Genotype–activity relationship for Mn-superoxide dismutase, glutathione peroxidase 1 and catalase in humans

Maria Bastaki[a], Karen Huen[a], Paolo Manzanillo[a], Neha Chande[a], Connie Chen[a], John R. Balmes[a,c], Ira B. Tager[b] and Nina Holland[a]

Objectives **This study examined the association between genetic polymorphisms and enzyme activity for antioxidant enzymes that share a common detoxification pathway: manganese superoxide dismutase (MnSOD), glutathione peroxidase-1 (GPX1) and catalase.**

Methods **MnSOD, GPX1, and catalase activities were measured in isolated erythrocytes of 231 healthy, non-smoking student volunteers (55% women, ages 17–21, majority Asian or Caucasian). DNA from blood clots was genotyped by Taqman PCR (C47T : MnSOD and C593T : GPX1) and standard PCR (−262C>T : catalase). Associations between genotype and enzyme activity were analyzed by multiple linear regression, adjusted for baseline factors including gender and ethnicity.**

Results **Minor allele frequencies ranged from 13% for catalase (T) to 18% for GPX1 (T), and 33% for MnSOD(C) with significant variation between ethnicities. Median GPX1 activity was 13.2 U/g Hb with a six-fold difference between lowest and highest levels. Catalase activity ranged eight-fold (median: 86.3 k/g Hb), while median MnSOD activity was 2.8 U/mg Hb with a 56-fold range of values. MnSOD enzyme activity was 15% higher in females than males (95%CI : −1%, 32%), and 33% higher in CT or TT individuals (C47T) versus CC individuals (95%CI : 7–59%). On average, catalase activity was 18.1 k/g Hb lower for TT subjects (−262C>T) versus CC subjects (95% CI: −32.3, −4.0). All enzyme activities were correlated (r=0.3–0.4, P<0.001).**

Conclusions **Interindividual variability of antioxidant enzyme activity in healthy young adults was partially explained by significant associations with three known genetic polymorphisms, and was further modified by gender and ethnicity. A substantial component of this variability may be attributable to differences in diet, environmental exposures, and additional genetic factors. *Pharmacogenetics and Genomics* 16:279–286 © 2006 Lippincott Williams & Wilkins.**

Pharmacogenetics and Genomics 2006, **16**:279–286

Keywords: superoxide dismutase, glutathione peroxidase, catalase, polymorphism, oxidative stress, enzyme activity, erythrocytes, healthy adults

[a]Division of Environmental Health Sciences, [b]Division of Epidemiology, School of Public Health, University of California, Berkeley CA, USA and [c]Lung Biology Center, University of California, San Francisco, CA, USA

Correspondence and requests for reprints to Dr Nina Holland, 140 Warren Hall, School of Public Health, University of California, Berkeley CA 94608-7376, USA
Tel: +1 510 642 8781; fax: +1 510 643 5426;
e-mail: ninah@berkeley.edu

Received 19 October 2005 **Accepted** 5 December 2005

Introduction

Oxidative stress, along with the formation of reactive oxygen species (ROS), is a key component of inflammation and is believed to underlie a wide array of health conditions that range from respiratory illness to cardiovascular diseases and cancer [1–3]. ROS are generated by regular metabolic processes *in vivo* and can initiate a cascade of free radical formation and damage to macromolecules. Antioxidant defences are available to combat ROS, although individuals vary in their capacity to deal with the oxidant burden. The effects of single nucleotide polymorphisms (SNPs) on antioxidant enzymes are not clearly established, although expression of these polymorphic genes may present as altered enzymatic levels or activities that could lead to reduced protection from oxidative stress. Among the enzymes involved in antioxidant protection, manganese superoxide dismutase (MnSOD), glutathione peroxidase-1 (GPX1), and catalase all play primary roles in the removal of ROS [1]. These three enzymes are linked via a common detoxification pathway through which MnSOD catalyzes the dismutation of the superoxide anion into peroxide and then catalase and GPX1 further metabolize peroxide to H_2O and O_2 [4]. Thus, overall antioxidant capacity depends upon the activities of these three enzymes.

While there are several forms of SOD in humans, we chose to measure mitochondrial MnSOD activity rather than cytosolic CuZnSOD, since a SNP with functional consequences has been identified in this gene. MnSOD, a metalloenzyme that contains a manganese cofactor, is the only enzyme in the mitochondria to convert superoxide to hydrogen peroxide and thus has a key role in quenching free radicals generated by the electron transport chain. Studies have reported several factors that may affect enzyme activity levels, which include

1744-6872 © 2006 Lippincott Williams & Wilkins

gender (higher in females) [5] and age (decrease with age) [6]. The MnSOD gene (locus 6q25) contains a C47T SNP that results in an Ala16Val amino acid substitution [7]. The Ala16Val polymorphism occurs within the mitochondrial targeting sequence of MnSOD and has been proposed to affect transport of the enzyme through the inner mitochondrial space [8]; the 16Ala variant targets into the mitochondrial matrix, while the 16Val variant is partially arrested in the inner mitochondrial space. While the 16Val allele has been associated with oxidative stress-related conditions such as aging, lung cancer [9], and cardiomyopathy [10], the 16Ala allele has been associated with breast cancer [11] and hypertension [12].

