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Well-done meat consumption, *NAT1* and *NAT2* acetylator genotypes and prostate cancer risk: The Multiethnic Cohort study

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Abstract

Background—Prostate cancer (PC) is the most common male malignancy in the U.S. and disparities in risk exist among ethnic/racial groups. A high intake of well-done meat and the presence of the rapid *NAT1* and slow *NAT2* acetylator genotypes, as modifiers of the carcinogenic effect of heterocyclic amines, were hypothesized to increase PC risk and possibly explain these ethnic differences in risk.

Methods—This study examined the associations between well-done (red) meat consumption, *NAT1* and *NAT2* acetylator genotypes and PC risk among five ethnicities (African American, Native Hawaiian, Japanese American, Latino and Caucasian) in a case-control study of PC nested within the Multiethnic Cohort study. Cases (n=2,106) and controls (n=2,063) were genotyped for eight single nucleotide polymorphisms (SNPs) in *NAT1* and seven SNPs in *NAT2* that characterize all common alleles for these genes. Well-done meat intake was computed based on responses to a detailed food frequency questionnaire including a question on meat preference. Conditional logistic regression was used in the analysis.

Results—There was no evidence of an increased risk associated with preference for well-done meat, intake of well-done meat and *NAT1* or *NAT2* genotypes (jointly or separately).

Conclusions—These results do not support the hypothesis that exposure to heterocyclic amines is associated with risk of PC. However, additional studies with more precise exposure measures are needed.

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Keywords

prostate cancer; well-done meat; N-acetyltransferase 1 (NAT1); N-acetyltransferase 2 (NAT2); the Multiethnic Cohort

Introduction

Prostate cancer (PC) is the most common male malignancy in the U.S. and risk varies by ethnicity which could partially be due to differential exposure to heterocyclic aromatic amines (HAAs), a class of carcinogens formed when meat is cooked at high temperature (1-8). The rapid *NAT1* and the slow *NAT2* genotypes are suspected to increase PC risk due to their effect on HAA activation by *O*-acetylation in the prostate and decreased detoxification of HAAs in the liver, respectively (9-11). We examined associations between well-done meat and PC risk, and the modifying effects of *NAT1* and *NAT2* acetylator genotypes, among five ethnic/racial groups.

Materials and Methods

This case-control study nested in the Multiethnic Cohort (MEC) was approved by the Institutional Review Boards at the University of Hawaii and the University of Southern California. Participants (N>215,000) were recruited from Hawaii and Los Angeles in 1993-1996, were aged 45-75 years at entry and were primarily comprised of African American, Native Hawaiian, Japanese American, Latino and Caucasian men and women (12,13). Incident PC cases since January 1995 were identified through Surveillance Epidemiology and End Results cancer registries (14). Blood samples were generally obtained after diagnosis (15). Controls were frequency-matched by ethnicity and age.

NAT1 and *NAT2* were determined using TaqMan allele discrimination assays (Applied Biosystems) (16,17) with a successful genotyping rate of ≥98.7% and genotype concordance (among 5% blind QC duplicates) of ≥98.5%. The genotype distributions among controls were in Hardy-Weinberg equilibrium (p>0.05) for each ethnic group. Through genotyping of seven single nucleotide polymorphisms (SNPs) occurring with >1% frequency in at least one ethnicity [G191A (R64Q), C282T, T341C (I114T), C481T, G590A (R197Q), A803G (K268R), G857A (G286T)], 26 of the common *NAT2* allelic variants can be detected (*NAT2*4; NAT2*5A,B,C,D,E,G,J; NAT2*6A,B,C,E; NAT2*7A,B; NAT2*11A; NAT2*12A,B,C; NAT2*13; NAT2*14A,B,C,D,E,F,G*) (18). Similarly, all common *NAT1* allelic variants (*NAT1*3; NAT1*4; NAT1*10; NAT1*11A,B,C; NAT1*14A,B; NAT1*15; NAT1*17; NAT1*19; NAT1**22) can be characterized by genotyping eight SNPs [C97T (R33Stop), C1095A (3′-UTR), C190T (R64W), G445A (V149I), C559T (R187Stop), G560A (R187Q), A752T (D251V), T1088A (3′-UTR)] (16,17). Individuals with two "rapid" alleles (*NAT2*4, NAT2*11A, NAT2*12A,B,C and NAT2**13), two "slow" phenotypes and with one "rapid" and one "slow" allele were assigned to the "rapid", "slow" and "intermediate" *NAT2* genotype, respectively. The *NAT1*10* allele was designated as the "at risk" phenotype. *NAT1* was categorized as "*NAT1*10*", "*NAT1*10*/other *NAT1* allele" and "any combination of other *NAT1* alleles", represented as "2", "1" and "0 copies", respectively. Missing SNP results were imputed when certainty was ≥95% using PHASE (version 2.1) (18,19).

