Associations between Catalase Phenotype and Genotype: Modification by Epidemiologic Factors

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Abstract

Catalase is an endogenous antioxidant enzyme that neutralizes hydrogen peroxide and is induced by oxidative challenge. A −262C → T polymorphism in the promoter region of the gene (*CAT*) is associated with risk of several conditions related to oxidative stress. We sought to determine the functional effects of the *CAT* polymorphism on enzyme activity in erythrocytes and the potential modifying effects of demographic and lifestyle factors on genotype/phenotype relationships, using specimens and data from controls from breast and prostate cancer studies in Arkansas (n = 420). There was a dose-response reduction in catalase activity by genotype, with geometric means of 115.4 units/mg hemoglobin for those with CC genotypes, 82.1 units/mg for those with CT genotypes, and 73.5 units/mg for those with TT genotypes. Associations were only observed among Caucasians (P < 0.0001), with no effects among African Americans (P = 0.91), and were stronger among women than men, although numbers in stratified analyses were small. Differences in catalase activity by genotype were most pronounced among those in the highest tertiles of consumption of fruits and vegetables (−35%, P = 0.003), with weaker relationships among those who were lower consumers (−21.8%, P = 0.16). Among those with CC genotypes, there was no change in activity by consumption, but there were notable decreases in activity by tertiles of consumption for those with at least one T allele. These data indicate that the *CAT* −262C → T polymorphism predicts a portion of catalase phenotype, which may be limited to Caucasians. Associations between genotype and phenotype were modified by dietary factors, illustrating the biochemical complexity of studies of genetic polymorphisms and disease risk. (Cancer Epidemiol Biomarkers Prev 2006;15(6):1217–22)

Introduction

Oxidative stress may play a significant role in the risk of chronic diseases (1-4). Although reactive oxygen species can cause oxidative damage to cellular macromolecules, such as DNA and lipids, multiple antioxidant defenses can neutralize reactive oxygen species (5-8). Variability in endogenous and exogenous pro-oxidants and antioxidants will determine levels of oxidative stress in the organism. Enzymes responsible for neutralizing reactive oxygen species endogenously include catalase, superoxide dismutase, and glutathione peroxidase as well as the glutathione *S*-transferases (1, 9). Fruits and vegetables are rich in vitamin C, vitamin E, selenium, and phytochemicals, such as flavonoids, which are potent exogenous antioxidants. It is not clear how these endogenous and exogenous modulators of oxidative stress interact *in vivo*; it is possible that endogenous and exogenous factors may act synergistically or that high levels of dietary antioxidants derived from consumption of fruit and vegetables may down-regulate endogenous antioxidant enzymes through feedback mechanisms.

Catalase is a heme enzyme that has a major role in controlling H_2O_2 concentrations in human cells, by converting H_2O_2 into H_2O and O_2. Working together with other antioxidant enzymes, catalase plays an integral role in the primary defense against oxidative stress. Catalase enzymatic activity was induced by exposure to H_2O_2 in hamster tracheal epithelial cells (10) and in human retinal pigment epithelial cells (11), indicating a key role for catalase in antioxidant defense and its inducibility in response to oxidative stress. A common polymorphism in the promoter region of the *CAT* gene consists of a C → T substitution at position −262 in the 5′ region (12), which is thought to result in reduced activity. Reduction in catalase activity may play a role in host response to oxidative stress and, indeed, variant *CAT T* alleles have been associated with increased risk of hypertension (13, 14) and vitiligo (15), both conditions that are related to oxidative stress. We previously showed that women with the common *CAT CC* genotype had a 17% reduction in risk of breast cancer compared with those with at least one T allele (*CT* and *TT* genotypes) in the Long Island Breast Cancer Study Project, a large population based case-control study (1,008 cases and 1,056 controls; ref. 16). We also observed a statistically significant multiplicative interaction between genotype and fruit consumption (P = 0.02) among women who did not use antioxidant supplements, with lowest risk observed for women who were high fruit consumers and had *CC* genotypes (odds ratio, 0.59; 95% confidence interval, 0.38-0.89). Although our preliminary study with specimens from healthy volunteers (n = 18) indicated that the polymorphism was associated with reduced catalase activity (16), the sample size was small, and the results need to be replicated.