The GPX1 enzyme functions as a homotetramer with a selenium (Se) cofactor [13–15]. It detoxifies hydrogen and lipid peroxides with glutathione (GSH), and protects cells from propagation of lipid peroxidation damage [15]. Factors which affect enzyme activity include gender, dietary supplementation, and tobacco consumption [5,6]. The GPX1 gene (locus 3p21.3) contains a SNP, C593T, that results in a Pro197Leu amino acid substitution [16,17]. While the Leu allele did not alter enzymatic activity in the red blood cells of Finnish/Swedish patients [18], recent in-vitro studies reported a decreased enzyme activity for this allele [19]. Similarly, responsiveness to Se stimulation of GPX1 activity in breast cancer cell lines was decreased for cells which expressed the Leu allele [20]. Other health effects associated with this SNP include lung cancer [21], sensitivity to oxidative damage by H_2O_2 and paraquat [14], and increased risk of cardiovascular and peripheral vascular diseases in patients with type 2 diabetes mellitus [19].

Catalase, an antioxidant enzyme found primarily in peroxisomes, catalyzes the conversion of hydrogen peroxide into water. Prior studies report higher catalase activities in women and no difference by age [5]. In addition, female smokers had lower catalase enzyme activities than non-smokers [5]. The catalase gene (locus 11p13) bears a C/T SNP at position −262 in the promoter region. The variant allele (T) increases gene expression both at the mRNA level and enzyme protein levels in red blood cells (RBCs) [22]. There is some evidence, however, that suggests that the variant allele confers lower activity [23]. Thus, the relation between the promoter polymorphism and catalase activity remains unclear.

While some polymorphisms of these three enzymes have been associated with increased risk of oxidative damage, it is not yet clear how each genotype affects baseline enzyme activity levels in humans. These SNPs have also been linked to several diseases that likely involve oxidative stress [14], but genotype associations with health outcomes lack biological plausibility unless the genetic variants impact enzyme protein level and/or activity.

Here we examine the relation between genotype and baseline enzymatic activity for three antioxidant enzymes: MnSOD, catalase, and GPX1 in a cohort of healthy young adults who participated in a study of the effects of ambient ozone concentrations on lung function [24]. We also explore additional factors that may affect baseline enzyme activity levels such as gender and ethnicity.

Methods

Study subjects and sample collection

A convenience sample of University of California, Berkeley (UCB) freshmen was recruited, as previously described by Tager *et al.* [24]. Briefly, healthy and non-smoking study subjects included 102 men and 129 women (56%) between 17 and 21 years of age. The majority of participants were Asian (52.6%) or Caucasian (29.6%). Lifetime residents of the LA basin composed 54% of the subjects. Lifetime residents of the San Francisco Bay Area made up 42% of the subjects, while the remaining subjects had spent equal residence time in both areas. Specimen collection took place in the spring when all subjects had spent the previous 6 months in the Bay Area; this was a period of low ambient ozone.

In the present study, we included 231 students who had red blood cell (RBC) samples available for enzymatic activity measurements and clots for DNA analysis. All samples were stored in aliquots at −80°C until use.

All protocols and procedures were approved by the Committee for the Protection of Human Subjects, University of California, Berkeley, and by the Committee on Human Research, University of California, San Francisco. Written consent was obtained for all subjects.

DNA isolation

DNA was isolated from stored (−80°C) blood clot samples, using a Qiamp Blood DNA Maxi kit (Qiagen Inc., Santa Clarita, California, USA) and isolation was performed in accordance with the manufacturer's instructions.

Genotyping

Taqman-based Assays-by-Design (Applied Biosystems Inc., Foster City, California) PCR were used for the MnSOD (C47T) and GPX1 (C593T) polymorphisms with the primers shown in Table 1.

Reactions were carried out based on optimized conditions provided by the manufacturer (Applied Biosystems, Inc.,

Table 1 Primers used in assays for MnSOD and GPX1

Primers[a]	SOD2	GPX1
Forward	GGCTGTGCTTTCTCGTCTTCA	TCGAAGCCCTGCTGTCTCA
Reverse	TCTGCCTGGAGCCCAGATAC	GAGACAGCAGCACTGCAACTG
VIC probe[b]	CCCAAAGCCGGAGC[c]	AGGGCCCAGCTGT[d]
FAM probe	CCAAAACCGGAGCCA[c]	AGGGCTCAGCTGTG[d]

[a]All primers are listed 5′ to 3′.
[b]The polymorphism nucleotide is underlined.
[c]Reverse sequence.
[d]Forward sequence.

Foster City, California, USA). Cycling was performed in 384-well plates on an ABI 7900 HT.

For the catalase polymorphism (−262C > T), we used a restriction fragment length PCR method as previously described [14]. The reaction was performed with QIAmp Taq polymerase (Qiagen) and with the primers: 5′-TAAGAGCTGAGAAAGCATAGCT-3′ (forward), 5′-AGAGCCTCGCCCCGCCGGACCG-3′ (reverse).

Quality assurance procedures included assessment of randomly distributed blank samples in each plate, duplicates of randomly selected samples, manual calls assisting automated calling, and repeated analysis. Repeated analysis showed a high degree of concordance ($\geq$ 95%), and the most robust call was selected in the case of discordance. Assays were repeated for all low confidence samples until a reliable call was obtained, using a combination of the TaqMan and Amplifluor methods for a subset of samples. Additionally, several catalase heterozygotes and homozygotes were sequenced to confirm their identity as determined by PCR analysis.

