caucasians in Iowa, and heterocyclic amine exposure was determined indirectly via dietary survey. Because the dietary information was obtained retrospectively, problems of differential recall between cases and controls in this cohort are possible. Nevertheless, the results of this study, in addition to our recent study investigating the *NAT1* acetylation polymorphism and well-done meat consumption in breast cancer risk (32), provide mechanistic support for the role of well-done meat consumption in breast cancer risk (4). These results, as well as additional gene-gene-environmental interactions, need to be confirmed in studies with a larger sample size and other ethnic populations.

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References

1. Ito, N., Hasegawa, R., Sano, M., Tamano, S., Esumi, H., Takayama, S., and Sugimura, T. A new colon and mammary carcinogen in cooked food, 2-amino-1-methyl-6- phenylimidazo[4,5-*b*]pyridine (PhIP). Carcinogenesis (Lond.), *12:* 1503–1506, 1991.

2. Ito, N., Hasegawa, R., Imaida, K., Tamano, S., Hagiwara, A., Hirose, M., and Shirai, T. Carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in the rat. Mutat. Res., *76*: 107–114, 1997.

3. Snyderwine, E. G. Mammary gland carcinogenesis by 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine in rats: possible mechanisms. Cancer Lett., *143:* 211–215, 1999.

4. Zheng, W., Gustafson, D. R., Sinha, R., Cerhan, J. R., Moore, D., Hong, C-P., Andersen, K. E., Kushi, L. H., Sellers, T. A., and Folsom, A. R. Well-done meat intake and risk of breast cancer. J. Natl. Cancer Inst., *90:* 1724–1729, 1998.

5. Minchin, R. F., Reeves, P. T., Teitel, C. H., McManus, M. E., Mojarrabi, B., Ilett, K. F., and Kadlubar, F. F. *N*- and *O*-Acetylation of aromatic and heterocyclic amine carcinogens by human monomorphic and polymorphic acetyltransferases expressed in COS-1 cells. Biochem. Biophys. Res. Commun., *185:* 839–844, 1992.

6. Hein, D. W., Doll, M. A., Rustan, T. D., Gray, K., Feng, Y., Ferguson, R. J., and Grant, D. M. Metabolic activation and deactivation of arylamine carcinogens by recombinant human NAT1 and polymorphic NAT2 acetyltransferases. Carcinogenesis (Lond.), *14:* 1633–1638, 1993.

7. Hein, D. W., Rustan, T. D., Ferguson, R. J., Doll, M. A., and Gray, K. Metabolic activation of aromatic and heterocyclic *N*-hydroxyarylamines by wild-type and mutant recombinant human NAT1 and NAT2 acetyltransferases. Arch. Toxicol., *68:* 129–133, 1994.

8. Hein, D. W., Doll, M. A., Rustan, T. D., and Ferguson, R. J. Metabolic activation of *N*-hydroxyarylamines and *N*-hydroxyarylamides by 16 recombinant human NAT2 allozymes: effects of seven specific NAT2 nucleic acid substitutions. Cancer Res., *55:* 3531–3536, 1995.

9. Hein, D. W., Doll, M. A., Fretland, A. J., Leff, M. A., Webb, S. J., Xiao, G. H., Devanaboyina, U-S., Nangju, N. A., and Feng, Y. Molecular genetics and epidemiology of the *NAT1* and *NAT2* acetylation polymorphisms. Cancer Epidemiol. Biomark. Prev., *9:* 29–42, 2000.

10. Weber, W. W., and Hein, D. W. *N*-Acetylation pharmacogenetics. Pharmacol. Rev., *37:* 25–79, 1985.

11. Ladero, J. M., Fernandez, M. J., Palmeiro, R., Munoz, J. J., Jara, C., Lazaro, C., and Perez-Manga, G. Hepatic acetylator polymorphism in breast cancer patients. Oncology (Basel), *44:* 341–344, 1987.

