

Effects of Antioxidant Enzyme Polymorphisms on Ozone-induced Lung Function Changes

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Supported by (1) the National Institutes of Health (R01 HL60689), and (2) the American Lung Association (Research Training Fellowship)

Running head: Antioxidant enzyme genes, O₃, lung function

Abstract word count: 198

Body of the manuscript word count: 3,478 words

Keywords: antioxidant enzymes, lung function, oxidative injury, ozone

ABSTRACT

Chronic exposure to O₃ can cause changes in lung function that may reflect remodeling of small airways. Antioxidant enzyme function likely affects susceptibility to O₃. The aim of this study was to determine whether polymorphisms in antioxidant enzyme (*GSTM1*, *GSTP1*, *NQO1*) genes affect the risk of lung function changes related to chronic exposure to O₃.

We genotyped 210 young adults who participated in a previous study that showed a relationship between lifetime exposure to O₃ and decreased lung function. We used multivariable linear regression to model sex-specific associations between genotypes and O₃-related lung function changes, adjusting for height, weight, lifetime exposure to NO₂, PM₁₀, and self-identified race/ethnicity.

The *GSTM1*-null/*NQO1* Pro187Pro-combination genotype was significantly associated with increased risk of an O₃-related decrease in FEF₂₅₋₇₅ in females [parameter estimate (SE): -75 (35) ml/s], while the *GSTP1* Val105 variant genotypes were significantly associated with greater risk of an O₃-related decrease in FEF₇₅ in males [-81 (31) ml/s]. *GSTM1*-null status was not significantly associated with any O₃-related changes in lung function in either sex.

We conclude that the effects of antioxidant enzyme gene polymorphisms on risk of decreased lung function related to chronic exposure to O₃ may be modified by sex-specific factors.

INTRODUCTION

Ozone (O_3), a major component of air pollution, is a potent oxidant gas that causes airway injury in human lungs (1). A large percentage of inhaled O_3 (up to 90%) is absorbed in the respiratory tract along the entire tracheobronchial tree (2), with the greatest dose being delivered to the peripheral airways at the junction between the conducting and respiratory airways (3). Ozone reacts with respiratory tract lining fluid (RTLFL) constituents and cellular membrane components to generate lipid ozonation products (LOP) and reactive oxygen species (ROS), which in turn can cause oxidative damage to other biological molecules (4-6). Acute exposure to ambient levels of O_3 can induce short-term lung function abnormalities and airway inflammation, while chronic exposure may lead to remodeling of the small airways where deposition is the greatest (7-12).

To minimize the potential for oxidative injury, the human lung has an integrated system of antioxidant enzymes and expendable soluble molecules. This system includes several mechanisms by which ROS are converted to products that are further detoxified by other enzymes. If the oxidant burden is sufficiently great, ROS may overwhelm the antioxidant system leading to a state of "oxidative stress," which is thought to contribute to the pathogenesis of a number of respiratory diseases (13-15). Although antioxidant defenses are available to decrease oxidative stress in the airways, individuals differ in their ability to deal with an oxidant burden; and such differences, in part, are determined genetically (16). This genetic variability may account for the considerable between-subject variability seen in both the lung function and airway inflammatory responses to O_3 (17, 18).

Glutathione *S*-transferase (GST) enzymes, a superfamily of dimeric phase II metabolic enzymes, play an important role in the antioxidant defense system. GST enzymes catalyze the

conjugation of toxic electrophilic molecules with glutathione and thereby protect cellular macromolecules from damage due to LOP and ROS. The specific GST enzymes that have been proposed as important for antioxidant defense are those of the mu (GSTM), theta (GSTT), and pi (GSTP) classes, each with functional polymorphisms that affect protein expression or function (19).

A common polymorphism in the GSTM1 gene locus, which exists in 30-50% of the general population (19, 20), involves a null allele that results in a complete lack of GSTM enzymatic function. Therefore, the GSTM1 null genotype would be expected to affect the individual's response to O₃ exposure, possibly causing increased susceptibility to oxidative injury. Recently, the results of several field studies showed that the GSTM1 null genotype is associated with greater acute lung function and/or respiratory symptom responses to O₃-induced oxidative stress (21-23). Other studies have suggested that the GSTM1 null genotype may play a significant role in development of asthma in response to oxidative stress (24, 25). In two of these studies, a polymorphism (Ser187) of a second antioxidant enzyme, NAD(P)H:Quinone Oxidoreductase (NQO1), provided a protective effect among GSTM1 null subjects (21, 25).

Another gene of interest with regard to responses to oxidant pollutants is GSTP1, which is the most abundant GST in lung tissue and has a common A105G polymorphism that results in a Ile105Val amino acid substitution. In the Children's Health Study (CHS), children who were homozygotes for GSTP1 Val105 variant allele had a lower rate of respiratory infections than those with the GSTP1 Ile/Ile105 wild type, but somewhat surprisingly, they also had a slower rate of lung function growth (26, 27). A recent study showed that the GSTP1 Val/Val105 genotype was associated with increased O₃-related respiratory symptoms (23). In contrast, a small (n=19) controlled exposure study of sensitized allergic rhinitic adults in which nasal

instillation of diesel exhaust particles, known to cause oxidative stress, enhanced specific allergic responses to ragweed, Gilliland and coworkers showed that the GSTM1 null genotype increased susceptibility and the GSTP1 Val105 variant had a protective effect (28).