Red blood cell sample preparation

RBC pellets were diluted 4 × in ice-cold ddH_2O containing protease inhibitors (Protease Inhibitor Cocktail for Mammalian Tissues, Sigma, St Louis Missouri, USA), lysed on ice, and centrifuged at 1500 *g*, 4°C, for 5 min. The supernatant (RBC extract) was divided into two aliquots, one was stored for GPX1 activity assays and the other was centrifuged at 13 000 *g*, 4°C, for 10 min to separate cytosol (supernatant) and mitochondria (pellet) [25]. The mitochondrial pellet was washed twice with 20 mmol/l Hepes, pH 7.2, resuspended in 250 μl mitochondrial lysis solution (20 mmol/l Hepes pH 7.2, 70 mmol/l sucrose, 210 mmol/l mannitol and protease inhibitors) [25], and sonicated on ice using an ultrasonicator (Branson SLPt). All samples were stored at −80°C in small aliquots until use.

Hemoglobin content was measured in RBC extracts using Drabkin's reagent, (11.9 mmol/l sodium bicarbonate, 0.8 mmol/l potassium cyanide, 0.6 mmol/l potassium ferricyanide, or 100 : 20 : 5 parts respectively), as described previously by Drabkin *et al.* [26].

Enzyme activity assays

MnSOD activity

MnSOD activity was measured using a commercially available assay kit (Kamiya Biomedical Company, Seattle, Washington, USA). The experiments were performed in isolated mitochondria according to the manufacturer's protocol. Samples were incubated with 2 mmol/l KCN for 30–45 min prior to analysis, in order to inhibit traces of possible contamination by CuZn-SOD (cytosolic form) [27]. Activity was normalized for hemoglobin content in the sample and expressed as U/mg Hb.

GPX1 activity

GPX1 activity was determined with a commercially available assay (Calbiochem, EMD Biosciences, Inc., San Diego, California, USA), based on a coupled reaction with glutathione reductase, in which oxidized GSH produced by GPX1 oxidation of *tert*-butyl-peroxide is reduced with parallel oxidation of $NADPH^+$ to $NADP^+$ [28]. The GPX1 activity was measured as the rate of decrease in $NADPH^+$ absorbance at 340 nm for 3 min, and 1 unit is defined as the amount of enzyme that results in oxidation of 1 μmol/l $NADPH^+$ per minute. Activity in samples was normalized for hemoglobin content and is expressed in U/g Hb.

Catalase activity

Catalase activity was determined in RBC extracts as previously described by Aebi *et al.* [29]. Units of activity were normalized for hemoglobin content (k/g Hb).

Statistical methods

Statistical analyses were performed with STATA 8 software (Statacorp, College Station, Texas, USA). Analysis of variance tests and post hoc comparison tests revealed no significant differences in GPX1 activity between the CC and CT genotypes and also no significant differences in MnSOD activity between the CT and TT genotypes. Thus, in subsequent statistical analyses, these groups (CC and CT for GPX1 and CT and TT for MnSOD) were combined.

We used multiple linear regression to examine which factors were associated with the outcome variable enzyme activity in three models: one for GPX1 activity, one for MnSOD activity, and one for catalase activity. GPX1 and

Table 2 Genotype and allele frequencies by ethnicity

	Caucasians	Asians/PI	Hispanics	Others*	Total
MnSOD *n*	67	122	20	19	228
Genotypes % (*n*)					
CC	26.9 (18)	4.1 (5)	25.0 (5)	–	12.3 (28)
CT	53.7 (36)	29.5 (36)	60.0 (12)	63.2 (12)	42.1 (96)
TT	19.4 (13)	66.4 (81)	15.0 (3)	36.8 (7)	45.6 (104)
Alleles					
C	0.54	0.19	0.55	0.32	0.33
T	0.46	0.81	0.45	0.68	0.67
GPX1 *n*	63	115	20	19	217
Genotypes % (*n*)					
CC	46.0 (29)	86.1 (99)	60.0 (12)	57.9 (8)	69.6 (151)
CT	46.0 (29)	11.3 (13)	35.0 (7)	21.1 (4)	24.4 (53)
TT	8.0 (5)	2.6 (3)	5.0 (1)	21.1 (4)	6.0 (13)
Alleles					
C	0.69	0.92	0.78	0.68	0.82
T	0.31	0.08	0.22	0.32	0.18
Catalase *n*	68	118	21	20	227
Genotypes % (*n*)					
CC	64.7 (44)	83.9 (99)	85.7 (18)	70.0 (14)	77.1 (175)
CT	30.9 (21)	14.4 (17)	9.5 (2)	20.0 (4)	19.4 (44)
TT	6.8 (3)	1.7 (2)	4.8 (1)	10.0 (2)	3.5 (8)
Alleles					
C	0.80	0.91	0.90	0.80	0.87
T	0.20	0.09	0.10	0.20	0.13

*For statistical analysis, African-Americans (4), Native-Americans (5), people of mixed origin (5), and other unlisted ethnicities (10) shown as 'others'.