Thus, we sought to confirm our findings in a much larger sample size and to evaluate if relationships between *CAT* genotype and phenotype were modified by demographic characteristics, fruit and vegetable consumption, and other lifestyle factors.

Materials and Methods

Study Population. For these analyses, we used samples and data from healthy men and women who participated in

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Table 1. Catalase activity by characteristics of study population (n = 420)

Characteristics	n	Unadjusted geometric mean (95% confidence interval)	Adjusted geometric mean* (95% confidence interval)	P †
Age (y)				0.91
20-30	13	72.1 (50.8-102.3)	81.6 (31.5-211.0)	
31-40	32	113.7 (86.1-150.2)	120.6 (68.5-212.5)	
41-50	90	95.2 (79.9-113.5)	113.1 (69.5-184.1)	
51-60	131	103.5 (91.3-117.3)	118.1 (72.4-192.4)	
61-70	86	111.1 (96.4-128.1)	122.9 (74.3-203.1)	
71-85	68	107.5 (93.8-123.2)	119.2 (71.8-198.1)	
Race ‡				0.61
Caucasian	266	108.4 (99.7-117.9)	118.7 (87.8-160.4)	
African American	109	100.5 (87.5-115.4)	116.0 (83.3-161.4)	
Sex				0.16
Male	210	112.9 (103.3-123.5)	117.6 (70.6-195.9)	
Female	210	95.0 (85.8-105.3)	105.8 (64.3-174.0)	
Smoking status§				0.33
Current	91	96.8 (82.1-114.2)	106.4 (63.1-179.3)	
Never or former	327	106.2 (98.6-114.5)	116.9 (71.5-191.1)	
Fruit and vegetable consumption (servings per week)‖				0.49
0-18.2 (tertile 1)	120	106.9 (95.0-120.3)	118.1 (71.6-194.8)	
18.3-31.7 (tertile 2)	131	104.5 (91.8-119.1)	109.7 (65.8-183.0)	
37.8- (tertile 3)	131	98.0 (86.7-110.9)	107.1 (64.0-179.1)	
Vitamin supplement use‖				0.50
Never	132	104.0 (91.0-118.9)	90.9 (58.5-141.4)	
Occasional	55	103.9 (83.9-128.7)	94.4 (59.8-149.1)	
Regular	205	101.3 (92.5-110.9)	91.4 (58.7-142.4)	

NOTE: Catalase activity units are units/mg hemoglobin.
*Adjusted age, race, sex, current smoking status, fruit and vegetable consumption, and vitamin supplement use.
†P for significance of the mean difference of catalase activity by demographic and lifestyle characteristics.
‡Thirty-nine were missing data on race, and six were deleted due to ''other'' race.
§Two were missing data on smoking status.
‖Twenty-eight were missing data on fruit and vegetable consumption and vitamin supplement use.

case-control studies of breast and prostate cancer conducted in Arkansas. A detailed description of the study design, participant recruitment, and methodology has been reported previously (17). Control participants were identified from the Arkansas state driver's license/identity card records for those under 65 years of age and the Health Care Finance Administration lists for those 65 years and older and were matched to cases on race, age (within 10 years), sex, and county of residence. Informed consent from each participant was obtained according to an institutional review board–approved protocol. We calculated sample size to be able to detect a difference in catalase activity of 20% SD, with 80% power and 0.05 α level, and thus included 420 control participants (50% male and 50% female).

Measurements

Erythrocyte Catalase Activity Assay. Stored erythrocytes from blood samples collected from study participants were assayed for catalase activity (18) using a commercial kit from *Oxis*Research (Oxis Research, Portland, OR), with modifications to accommodate a microtiter plate format. The erythrocytes were washed thrice with ice-cold 0.9% NaCl, centrifuging between each wash. The cell pellet was then resuspended in 4 volumes of ice-cold water, incubated on ice for 10 minutes to lyse the cells, and centrifuged at 3,000 × g for 10 minutes at 4°C. For the assay, the lysate was diluted 1:400 with sample diluent before use. Triplicates of 30 μL of diluted erythrocyte lysate were delivered to a 96-well microplate. The samples were incubated with 10 mmol/L H_2O_2 at room temperature for exactly 1 minute. The reaction was stopped with the provided stop reagent, and an aliquot was removed and added to a microplate containing horseradish peroxidase/Chromagen reagent and allowed to develop for 10 minutes. Absorbance was read at 520 nm on a SpectraMax UV-VIS microplate reader (Molecular Devices, Sunnyvale, CA), and activity units were assigned based on a calibration curve of known amounts of H_2O_2. Multiple aliquots of a control erythrocyte sample were made and processed identically to the study samples to assess variability of the assay. The reproducibility of the assay was high, with a coefficient of variation of 8.7%. Activity was normalized to hemoglobin content in the sample.