12. Webster, D. J., Flook, D., Jenkins, J., Hutchings, A., and Routledge, P. A. Drug acetylation in breast cancer. Br. J. Cancer, *60:* 236–237, 1989.

13. Ilett, K. F., Detchon, P., Ingram, D. M., and Castleden, W. M. Acetylation phenotype is not associated with breast cancer. Cancer Res., *50:* 6649–6651, 1990.

14. Bulovskaya, L. N., Krupkin, R. G., Bochina, T. A., Shipkova, A. A., and Pavlova, M. V. Acetylator phenotype in patients with breast cancer. Oncology (Basel), *35:* 185–188, 1978.

15. Cartwright, R. A. Epidemiological studies on *N*-acetylation and C-center ring oxidation in neoplasia. *In:* G. S. Omen and H. V. Gelboin (eds.), Genetic Variability in Responses to Chemical Exposures, Vol. 16, pp. 359–365. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1984.

16. Sardas, S., Cok, I., Sardas, O. S., Ilhan, O., and Karakaya, A. E. Polymorphic *N*-acetylation capacity in breast cancer patients. Int. J. Cancer, *46:* 1138–1139, 1990.

17. Philip, P. A., Rogers, H. J., Millis, R. R., Rubens, R. D., and Cartwright, R. A. Acetylator status and its relationship to breast cancer and other diseases of the breast. Eur. J. Cancer Clin. Oncol., *23:* 1701–1706, 1987.

18. Dunning, A. M., Healey, C. S., Pharoah, P. D. P., Teare, M. D., Ponder, B. A. J., and Easton, D. F. A systematic review of genetic polymorphisms and breast cancer risk. Cancer Epidemiol. Biomark. Prev., *8:* 843–854, 1999.

19. Ambrosone, C. B., Freudenheim, J. L., Graham, S., Marshall, J. R., Vena, J. E., Brasure, J. R., Michalek, A. M., Laughlin, R., Nemoto, T., Gillenwater, K. A., and Shields, P. G. Cigarette smoking, *N*-acetyltransferase 2 genetic polymorphisms, and breast cancer risk. J. Am. Med. Assoc., *276:* 1494–1501, 1996.

20. Hunter, D. J., Hankinson, S. E., Hough, H., Gertig, D. M., Garcia-Closas, M., Spiegelman, D., Manson, J. E., Colditz, G. A., Willett, W. C., Speizer, F. E., and Kelsey, K. A prospective study of NAT2 acetylation genotype, cigarette smoking, and risk of breast cancer. Carcinogenesis (Lond.), *18:* 2127–2132, 1997.

21. Ambrosone, C. B., Freudenheim, J. L., Sinha, R., Graham, S., Marshall, J. R., Vena, J. E., Laughlin, R., Nemoto, T., and Shields, P. G. Breast cancer risk, meat consumption and *N*-acetyltransferase (*NAT2*) genetic polymorphisms. Int. J. Cancer, *75:* 825–830, 1998.

22. Gertig, D. M., Hankinson, S. E., Hough, H., Spiegelman, D., Colditz, G. A., Willett, W. C., Kelsey, K. T., and Hunter, D. J. *N*-Acetyltransferase 2 genotypes, meat intake and breast cancer risk. Int. J. Cancer, *80:* 13–17, 1999.

23. Millikan, R. C., Pittman, G. S., Newman, B., Tse, C-K. J., Selmin, O., Rockhill, B., Savitz, D., Moorman, P. G., and Bell, D. A. Cigarette smoking, *N*-acetyltransferases 1 and 2, and breast cancer risk. Cancer Epidemiol. Biomark. Prev., *7:* 371–378, 1998.

24. Huang, C-S., Chern, H-D., Shen, C-Y., Hsu, S-M., and Chang, K-J. Association between *N*-acetyltransferase 2 (NAT2) genetic polymorphism and development of breast cancer in post-menopausal Chinese women in Taiwan, an area of great increase in breast cancer incidence. Int. J. Cancer, *82:* 175–179, 1999.