MnSOD activity were log transformed to normalize their distributions. Independent variables used included gender, genotype for each particular enzyme, and ethnicity. We did not adjust for the effect of age in our models because our cohort included only young adults, aged 17–21. We used dichotomous variables to group subjects by genotype for GPX1 and MnSOD models. For GPX, the reference group included subjects with the CC or CT genotype. For MnSOD, the genotype variable was divided into those with the CC genotype (reference group) versus all others (CT or TT). In the catalase model, we examined enzyme activities of the CT and TT groups separately and compared them to the CC reference group. Ethnic groups were coded as dummy variables and Caucasians were used as the reference group. African-Americans ($n = 4$), Native-Americans ($n = 1$), people of mixed origin ($n = 5$), and subjects with other ethnic backgrounds ($n = 10$) were pooled together as 'others' for statistical analysis. Backwards deletion was used to select variables used in the final multiple regression model. To test the significance of the interaction between gender and genotypes (GPX1), we used a simultaneous test based on the sums of squares from full and reduced linear regression models to compare models with interaction variables to the baseline model without these interaction variables (*F*-tests). The same test was used to examine possible interactions between gender and ethnicity and between genotypes and ethnicity.

We also verified that the area of residence did not affect baseline enzyme activity levels ($P = 0.972$ for GPX1, $P = 0.268$ for MnSOD). Area of residence appeared initially to be associated significantly with catalase activity; those who spent equal time in the Bay Area and LA had higher catalase activity than lifetime residents of either the Bay Area or LA. However, upon further inspection, the effect appeared to be due to a small number of outliers with high catalase activity. Dropping the area variable from the regression model did not change the coefficients significantly for the baseline variables. Thus, the area variable was not included in the regression model for catalase activity.

Results

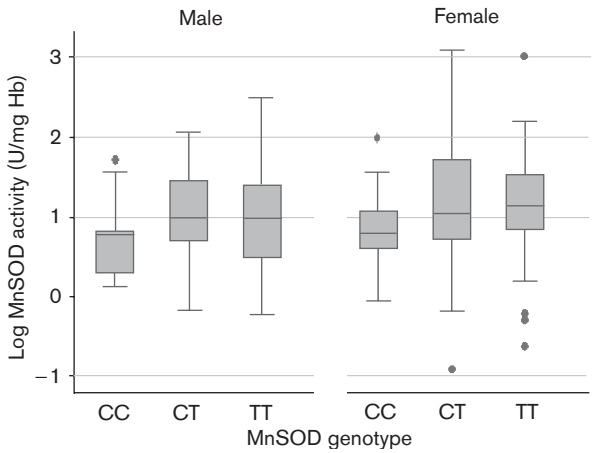
Allele and genotype frequencies

Genotype distributions did not differ significantly from Hardy–Weinberg equilibrium for the total cohort and within ethnic groups with the exception of the GPX1 genotype in the Asian/Pacific Islander group ($P = 0.02$); this is likely due to the ethnic heterogeneity of this particular subgroup classification. Overall, genotype frequencies for MnSOD were 12.3% for CC, 42.1% for CT, and 45.6% for TT respectively. For GPX1, genotype frequencies were 69.6% for CC, 24.4% for CT, and 6.0% for TT respectively. Allelic and genotype frequencies in Asians differed from both Caucasians and Hispanics for both MnSOD and GPX1, while frequencies were similar between the latter two groups (Table 2). C allele frequencies for MnSOD in Asian /Pacific Islanders were much lower as compared to Hispanics and Caucasians. For catalase, however, Asian/Pacific Islanders and Hispanics had similar allele and genotype frequencies and Caucasians differed from those two groups (CC: 83.9%, 64.7%; CT: 14.4%, 30.9%; TT: 1.7%, 6.8% for Asians and Caucasians respectively).

MnSOD activity

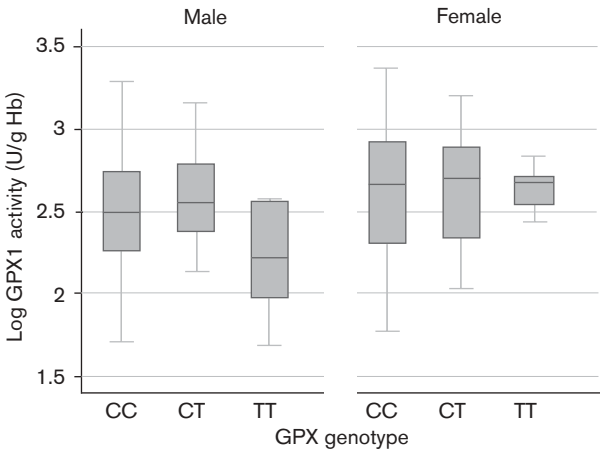
MnSOD activity ranged from 0.40 to 22.31 U/mg Hb with a median of 2.81 U/mg Hb. Figure 1 shows the distribution of log MnSOD activity by gender and genotype. For

Fig. 1



Distributions of log MnSOD activity by gender and genotype. Using descriptive statistics without adjusting for ethnicity, mean log MnSOD activity was 0.73 (95%CI: 0.39, 1.07) for genotype CC and 1.02 (95%CI: 0.90, 1.15) for genotypes CT or TT in males ($n=102$). For females ($n=129$), mean log MnSOD activity for CC was 0.87 (95%CI: 0.62, 1.12) and 1.16 (95%CI: 1.03, 1.29) for CT or TT. In the multiple regression model, MnSOD activity was 16% higher in females than males (95%CI: −1%, 38%) after adjusting for ethnicity. Those with the CC genotype had 39% lower MnSOD activity than those with CT or TT (95%CI: 7%, 80%), but there is substantial overlap in the distributions.