Hemoglobin Assay. Stored erythrocytes were assayed for hemoglobin content. The erythrocyte pellet was resuspended in 50 volumes of ice-cold water, incubated on ice for 10 minutes to lyse the cells, and centrifuged at 3,000 × g for 10 minutes at 4°C. For the assay, triplicates of 20 μL of erythrocyte lysate were delivered to a 96-well microplate. The samples were incubated with Drabkin's reagent at room temperature for exactly 15 minutes. Absorbance was read at 540 nm on a SpectraMax UV-VIS microplate reader, and hemoglobin concentration was assigned based on a calibration curve of known amounts of Randox Cyanmethemoglobin Standard Set. Multiple aliquots of a control hemoglobin sample were made and processed identically to the study samples to assess variability of the assay.

CAT *Genotype Determination.* Genomic DNA was extracted from lymphocytes using a commercial kit (Qiagen, Inc., Valencia, CA). The *CAT* −262C → T polymorphism (rs#1001179) was determined by direct sequencing on the Beckman CEQ genetic analysis system as described previously (16), using primers 5′- ACGTTGGATGTCTGGCCCAG-CAATTGGAGAG-3′ and 5′-ACGTTGGATGAGGATGCTGA-TAACCGGGAG-3′. All genotyping results were reviewed manually for quality control. Controls for genotype and two nontemplate controls were included on each plate. Laboratory personnel1 were blinded to catalase activity level.

Exposure Assessment. Data on demographic characteristics, diet, and other lifestyle factors were obtained in an in-person interview, using the Block food frequency questionnaire to assess dietary intake 12 months before the interview. The instrument queried intake of seven fruits and fruit juices and 11 vegetables (excluding French fries). Use of multiple

vitamins (i.e., one-a-day, therapeutic, and Stress tab type) and single supplements of vitamin A, β-carotene, vitamin E, calcium or Dolomite, vitamin C, and iron were also included in the food frequency questionnaire. For food items and supplements, data were collected on frequency of consumption and use; and for food items, responses for portion sizes were relative to a stated average serving size with options of small, medium, or large. Frequency and portion size data were translated to servings per weeks.

Statistical Analyses. All analyses were conducted using SAS version 8.2 (SAS Institute, Inc., Cary, NC). We analyzed the association between *CAT* genotype and phenotype using linear regression models as implemented by SAS PROC GLM. Catalase activities were natural logarithm transformed to approximate normal distributions. Least-square geometric mean activity and 95% confidence intervals within each genotype were calculated, with adjustment for age at the time of interview (continuous), sex, race (Caucasian and African American), smoking status, fruit and vegetable consumption, and vitamin supplement use. Percentage differences and *P*s were calculated for the mean difference associated with the variant genotype (*CT* or *TT* genotype) compared with the common genotype (*CC* genotype).

To evaluate whether associations between *CAT* genotype and activity were modified by demographic, diet, and lifestyle factors, joint categories of *CAT* genotype and those factors were used in the multivariate model. Percentage differences and *P*s were calculated for the mean difference associated with the variant genotype (*CT* or *TT* genotype) compared with the common genotype (*CC* genotype), within each demographic, diet, or lifestyle strata. For test for trend, we used the medians of the categories for fruit and vegetable intake modeled as continuous variables. Multiplicative interactions were tested by adding cross-product terms in the model. All statistical tests were two sided.

Results

Population Characteristics. Population characteristics and their relationships with catalase activity are shown in Table 1. There were no significant differences in the geometric mean of catalase activity by age, race, sex, current smoking status, fruit and vegetable consumption, and vitamin supplement use. However, geometric means for catalase activity were somewhat lower among females than males, current smokers than never or former smokers, and higher consumers of fruits and vegetables than lower consumers. Dietary data and racial information were only available on a portion of the population [n = 392 (93%) and n = 381 (91%), respectively]; however, there were no differences in the characteristics of the study population between the entire sample with genotype and phenotype data and those who with missing data (data not shown).