The validated food frequency questionnaire (FFQ) included questions on preference for well-done meat and the amount and frequency of consumption of different types of meat over the past year (12,13). The meat groups were computed as the sum of all corresponding food items and the relevant proportion from mixed dishes.

Conditional logistic regression stratified by 5-year age groups and ethnicity and adjusted for energy, BMI, education, family history and smoking was used to estimate odds ratios (ORs) and 95% confidence intervals (95% CI) using SAS, version 9.1 (SAS, Cary, NC, USA). Adjustment for fat was not included because fat intake was not found to have any effect on PC risk in the MEC. Interactions between ethnicity, well-done red meat, *NAT1* and *NAT2* were examined by a Wald test of cross-product terms. Results for Native Hawaiians are not presented separately because of the small sample size, although they were included in the combined group.

Results

Among cases and controls, more African Americans and Latinos consumed well-done meat than other ethnicities (Table 1). African Americans had a higher prevalence than Caucasians for the high risk *NAT1*10* allele but not for the *NAT2* slow genotype.

The age- and ethnicity-adjusted and multivariate-adjusted ORs were similar in all models. No statistically significant association was observed between meat preference ($p_{heterogeneity}$=0.72) (Table 1) or types of meat by level of doneness and PC risk. There was no association with PC risk for 1 copy or 2 copies of *NAT1*10* compared to 0 copies, the intermediate or slow *NAT2* compared to the rapid genotype ($p_{heterogeneity}$=0.37 for *NAT1* and 0.25 for *NAT2*) (Table 1) or *NAT1* and *NAT2* jointly (data not shown). The OR for men with 2 copies of *NAT1*10* and the slow *NAT2* genotype was 0.81 (0.54-1.21) compared to those with 0 copies and the rapid genotype ($p_{heterogeneity}$=0.22). The two-way (Table 2) and three-way interactions of *NAT1**10, *NAT2* and preference for well-done meat were not significant. All results were also null in an analysis of advanced PC.

Discussion

This study did not find significant associations for well-done meat, *NAT1* and *NAT2* with PC risk overall, by ethnicity or among advanced PC cases. Our null findings for meat and PC risk agree with a previous cohort study (20). In another study, high consumption of red meat doubled the PC risk for African Americans (21), while in two largely Caucasian cohorts a direct association was observed for high intake of red meat and well-done meat with PC risk (4,22). The slow *NAT2* genotype has been associated with a lowered PC risk while the rapid *NAT2* genotype has been associated with a non-significantly elevated PC risk (23,24). Among Japanese, the *NAT1*10* was related to a higher PC risk (25) and the slow *NAT2* genotype was more common in PC cases than controls (26). In agreement with our results, other studies also found no relationship between *NAT2* and PC (27,28). The combination of the *NAT1*10* and the slow *NAT2* genotype has been associated with a five-fold higher PC risk and the very slow *NAT2* genotype with a seven-fold elevated PC risk (11). In one small case-control study, the associations of meat and *NAT1/NAT2* with PC were also not significant (29).

This study is the first large nested case-control study to investigate the ethnic-specific effect of well-done meat, *NAT1* and *NAT2* on PC risk. A FFQ developed specifically for this population was used to ensure standardized data collection, and a comprehensive number of *NAT1* and *NAT2* SNPs were genotyped. Since exposure to dietary HAAs is difficult to measure, as it depends on the type of meat, as well as the duration and temperature of cooking, additional studies with more direct measurement of HAAs would be useful.

In conclusion, these data do not support the hypothesis that consumption of well-done meat, *NAT1*, *NAT2* or their interactions are associated with PC risk.

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Table 1

Odds ratios (ORs) and 95% confidence intervals (CIs) for risk of prostate cancer associated with meat preference, *NAT1* and *NAT2* genotype