Fig. 2



Distributions of log GPX1 activity by gender and genotype. In males ($n=102$), mean log GPX1 activity was 2.52 (95%CI: 2.45, 2.59) for those with genotypes CC or CT and 2.20 (95%CI: 1.83, 2.57) for those with the TT genotype (as determined by descriptive statistics). In females ($n=129$) mean log GPX1 activity was 2.62 (95%CI: 2.55, 2.69) for those with genotypes CC or CT and 2.68(95%CI: 2.43, 2.93) for those with the TT genotype. TT males had lower activity than males CC or CT.

all three genotypes, activity levels were greater in females than in males. Multiple linear regression analysis revealed a marginally significant association between gender and MnSOD activity, where activity in females was 16% greater than in males (95%CI : 1%, 38%). By genotype, subjects with CT or TT had 39% greater activity than subjects with the CC genotype (95%CI : 7%, 80%). MnSOD activity also differed by ethnic group after adjusting for gender and genotype; it was 24% lower in Hispanics than Caucasians (95%CI: −45%, 3%), although this association was only suggestive. The 'others' group was also significantly lower than Caucasians (−28%, 95% CI: −48%, −1%), however, this group was heterogeneous thus this association may be due to the lack of CC genotypes in the group.

GPX1 activity

GPX1 activity in this population ranged from 5.09 to 28.93 U/g Hb with a median of 13.16 U/g Hb. Mean log GPX1 activity was lower in TT males (2.20 U/g Hb) than in CT or CC males (2.60 U/g Hb and 2.49 U/g Hb respectively), while mean log GPX activity in females was similar for all three genotypes (TT: 2.63 U/g Hb; CT: 2.65 U/g Hb; CC: 2.62 U/g Hb) (Fig. 2). Multiple linear regression provided suggestive evidence for an interaction ($P=0.096$) between gender and genotype (CC or CT versus TT), where males with the TT genotypes had the lowest GPX1 activity. Ethnicity did not affect baseline activity of GPX1 in this population.

Catalase activity

Catalase activity in this cohort had a mean level of 86.27 k/g Hb and ranged from 18.93 to 152.58 k/g Hb (Fig. 3). Gender and ethnicity did not affect catalase activity in the analysis. However, the regression model did indicate an association between genotype and catalase activity. Subjects with CT had on average 7.74 k/g Hb (95%CI: −17.64, 2.17) lower activity than subjects with CC (89.47 k/g Hb, 95%CI: 85.38, 93.56), while subjects with TT had on average 18.13 k/g Hb (95%CI: −32.26, −4.00) lower activity than CC subjects.

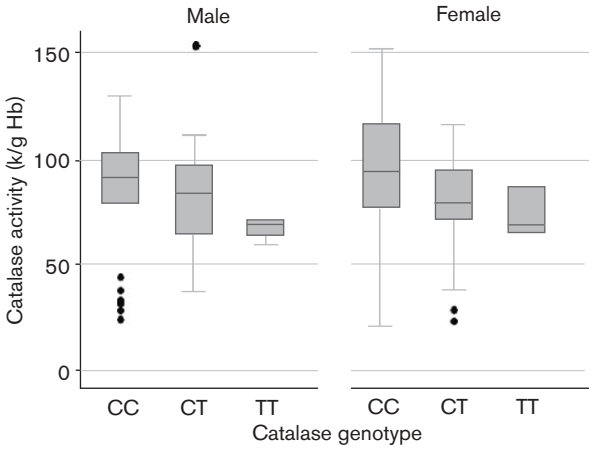
Correlations between enzyme activities

Overall, the activity levels of the three enzymes were positively correlated with each other (Table 3). The Pearson's correlation coefficients (r) were 0.32 for log transformed GPX1 activity versus log transformed MnSOD activity, 0.29 for log transformed GPX1 versus catalase, and 0.39 for log transformed MnSOD versus catalase. All three correlations were highly significant ($P<0.001$).

Discussion

This study examined the genotype–activity relationships for three metabolically linked antioxidant enzymes directly involved in ROS detoxification: MnSOD (C47T), GPX1 (C593T), and catalase (−262C > T). We used a considerably larger sized cohort ($n=231$) of healthy young adults as compared to previously published

Fig. 3



Distributions of catalase activity by gender and genotype. Mean catalase activity as determined by descriptive statistics, was 89.47(95%CI: 85.20, 93.74) for those with the CC genotype and was higher than mean catalase activity for those with either the CT or TT genotypes: 78.5 (95%CI: 72.10, 84.97).

studies. Since this cohort was ethnically diverse, we also examined to some extent how ethnicity might affect baseline enzyme activity levels. Genotype frequencies for Caucasians and Asians were similar to those published in the literature for all three genes [12,14,19,30].