***CAT* Genotype and Catalase Enzyme Activity.** Associations between *CAT* genotypes and catalase activity are shown in Table 2. Frequencies of the *CAT T* allele were 17% and 5% for Caucasians and African Americans, respectively, and genotype distributions followed Hardy-Weinberg equilibrium (P = 0.10 for Caucasian and P = 0.13 for African American). Allele frequencies were similar to those reported in national databases (19).

There was a dose-response reduction in catalase activity by genotypes, with geometric means of 115.4 units/mg hemoglobin for those with CC genotypes, 82.1 units/mg hemoglobin for CT genotypes, and 73.5 units/mg hemoglobin for TT genotypes [% activity difference = −28.8% (P = 0.002) for CT genotype versus CC genotype; % activity difference = −36.3% (P = 0.02) for TT genotype versus CC genotype]. Associations between genotypes and activity were most apparent among Caucasians, with a 33.7% difference in activity between those with CC genotypes and those with CT and TT genotypes combined. Among African Americans, there was little difference in activity by genotype (−2.6%), although there were only 10 individuals with variant alleles.. There also seemed to be differential relationships by sex. Comparing those with any T alleles to those with CC genotypes, there seemed to be somewhat greater reductions in activity among women than men (% activity difference = −35.7% for women and −24.6% for men). Relationships between *CAT* genotype and phenotype did not vary by smoking status. There were also no activity differences by *CAT* genotype among age groups (data not shown).

***CAT* Genotype and Catalase Enzyme Activity by Fruit and Vegetable Consumption.** Catalase activities were consistently higher in individuals with *CAT CC* genotype compared with those with at least one variant T allele, regardless of dietary

Table 2. Geometric mean of catalase activity by *CAT* −262C → T polymorphism (n = 420)

	n	Geometric mean* (95% confidence interval)	% Difference† (P‡)
Total participants (N = 420)			
CAT CC	328	115.4 (106.5-125.0)	Reference
CAT CT	83	82.1 (70.3-96.0)	−28.8 (0.002)
CAT TT	14	73.5 (50.3-107.4)	−36.3 (0.02)
CAT CC	328	115.4 (106.5-125.0)	Reference
CAT CT + TT	97	80.8 (69.9-93.5)	−29.9 (0.0001)
Caucasian§ (N = 266)			
CAT CC	185	122.2 (111.1-134.5)	Reference
CAT CT	69	82.6 (70.6-96.6)	−32.4 (0.01)
CAT TT	12	72.8 (49.8-106.5)	−40.4 (<0.0001)
CAT CC	185	122.2 (111.1-134.5)	Reference
CAT CT + TT	81	81.1 (70.2-93.7)	−33.7 (<0.0001)
African American§ (N = 109)			
CAT CC	99	102.9 (64.9-115.8)	Reference
CAT CT	9	105.3 (64.6-171.8)	2.4 (0.81)
CAT TT	1	83.63 (19.51-358.53)	−18.7 (0.85)
CAT CC	99	102.9 (64.9-115.8)	Reference
CAT CT + TT	10	100.2 (86.8-115.8)	−2.6 (0.91)
Male (N = 210)			
CAT CC	165	119.4 (108.0-132.0)	Reference
CAT CT	42	88.8 (72.3-109.1)	−25.6 (0.01)
CAT TT	3	107.5 (50.9-226.8)	−10.0 (0.78)
CAT CC	166	119.4 (108.0-132.0)	Reference
CAT CT + TT	45	90.0 (73.9-109.8)	−24.6 (0.01)
Female (N = 210)			
CAT CC	158	110.9 (97.5-126.2)	Reference
CAT CT	41	74.7 (58.8-95.0)	−32.6 (0.005)
CAT TT	11	60.3 (38.2-95.1)	−45.6 (0.01)
CAT CC	159	110.9 (97.5-126.2)	Reference
CAT CT + TT	52	71.4 (57.6-88.4)	−35.7 (0.0007)
Current smokers‖ (N = 91)			
CAT CC	76	102.5 (85.6-122.7)	Reference
CAT CT	15	72.7 (48.3-109.3)	−28.9 (0.13)
CAT TT	0	—	—
CAT CC	76	102.2 (85.5-122.1)	Reference
CAT CT + TT	15	72.7 (48.4-109.0)	−28.9 (0.13)
Never or former smokers‖ (N = 327)			
CAT CC	246	113.7 (104.4-123.8)	Reference
CAT CT	67	87.9 (74.7-103.5)	−22.7 (0.006)
CAT TT	14	77.0 (53.9-109.8)	−32.3 (0.04)
CAT CC	246	113.4 (104.2-123.4)	Reference
CAT CT + TT	81	85.7 (73.9-99.2)	−24.2 (0.001)