25. Agundez, J. A. G., Ladero, J. M., Olivera, M., Abildua, R., Roman, J. M., and Benitez, J. Genetic analysis of the arylamine *N*-acetyltransferase polymorphism in breast cancer patients. Oncology (Basel), *52:* 7–11, 1995.

26. Hein, D. W., Grant, D. M., and Sim, E. Update on consensus arylamine *N*-acetyltransferase gene nomenclature. Pharmacogenetics, *10:* 291–292, 2000.

27. Hein, D. W., Doll, M. A., Fretland, A. J., and Leff, M. A. Metabolic activation of *N*-hydroxy-2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (N-OH-PhIP) by twenty-three recombinant human NAT2 allozymes. Proc. Am. Assoc. Cancer Res., *39:* 455, 1998.

28. Lang, N. P., Butler, M. A., Massengill, J., Lawson, M., Stotts, R. C., Hauer-Jensen, M., and Kadlubar, F. F. Rapid metabolic phenotypes for acetyltransferase and cytochrome P4501A2 and putative exposure to food-borne heterocyclic amines increase the risk for colorectal cancer or polyps. Cancer Epidemiol. Biomark. Prev., *3:* 675–682, 1994.

29. Roberts-Thomson, I. C., Ryan, P., Khoo, K. K., Hart, W. J., McMichael, A. J., and Butler, R. N. Diet, acetylator phenotype, and risk of colorectal neoplasia. Lancet, *347:* 1372–1374, 1996.

30. Welfare, M. R., Cooper, J., Bassendine, M. F., and Daly, A. K. Relationship between acetylator status, smoking, diet and colorectal cancer in the northeast of England. Carcinogenesis (Lond.), *18:* 1351–1354, 1997.

31. Chen, J., Stampfer, M. J., Hough, H. L., Garcia-Closas, M., Willett, W. C., Hennekens, C. H., Kelsey, K. T., and Hunter, D. J. A prospective study of *N*-acetyltransferase genotype, red meat intake, and risk of colorectal cancer. Cancer Res., *58:* 3307–3311, 1998.

32. Zheng, W., Deitz, A. C., Campbell, D. R., Wen, W-Q., Cerhan, J. R., Sellers, T. A., Folsom, A. R., and Hein, D. W. *N*-Acetyltransferase 1 (*NAT1*) genetic polymorphism, cigarette smoking, well-done meat intake, and breast cancer risk. Cancer Epidemiol. Biomark. Prev., *8:* 233–239, 1999.

33. Kushi, L. H., Sellers, T. A., Potter, J. D., Nelson, C. L., Munger, R. G., Kaye, S. A., and Folsom, A. R. Dietary fat and postmenopausal breast cancer. J. Natl. Cancer Inst., *4:* 1092–1099, 1992.

34. Folsom, A. R., Kaye, S. A., Sellers, T. A., Hong, C. P., Cerhan, J. R., Potter, J. D., and Prineas, R. J. Body fat distribution and 5-year risk of death in older women. J. Am. Med. Assoc., *269:* 483–487, 1993.

35. Bisgard, K. M., Folsom, A. R., Hong, C. P., and Sellers, T. A. Mortality and cancer rates in nonrespondents to a prospective study of older women: 5-year follow-up. Am. J. Epidemiol., *139:* 990–1000, 1994.

36. Doll, M. A., Fretland, A. J., Deitz, A. C., and Hein, D. W. Determination of human NAT2 acetylator genotype by restriction fragment-length polymorphism and allele-specific amplification. Anal. Biochem., *231:* 413–420, 1995.

37. Cascorbi, I., and Roots, I. Pitfalls in *N*-acetyltransferase 2 genotyping. Pharmacogenetics, *9:* 123–127, 1999.

38. Butler, M. A., Iwasaki, M., Guengerich, F. P., and Kadlubar, F. F. Human cytochrome P-450$_{PA}$ (P-4501A2), the phenacetin *O*-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and *N*-oxidation of carcinogenic arylamines. Proc. Natl. Acad. Sci. USA, *86:* 7696–7700, 1989.