It is difficult to compare the results of our MnSOD data to other studies as most studies have examined the effects of MnSOD genotype on disease outcomes rather than enzyme activity. The only other study looking at MnSOD activity reported 40% greater activity for the CC genotype in a human hepatoma cell line [31]. However, this hepatoma cell line study looked at the intensity bands on activity gels to measure functional activity, a method which has been questioned in the past because of potential limitations of accuracy [32]. The in-vitro results, however, may have some biological plausibility given the experimental evidence, which suggested that transport of the Val variant may be partially arrested in the inner mitochondrial membrane, while the Ala variant targets successfully into the mitochondria [33]. In contrast to this transformed cell line study [31], we found that the TT and CT genotypes for the MnSOD polymorphism (C47T) conferred higher enzyme activity in erythrocytes of humans than the CC genotype.

Two studies that examined the effects of this MnSOD polymorphism on markers of oxidative stress found that the Val variant was actually protective. Hong *et al.* [34] reported an increased formation of 8-OHdG, a common biomarker of ROS-induced DNA damage for Val/Ala (CT) and Ala/Ala (CC) variants. Similarly, Park *et al.* [35] saw that the Val/Ala and Ala/Ala polymorphisms modulated the effect of 1-OHP (a biomarker of exposure to PAHs) on 8-OHdG where those two variants experienced greater oxidative injury than the Val/Val (TT) variant. Thus, although these studies did not measure functional enzyme activity, their results were consistent with those of our study, pointing to an increased protection from oxidative stress for the TT genotype.

Table 3 Multiple linear regression analysis of enzyme activity

	n	Variable	β[c]	95% CI	*P*-value	R^{2d}
MnSOD[a]	228	Intercept	0.80	(0.29, 0.99)		0.06
		Gender				
		Males[b]	–			
		Females	0.15	(−0.01, 0.32)	0.07	
		SOD2 genotype				
		CC[b]	–			
		CT or TT	0.33	(0.07, 0.59)	0.01	
		Ethnicity				
		Caucasian[b]	–			
		Asian	−0.09	(−0.28, 0.11)	0.38	
		Hispanic	−0.28	(−0.59, 0.03)	0.07	
		Others	−0.33	(−0.66, −0.01)	0.04	
GPX1[a]	217	Intercept	2.52	(2.45, 2.59)		0.05
		Gender				
		Males[b]	–			
		Females	0.10	(0.01, 0.20)	0.04	
		GPX genotype				
		CC or CT[b]	–			
		TT	−0.65	(−1.27, −0.02)	0.04	
		Gender *GPX genotype	0.33	(−0.06, 0.71)	0.10	
Catalase	227	Intercept	89.47	(85.38, 93.56)	0.03	0.03
		Catalase genotype				
		CC[b]	–			
		CT	−7.74	(−17.64, 2.17)	0.13	
		TT	−18.13	(−32.26, −4.00)	0.01	

[a]Activities were log transformed to normalize the distribution.
[b]Reference group.
[c]β represents the regression coefficient for each particular variable in the model.
[d]R^2 describes the goodness of fit of the regression model and is frequently interpreted as the fraction of variability explained by the independent variables.

We also found suggestive evidence that gender modifies the effect of genotype (C593T polymorphism) on GPX1 enzyme activity. The first study to investigate the genotype–activity relationship of the C593T polymorphism reported no difference in activity by genotype; however, their sample size was smaller ($n = 66$) and no information on gender was reported [18]. In contrast, more recent studies corroborate our results. Hamanishi *et al.* [19] performed in-vitro functional analyses, which indicated a 40% decrease in enzyme activity for the T variant versus the C variant. Massafra *et al.* [36] also reported gender-related differences in GPX1 activity (erythrocytes), where activity in premenopausal women was about 10% higher than in age-matched men.

The effect of the C593T polymorphism on enzymatic activity and phenotype remains unclear, but dietary antioxidant intake may contribute to the gender differences seen in GPX1 activity, since GPX1 is selenium dependent [13]. In a previous study of this cohort, we analyzed dietary antioxidants and lung function and reported that the males consumed fewer fruits and vegetables and had a lower intake of dietary antioxidant nutrients than the females [37]. The differences between the C and T allele may only be apparent when dietary differences or correlated variables (e.g. gender in our study) are considered.

The $-262C > T$ polymorphism in the promoter region of the catalase gene affects transcription factor binding, and the T allele has been associated with higher blood catalase levels [22]. We have found the TT genotype to be associated with a decrease in catalase enzymatic activity, which agrees with another previous study of patients with breast cancer [38]. The CT genotype was also associated with decreased catalase activity, but it did not reach statistical significance. While the TT genotype may confer higher transcription of the catalase gene, it is not clear how this might affect enzymatic activity and function. One possibility is that the $-262C > T$ polymorphism in the promoter region could be in strong linkage disequilibrium with a polymorphism in the coding region that affects functional enzymatic activity.