NOTE: Catalase activity units are units/mg hemoglobin; $P_{\text{multiplicative interaction}}$ = 0.43 (race), 0.63 (sex), and 0.27 (smoking status).

*Geometric means are adjusted age, race, sex, current smoking status, fruit and vegetable consumption, and vitamin supplement use.

†% Difference = 100 × (mean catalase activity of CT or TT genotype − mean catalase activity of CC genotype) / (mean catalase activity of CC genotype).

‡*P*s are calculated for the mean difference associated with CT or TT genotype compared with CC genotype.

§Six were deleted due to ''other'' race, and 39 were missing racial information.

‖Two were missing data on smoking status.

Table 3. Geometric mean of catalase activity by *CAT*−262C → T polymorphism and tertiles of fruit and vegetable consumption (*n* = 392)

Diet	Tertile 1 (low consumption)*			Tertile 2 (intermediate consumption)*			Tertile 3 (high consumption)*			P_{trend}[†]
	n	Geometric mean[‡] (95% confidence interval)	% Difference[§] (P[‖])	*n*	Geometric mean[‡] (95% confidence interval)	% Difference[§] (P[‖])	*n*	Geometric mean[‡] (95% confidence interval)	% Difference[§] (P[‖])	
Total fruits and vegetables										
CAT CC	108	118.9 (103.0-137.2)	Reference	95	117.0 (100.8-135.7)	Reference	96	115.0 (99.0-133.7)	Reference	0.77
CAT CT + TT	22	93.0 (68.4-126.3)	−21.8 (0.16)	36	78.0 (61.3-99.2)	−33.3 (0.005)	35	74.7 (59.2-94.4)	−35.0 (0.003)	0.09

NOTE: Catalase activity units are units/mg hemoglobin; $P_{multiplicative\ interaction}$ = 0.38 (total fruits and vegetables). Twenty-eight were missing data on diet.
*Fruit and vegetable consumption based on tertiles: total fruits and vegetables (<18.2, 18.3-31.7, ≥31.8 servings/wk).
†P_{trend}: used the medians of the categories for fruit and vegetable modeled as continuous variables.
‡Geometric means are adjusted age, race, sex, current smoking status, and vitamin supplement use.
§% Difference = 100 × (mean catalase activity of CT or TT genotype − mean catalase activity of CC genotype) / (mean catalase activity of CC genotype).
‖*P*s are calculated for the mean difference associated with CT and TT genotype compared with the CC genotype, within each diet strata.

fruit and vegetable consumption (Table 3; Fig. 1). However, genotype/phenotype relationships were modified by fruit and vegetable consumption. Activity difference by *CAT* genotype was more pronounced among those in the highest tertile of fruit and vegetable consumption [% activity difference = −35.0% (P = 0.003) for the highest tertile; % activity difference = −21.8% (P = 0.16) for the lowest tertile]. Among those with at least one variant T allele, there was a notable reduction in activity levels by each increasing tertile of fruit and vegetable consumption. Those with T alleles who were in the lowest tertile of consumption had a geometric mean of 93.0 units/mg hemoglobin; levels were lower for those in the intermediate group (78.0 units/mg) and even lower in the high consumption tertile (74.2 units/mg). However, this trend was not statistically significant (P = 0.09). Among individuals with *CC* genotype, catalase activity remained unchanged by tertiles of fruit and vegetable consumption. Overall, the lowest catalase activity levels were among those with CT or TT genotypes and the highest consumption of fruits and vegetables.