	Total			African American		Japanese American		Latino		Caucasian	
	Cases/ controls	**OR (95% CI), adjusted for age and ethnicity***	**OR (95% CI), multivariate adjusted†**	**Cases/controls**	**OR (95% CI)‡**	**Cases/controls**	**OR (95% CI)‡**	**Cases/controls**	**OR (95% CI)‡**	**Cases/controls**	**OR (95% CI)‡**
Meat preference											
Rare / No meat	184 / 160	1.00	1.00	22/15	1.00	38/40	1.00	39/29	1.00	75/69	1.00
Medium	902 / 892	0.86 (0.68-1.09)	0.93 (0.76-1.14)	173/169	0.68 (0.34-1.36)	269/252	1.01 (0.62-1.65)	191/209	0.67 (0.40-1.13)	230/229	0.94 (0.64-1.37)
Well-done	1020 / 1011	0.85 (0.67-1.08)	0.84 (0.66-1.07)	404/387	0.73 (0.37-1.43)	117/128	0.87 (0.51-1.47)	366/350	0.72 (0.44-1.21)	116/123	0.84 (0.55-1.27)
p for trend		0.30	0.23		0.94		0.41		0.69		0.38
Genotypes											
NAT1*10											
0 copy	864 / 841	1.00	1.00	200/187	1.00	145/118	1.00	229/245	1.00	282/281	1.00
1 copy	878 / 873	0.98 (0.86-1.13)	0.98 (0.86-1.13)	278/265	1.00 (0.77-1.30)	179/204	0.72 (0.53-1.00)	262/257	1.09 (0.85-1.39)	125/121	1.04 (0.77-1.40)
2 copies	364 / 349	1.01 (0.84-1.21)	1.01 (0.84-1.22)	121/119	0.96 (0.70-1.33)	100/98	0.84 (0.58-1.21)	105/86	1.30 (0.92-1.82)	14/19	0.73 (0.36-1.49)
P for trend§		0.99	0.96		0.83		0.27		0.14		0.73
NAT2											
Rapid	379 / 355	1.00	1.00	65/48	1.00	202/204	1.00	68/65	1.00	27/21	1.00
Intermediate	909 / 894	0.93 (0.78-1.12)	0.92 (0.77-1.11)	254/275	0.68 (0.45-1.02)	169/175	0.97 (0.73-1.29)	284/268	1.02 (0.70-1.48)	167/147	0.88 90.48-1.62)
Slow	818 / 814	0.91 (0.75-1.11)	0.91 (0.75-1.11)	280/248	0.82 (0.55-1.24)	53/41	1.28 (0.81-2.01)	244/255	0.92 (0.63-1.35)	227/253	0.70 (0.38-1.27)
P for trend§		0.42	0.42		0.87		0.49		0.50		0.07
Intermediate	1288 / 1249	1.00	1.00	319/323	1.00	371/379	1.00	352/333	1.00	194/168	1.00
Slow	818 / 814	0.97 (0.85-1.10)	0.97 (0.95-1.11)	280/248	1.14 (0.90-1.43)	53/41	1.29 (0.84-1.99)	244/255	0.91 (0.72-1.15)	227/253	0.78 (0.60-1.03)

*Adjusted for age groups and ethnicity as strata in a conditional logistic regression model;

†Adjusted for age groups and ethnicity as strata in a conditional logistic regression model, and for energy, body mass index, years of education, family history of prostate cancer and smoking status (never/ former/current) as covariates;

‡Adjusted for age groups as strata in a conditional logistic regression model;

§Wald statistic for trend variables assigned the number of variant alleles for *NAT1* (0, 1 and 2 copies, respectively) and *NAT2* (slow, intermediate and rapid, respectively).

Table 2

Odds ratios (ORs) and 95% confidence intervals (CIs) for risk of prostate cancer associated with the two-way interaction between *NAT1/NAT2* and preference for well-done meat

NAT	**Preference for well-done meat**	**Cases / Controls**	**OR, adjusted for age and ethnicity ***	**95% CI**	**OR, multivariate adjusted †**	**95% CI**
***NAT1*10* (copies)**						
0	No	469 / 446	1.00		1.00	
0	Yes	395 / 395	0.94	0.77-1.14	0.92	0.76-1.12
1 or 2	No	617 / 606	0.97	0.81-1.26	0.96	0.80-1.15
1 or 2	Yes	625 / 616	0.95	0.79-1.14	0.93	0.78-1.13
p for interaction (1 df)‡				*0.72*		*0.67*
NAT2						
Intermediate/Rapid	No	693 / 638	1.00		1.00	
Intermediate/Rapid	Yes	595 / 611	0.88	0.75-1.04	0.88	0.74-1.03
Slow	No	393 / 414	0.86	0.72-1.04	0.87	0.72-1.05
Slow	Yes	425/ 400	0.95	0.79-1.15	0.94	0.78-1.14
p for interaction (1 df) ‡				*0.08*		*0.10*

*Adjusted for age groups and ethnicity as strata in a conditional logistic regression model.

†Adjusted for age groups and ethnicity as strata in a conditional logistic regression model and for energy, body mass index, years of education, family history of prostate cancer and smoking status (never/former/current) as covariates.

‡The p for interaction is based on a Wald test of cross-product terms.