While statistically significant differences in mean enzyme activities by genotype were detected, there was also considerable interindividual variability within genotypes. This is consistent with past studies which also reported a wide range of enzymatic activities among randomly selected subjects [6] and healthy subjects [5]. For example, MnSOD enzyme activities in our cohort (0.40–22.31 U/mg Hb) were similar to those in healthy subjects from Argentina (7.8–17.6 U/mg Hb) [5]. GPX1 activities ranged from 0.1–0.7 U/g Hb in one study [5] and 86.32–165.55 U/g Hb in another [6], while our GPX1 activities ranged from 5.09–28.93 U/g Hb. Finally, catalase activities in our cohort (18.93–152.58 k/g Hb) were somewhat lower than those in a prior study of randomly selected Caucasian subjects (130.36–192.27 k/g Hb) [6]. Differences in activities between studies may be attributable to a number of factors such as age, ethnicity, gender, and chance variation.

The variability within each genotype group suggests that in addition to the studied polymorphisms, other sources of interindividual variability contribute to enzymatic activity levels including combinations of multiple genetic variants, age, gender and environmental factors. This is consistent with our results, which showed an effect of gender on GPX1 activity and gender and ethnicity on MnSOD activity. Further, the low R^2 values of the linear regression models suggest that genotype accounts for only a small portion of enzyme activity variation. We postulate that the baseline activity of these three enzymes is under complex control and is likely driven by multiple factors including dietary intake of antioxidants, exercise, and environmental exposures. In summary, this study found that a wide range of activity levels for three antioxidant enzymes among a cohort of healthy young adults is partially explained by variation of genetic polymorphisms, and is further modified by gender and ethnicity. There may also be common regulatory mechanisms for all three enzymes since our analysis showed their activities were positively correlated.

Acknowledgements

The authors thank Jessie Murphy, Lucas Carlton, and Sarah Deamer for collection of study subject information, Laura Pfleger for hemoglobin measurements, and Dr. Kenny Beckman for assistance with genotyping. This work was supported by grant HL60689 from the Division of Lung Diseases, National Heart, Lung and Blood Institute.

References

1 Halliwell B, Cross CE. Oxygen-derived species: their relation to human disease and environmental stress. *Environ Health Perspect* 1994; **102(Suppl 10)**:5–12.
2 Van Remmen H, Qi W, Sabia M, Freeman G, Estlack L, Yang H, *et al.* Multiple deficiencies in antioxidant enzymes in mice result in a compound increase in sensitivity to oxidative stress. *Free Radic Biol Med* 2004; **36**:1625–1634.
3 MacNee W. Pulmonary and systemic oxidant/antioxidant imbalance in chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2005; **2**: 50–60.
4 Cerutti P, Larsson R, Krupitza G, Muehlematter D, Crawford D, Amstad P. Pathophysiological mechanisms of oxidants. In: Cerutti PA, ed. *Oxy-Radicals in Molecular Biology and Pathology*. New York: Alan R. Liss; 1988.
5 Bolzan AD, Bianchi MS, Bianchi NO. Superoxide dismutase, catalase and glutathione peroxidase activities in human blood: influence of sex, age and cigarette smoking. *Clin Biochem* 1997; **30**:449–454.
6 Andersen HR, Nielsen JB, Nielsen F, Grandjean P. Antioxidative enzyme activities in human erythrocytes. *Clin Chem* 1997; **43**:562–568.
7 Rosenblum JS, Gilula NB, Lerner RA. On signal sequence polymorphisms and diseases of distribution. *Proc Natl Acad Sci USA* 1996; **93**: 4471–4473.
8 Sutton A, Khoury H, Prip-Buus C, Cepanec C, Pessayre D, Degoul F. The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. *Pharmacogenetics* 2003; **13**:145–157.

9 Wang LI, Miller DP, Sai Y, Liu G, Su L, Wain JC, *et al.* Manganese superoxide dismutase alanine-to-valine polymorphism at codon 16 and lung cancer risk. *J Natl Cancer Inst* 2001; **93**:1818–1821.

10 Hiroi S, Harada H, Nishi H, Satoh M, Nagai R, Kimura A. Polymorphisms in the SOD2 and HLA-DRB1 genes are associated with nonfamilial idiopathic dilated cardiomyopathy in Japanese. *Biochem Biophys Res Commun* 1999; **261**:332–339.

11 Ambrosone CB, Freudenheim JL, Thompson PA, Bowman E, Vena JE, Marshall JR, *et al.* Manganese superoxide dismutase (MnSOD) genetic polymorphisms, dietary antioxidants, and risk of breast cancer. *Cancer Res* 1999; **59**:602–606.

12 Hsueh YM, Lin P, Chen HW, Shiue HS, Chung CJ, Tsai CT, *et al.* Genetic polymorphisms of oxidative and antioxidant enzymes and arsenic-related hypertension. *J Toxicol Environ Health A* 2005; **68**:1471–1484.

13 Arthur JR. The glutathione peroxidases. *Cell Mol Life Sci* 2000; **57**: 1825–1835.

14 Forsberg L, de Faire U, Morgenstern R. Oxidative stress, human genetic variation, and disease. *Arch Biochem Biophys* 2001; **389**:84–93.

15 Brown KM, Pickard K, Nicol F, Beckett GJ, Duthie GG, Arthur JR. Effects of organic and inorganic selenium supplementation on selenoenzyme activity in blood lymphoctyes, granulocytes, platelets and erythrocytes. *Clin Sci (Lond)* 2000; **98**:593–599.