Because use of antioxidant vitamin supplements is another exogenous source of antioxidants, we also evaluated the effect of supplement use on associations between genotype and enzyme activity. The relationship between *CAT* genotype and activity did not differ between supplement users and non-supplement users (data not shown). However, associations among *CAT* genotypes, catalase activity, and fruit and vegetable consumption seemed to be strongest among those who did not use vitamin supplements. Among non-vitamin supplement users, we observed a marginally significant multiplicative interaction (P = 0.07), with greatest activity difference by *CAT* genotype observed for those who were in the higher two tertiles of fruit and vegetable consumption (tertile 1: −6.3% activity difference; mean = 131.8; 95% confidence interval, 104.3-166.7 and mean = 123.5; 95% confidence interval, 101.4-150.3 for CC genotype and for CT + TT genotype, respectively; tertiles 2 and 3 combined: −54.6% activity difference; mean = 122.7; 95% confidence interval, 92.1-123.7 and mean = 55.7; 95% confidence interval, 63.1-98.4 for CC genotype and for CT + TT genotype, respectively). Among supplement users, however, there was no significant multiplicative interaction between fruit and vegetable consumption and *CAT* genotype (P = 0.73).

Discussion

In this large cross-sectional study, we found that *CAT* genotype was associated with catalase activity, with individuals with variant *CT* and *TT* genotypes having significantly lower activity than those with common *CC* genotypes. Associations between genotype and phenotype were only apparent among Caucasians, with no differences in activity noted by genotype among African Americans, and relationships seemed somewhat stronger for women than for men. Although smoking did not seem to affect associations, there was modification of relationships by consumption of fruits and vegetables, with catalase activity decreasing with increasing intake, only among those with CT or TT genotypes. These findings support our *a priori* hypothesis that CT and TT genotypes are associated with lower catalase activity and provide a biological basis for epidemiologic studies showing associations between *CAT* genotype and risk of diseases related to oxidative stress (13, 14, 16, 20).

In a small pilot study (n = 18), we had previously reported that women with variant *CT* and *TT* genotypes had significantly lower catalase activity compared with those with *CC* genotypes (P = 0.007; ref. 16). We had also found that women with common *CC* genotypes had a 17% reduction in breast cancer risk in the Long Island Breast Cancer Study Project data compared with those with at least one T allele, based on the hypothesis that oxidative stress plays a role in breast carcinogenesis and that higher catalase activity reduces oxidative stress (16). Ahsan et al. also showed that arsenic exposure resulted in hyperkeratosis to a much greater extent (4-fold) among those with *CAT TT* genotypes than among those with *CC* genotypes (20). The *TT* genotype was also associated with higher blood pressure compared with those

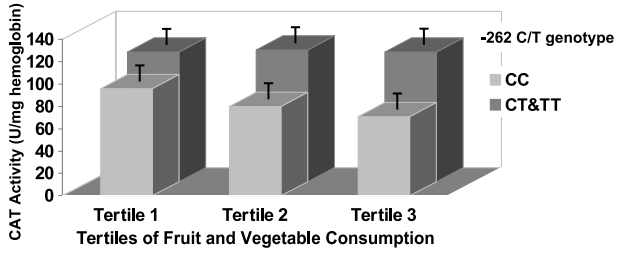

Figure 1. Catalase activity (geometric mean, with 95% confidence interval) in erythrocytes by catalase −262 *CC*, *CT*, and *TT* genotypes and total fruit and vegetable consumption (Arkansas, 2004). Differences in catalase activity by genotype were most pronounced among those in the highest tertiles of consumption of fruits and vegetables (−35%, P = 0.003), with weaker genotype/phenotype relationships among those who were lower consumers (−21.8%, P = 0.16). Among those with CC genotypes, there was no change in activity by consumption, but there were notable decreases in activity by tertiles of consumption for those with at least one T allele.

with *CC* genotype, and those with *CT* genotype had intermediate risk (13). Thus, our findings of genotype/phenotype relationships support the results from epidemiologic studies, providing a functional basis for the investigation of the −262C → T polymorphism in relation to risk of conditions associated with oxidative stress.