39. Turesky, R. J., Constable, A., Richoz, J., Varga, N., Markovic, J., Martin, M. V., and Guengerich, F. P. Activation of heterocyclic aromatic amines by rat and human liver microsomes and by purified rat and human cytochrome P4501A2. Chem. Res. Toxicol., *11:* 925–936, 1998.

40. Sadrieh, N., Davis, C. D., and Snyderwine, E. G. *N*-Acetyltransferase expression and metabolic activation of the food-derived heterocyclic amines in the human mammary gland. Cancer Res., *56:* 2683–2687, 1996.

41. Dubuisson, J. G., and Gaubatz, J. W. Bioactivation of the proximal food mutagen 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (N-OH-PhIP) to DNA-binding species by human mammary gland enzymes. Nutrition, *14:* 683–686, 1998.

42. Shimada, T., Hayes, C. L., Yamazaki, H., Amin, S., Hecht, S. S., Guengerich, F. P., and Sutter, T. R. Activation of chemically diverse procarcinogens by human cytochrome P-4501B1. Cancer Res., *56:* 2979–2984, 1996.

43. Crofts, F. G., Strickland, P. T., Hayes, C. L., and Sutter, T. R. Metabolism of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) by human cytochrome P4501B1. Carcinogenesis (Lond.), *18:* 1793–1798, 1997.

44. Crofts, F. G., Sutter, T. R., and Strickland, P. T. Metabolism of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine by human cytochrome P4501A1, P4501A2 and P4501B1. Carcinogenesis (Lond.), *19:* 1969–1973, 1998.

45. Lewis, A. J., Walle, U. K., King, R. S., Kadlubar, F. F., Falany, C. N., and Walle, T. Bioactivation of the cooked food mutagen *N*-hydroxy-2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine by estrogen sulfotransferase in cultured human mammary epithelial cells. Carcinogenesis (Lond.), *19:* 2049–2053, 1998.

46. Yamazoe, Y., Nagata, K., Yoshinari, K., Fujita, K., Shiraga, T., and Iwasaki, K. Sulfotransferase catalyzing sulfation of heterocyclic amines. Cancer Lett., *143:* 103–107, 1999.

47. Delfino, R. J., Sinha, R., Smith, C., West, J., White, E., Lin, H. J., Liao, S-Y., Gim, J. S. Y., Ma, H. L., Butler, J., and Anton-Culver, H. Breast cancer, heterocyclic aromatic amines from meat and *N*-acetyltransferase 2 genotype. Carcinogenesis (Lond.), *21:* 607–615, 2000.

48. Cascorbi, I., Brockmoller, J., Mrozikiewicz, P. M., Muller, A., and Roots, I. Arylamine *N*-acetyltransferase activity in man. Drug Metab. Rev., *31:* 489–502, 1999.

49. Debiec-Rychter, M., Land, S. J., and King, C. M. Histological localization of acetyltransferases in human tissue. Cancer Lett., *143:* 99–102, 1999.

50. Deitz, A. C., Doll, M. A., and Hein, D. W. Acetylation polymorphism in mammary tissue from Syrian hamsters congenic at the NAT2 locus. Proc. Am. Assoc. Cancer Res., *37:* 130–131, 1996.

51. Fretland, A. J., Devanaboyina, U. S., Nangju, N. A., Leff, M. A., Xiao, G. H., Webb, S. J., Doll, M. A., and Hein, D. W. DNA adduct levels and absence of tumors in female rapid and slow acetylator congenic hamsters administered the rat mammary carcinogen 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine. J. Biochem. Mol. Toxicol., *15:* in press, 2001.

52. Stone, E. M., Williams, J. A., Grover, P. L., Gusterson, B. A., and Phillips, D. H. Interindividual variation in the metabolic activation of heterocyclic amines and their *N*-hydroxy derivatives in primary cultures of human mammary epithelial cells. Carcinogenesis (Lond.), *19:* 873–879, 1998.

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