16 Forsberg L, de Faire U, Morgenstern R. Low yield of polymorphisms from EST blast searching: analysis of genes related to oxidative stress and verification of the P197L polymorphism in GPX1. *Hum Mutat* 1999; **13**:294–300.

17 Moscow JA, Schmidt L, Ingram DT, Gnarra J, Johnson B, Cowan KH. Loss of heterozygosity of the human cytosolic glutathione peroxidase I gene in lung cancer. *Carcinogenesis* 1994; **15**:2769–2773.

18 Forsberg L, de Faire U, Marklund SL, Andersson PM, Stegmayr B, Morgenstern R. Phenotype determination of a common Pro-Leu polymorphism in human glutathione peroxidase 1. *Blood Cells Mol Dis* 2000; **26**:423–426.

19 Hamanishi T, Furuta H, Kato H, Doi A, Tamai M, Shimomura H, *et al.* Functional variants in the glutathione peroxidase-1 (GPx-1) gene are associated with increased intima-media thickness of carotid arteries and risk of macrovascular diseases in Japanese type 2 diabetic patients. *Diabetes* 2004; **53**:2455–2460.

20 Hu YJ, Diamond AM. Role of glutathione peroxidase 1 in breast cancer: loss of heterozygosity and allelic differences in the response to selenium. *Cancer Res* 2003; **63**:3347–3351.

21 Ratnasinghe D, Tangrea JA, Andersen MR, Barrett MJ, Virtamo J, Taylor PR, *et al.* Glutathione peroxidase codon 198 polymorphism variant increases lung cancer risk. *Cancer Res* 2000; **60**:6381–6383.

22 Forsberg L, Lyrenas L, deFaire U, Morgenstern R. A common functional C-T substitution polymorphism in the promoter region of the human catalase gene influences transcription factor binding, reporter gene transcription and is correlated to blood catalase levels. *Free Radic Biol Med* 2001; **30**: 500–505.

23 Ahsan H, Chen Y, Kibriya MG, Islam MN, Slavkovich VN, Graziano JH, *et al.* Susceptibility to arsenic-induced hyperkeratosis and oxidative stress genes myeloperoxidase and catalase. *Cancer Lett* 2003; **201**:57–65.

24 Tager IB, Balmes J, Lurmann F, Ngo L, Alcorn S, Kunzli N. Chronic exposure to ambient ozone and lung function in young adults. *Epidemiology* 2005; **16**:751–759.

25 Mattiazzi M, D'Aurelio M, Gajewski CD, Martushova K, Kiaei M, Beal MF, *et al.* Mutated human SOD1 causes dysfunction of oxidative phosphorylation in mitochondria of transgenic mice. *J Biol Chem* 2002; **277**:29626–29633.

26 Drabkin D, Austin J. Spectrophotometric studies: preparation from washed blood cells, nitric oxide, hemoglobin and sulfhemoglobin. *J Biol Chem* 1935; **112**:51–65.

27 Flohe L, Otting F. Superoxide dismutase assays. *Methods Enzymol* 1984; **105**:93–104.

28 Flohe L, Gunzler WA. Assays of glutathione peroxidase. *Methods Enzymol* 1984; **105**:114–121.

29 Aebi H. Catalase *in vitro*. *Methods Enzymol* 1984; **105**:121–126.

30 Van Landeghem GF, Tabatabaie P, Kucinskas V, Saha N, Beckman G. Ethnic variation in the mitochondrial targeting sequence polymorphism of MnSOD. *Hum Hered* 1999; **49**:190–193.

31 Sutton A, Imbert A, Igoudjil A, Descatoire V, Cazanave S, Pessayre D, *et al.* The manganese superoxide dismutase Ala16Val dimorphism modulates both mitochondrial import and mRNA stability. *Pharmacogenet Genom* 2005; **15**:311–319.

32 St Clair D, Kasarskis E. Genetic polymorphism of the human manganese superoxide dismutase: what difference does it make? *Pharmacogenetics* 2003; **13**:129–130.

33 Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, Nakagawa-Hattori Y, Shimizu Y, Mizuno Y. Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. A predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinson's disease. *Biochem Biophys Res Commun* 1996; **226**:561–565.

34 Hong YC, Lee KH, Yi CH, Ha EH, Christiani DC. Genetic susceptibility of term pregnant women to oxidative damage. *Toxicol Lett* 2002; **129**:255–262.

35 Park SY, Lee KH, Kang D, Lee KH, Ha EH, Hong YC. Effect of genetic polymorphisms of MnSOD and MPO on the relationship between PAH exposure and oxidative DNA damage. *Mutat Res* (in press).

36 Massafra C, Gioia D, De Felice C, Muscettola M, Longini M, Buonocore G. Gender-related differences in erythrocyte glutathione peroxidase activity in healthy subjects. *Clin Endocrinol (Oxf)* 2002; **57**:663–667.

37 Tujague J, Bastaki M, Holland N, Balmes J, Tager I. Antioxidant intake, GSTM1 polymorphism and pulmonary function in healthy young adults. *Eur Respir J* (in press).

38 Ambrosone CB, Ahn J, Singh KK, Rezaishiraz H, Furberg H, Sweeney C, *et al.* Polymorphisms in genes related to oxidative stress (MPO, MnSOD, CAT) and survival after treatment for breast cancer. *Cancer Res* 2005; **65**: 1105–1011.