Although catalase activity normalized to hemoglobin content (units/mg hemoglobin) in erythrocytes was significantly influenced by genotype, we were unable to determine if this relationship extrapolates to catalase activity in other tissues. We were also unable to determine if this genetic polymorphism was related to altered expression levels of the protein. However, a transcription factor binding site search (21) indicates that the −262 C allele is located in close proximity to several binding sites for transcription factors, such as Sp1, and could potentially influence rates of transcription. Forsberg et al. (12) previously showed that the T allele was associated with greater catalase protein levels in blood than the C allele (n = 29, males). These results are in contrast to our findings for erythrocyte catalase activity, and it could be due to stability of the enzyme according to sample preparation methods or to the absence of females in the previous study. Nonetheless, the evidence that associations between T alleles and reduced activity were shown in two different study populations, and that studies have consistently noted associations between T variant allele and increased risks of oxidative stress related conditions, strengthen our confidence in these findings.

The activity differences by *CAT* genotype were more pronounced among Caucasians, whereas relationships were not observed among African Americans. Zhou et al. (14) reported that having at least one T allele was associated with an increase in risk of hypertension only among Caucasians, not among African Americans, which is consistent with these findings regarding racial difference by catalase activity. Our previous results of reduced breast cancer risk among women with *CAT CC* genotype was based on a population that consisted of 90% Caucasian women (16), which are also consistent with the associations by race. Nonetheless, the lack of association between genotypes and catalase activity could be due to chance due to the relatively small number of African Americans in this study. If additional studies replicate these findings, further research will be required to investigate the biological mechanisms responsible for these racial differences.

There were notable decreases in catalase activity with increasing fruit and vegetable consumption particularly for women with variant T allele. Because H_2O_2 concentrations positively regulate *CAT* expression (11), and because high consumption of fruits and vegetables, rich sources of exogenous antioxidants, may reduce H_2O_2 level *in vivo*, it is possible that high fruit and vegetable consumers have low *CAT* expression and thus have low catalase activity. Although it is not clear why the inverse trend was particularly noted among those with variant T alleles, it is possible that the effects of fruits and vegetables may be more important for those with low catalase activity (i.e., at least one T allele), and that individuals with high catalase activity (i.e., CC genotype) are less susceptible to dietary effects. It is also possible that if antioxidant status is high from dietary exposures, feedback inhibition could decrease the expression levels of catalase. It is also consistent with the view that higher antioxidant intake did reduce levels of oxidative stress biomarkers among susceptible populations (i.e., overweight smokers) but did not reduce markers among healthy populations (22). Nonetheless, despite lowest catalase activity among higher consumers with T alleles, it is interesting to note women with T alleles in our prior study did not have a higher risk of breast cancer than lower consumers with common CC genotypes (16).

Due to the cross-sectional nature of this study, we are only able to capture catalase activity at one point in time, which is unlikely to be representative of lifetime activity, although there is no evidence that catalase activity varies by age in our data. In addition, because we did not measure catalase expression, we are not able to directly compare Forsberg's results (12) with our findings. Although it would be valuable to assess differences in oxidative damage in participants by *CAT* genotype, we are unable to do that in this population due to the limitations of sample acquisition and processing and specimen availability. However, findings from this study provide a basis for future research to determine biological mechanisms by which *CAT* genotype may reduce total body oxidative stress and thus influence cancer etiology and other oxidative stress–related conditions.

In conclusion, this study provides an example of a gene-environment (diet) interaction in which catalase phenotype is notably affected by the individual genetic background and dietary practices. Moreover, because catalase activity is closely linked to endogenous antioxidant activity, these data provide a plausible biological explanation for our previous findings of the association between the *CAT* −262*CC* genotypes and the reduced risk for breast cancer and other disease risk. Importantly, the finding that associations between genotype and activity are modified by demographic and lifestyle factors (race, gender, and dietary habits) has implications for molecular epidemiology studies. When polymorphisms are evaluated in relation to disease risk, there is little accounting for the biochemical processes that underlie these associations. The demonstration that phenotype related to genotype is modified, in particular, by dietary habits, could lend support to the notion that some of the inconsistencies in findings from molecular epidemiologic studies could be due to differences in the populations studied and underlying characteristics that are not accounted for that mediate the relationship between genetic polymorphisms and the actual *in vivo* phenotypes. To advance the field of molecular epidemiology, studies such as these may be useful for elucidating the complex relationships among genetic polymorphisms, phenotypic effects, and cancer risk.

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