Considered together, these human studies provide suggestive evidence that polymorphisms of Phase II enzymes contribute to susceptibility to inhaled oxidant-induced toxicity. In a recent epidemiological study (11), our group showed an association between lifetime exposure to ambient O₃ and decreased lung function parameters consistent with small airway remodeling. To determine whether the GSTM1 null, GSTM1 null/NQO1 homozygous Pro187 combination, or GSTP1 Val105 variant genotypes had an effect on the observed relationship between lifetime exposure to O₃ and decreased lung function, we genotyped the subjects who participated in the previous study and assessed whether these genotypes affected risk of O₃-induced lung function changes. We selected these three genotypes for study on the basis of the previous literature reviewed above.

METHODS

The protocol for the study was approved by the Committee for the Protection of Human Subjects, University of California, Berkeley (UCB), and the Committee on Human Research, University of California, San Francisco. Written, informed consent was obtained from all study participants once eligibility was established.

Study Design

The overall design of the study has been previously presented in detail (11). Briefly, a convenience sample of 255 freshman undergraduates at the University of California, Berkeley

(UCB) was recruited in three waves that began on April 10, 2000, February 12, 2001 and February 6, 2002. All waves ended in the first week of June. Subjects were studied between February-May when students from Los Angeles (LA) would not have been exposed to the high summertime O₃ concentrations.

Students were eligible based on the following: 1) lifelong resident of the greater LA *or* San Francisco Bay (SF) area prior to enrollment at UCB; 2) lifetime never smoker, 3) no history of chronic respiratory disease (history of asthma before age 12 years was permitted, provided that student had no symptoms and had not taken any medication at any time after age 12 (n=6); and 4) no physical impairment that would hinder performance of spirometry. Location of all residences within the geographical boundaries for the study was confirmed by study personnel.

Ozone Exposure Assessment

A detailed description of the creation of lifetime cumulative O₃ exposure for each subject has been previously reported (10). Briefly, life-time residential history was reconstructed with a standardized questionnaire and air pollutant (O₃, NO₂, and PM₁₀) concentrations were assigned for each month of life to each residential location. Air quality data were acquired from the California Air Resources Board (ARB, CD No. PTSD-02-017-CD), the Aerometric Information Retrieval System (AIRS) and from special requests to ARB. Monthly mean measures of O₃ were interpolated spatially from air quality monitoring stations to the residence locations with inverse distance weighting and a maximum of three monitoring stations for each interpolation (maximum interpolation radius of 50 km). The details and reliability of the exposure assignment method have been published (10, 29, 30). Briefly, we fit two basic models to estimate life-time pollutant (O₃, NO₂, and PM₁₀) exposure. There was no significant difference in the association

between lifetime O₃ exposure and lung function between the two models. In this paper, we used the so-called “ecological” model which omitted estimates of time spent outdoors and used only the residence-specific monthly average, interpolated pollutant concentrations.

Subject Characteristics

Of the 255 enrolled subjects, 226 had sufficient DNA available for genotyping. The 29 subjects who were not genotyped were mostly female. Sixteen subjects did not self-identify with one of three main racial/ethnic groups (Asians/Pacific Islanders, Caucasians, and Hispanics) and were excluded from further analysis. Thus, 210 subjects were used in the final analysis (Table 1). Approximately 43% were male and 60% were lifelong residents of the LA area. Most of the participants were Asians (54% of males and 60% of females) or Caucasians (39% of males and 28% of females). There were no significant sex differences in lifetime exposure estimates of pollutants (O₃, PM₁₀, and NO₂). Although subjects who grew up in LA had higher median estimated lifetime exposures than those from the SF area, distributions between the two regions overlapped and represented a continuum of individual exposure.

Antioxidant Enzyme Genotyping

DNA was isolated from clot with a Qiaamp Blood DNA Maxi kit (Qiagen, Inc., Santa Clarita, CA, USA) in accordance with the manufacturer’ instructions, and stored in -80° until use. Genotyping for the GSTM1 polymorphism was carried out following a previously reported protocol (31). TaqMan real time PCR method was used to detect polymorphisms of GSTP1 (A105G) and NQO1 (C187T). Primers and probes for the SNPs were custom-designed by Applied Biosystems, Inc. (Foster City, CA) (see online supplement for primers’ sequences). The

reaction was carried out in TaqMan Universal Master Mix with a 7900 Real-Time PCR machine. Quality assurance procedures included assessment of randomly distributed blank samples, duplicates of randomly selected samples, manual calls assisting automated calling for Taqman analysis, and repeated additional analysis from independently isolated DNA samples from the same subjects. Assays were repeated for all low confidence samples until a reliable call was obtained. The genotype frequencies for GSTM1, GSTP1, and GSTM1 null/NQO1 did not deviate from Hardy-Weinberg equilibrium.

Spirometry

Forced expiratory volumes were obtained in the sitting position with nose clip with a Collins Survey rolling seal spirometer (Collins Survey; Warren E. Collins, Co. Braintree, MA) with two modifications to the standard criteria of the American Thoracic Society (32), details of which were previously reported (33). FVC, FEV₁, FEF₂₅₋₇₅, FEF₇₅ were recorded. The FEF₂₅₋₇₅/FVC ratio, an estimate of the reciprocal of the time constant of the lung (34) and a reflection of intrinsic airway size (33), was also calculated. This measure was used, in part, to control for racial/ethnic differences in airway size (11). Sex-specific models for each measure of lung function were fit based on height, weight and age as described previously (11, 31). There was no association between any measure of lung function and history of asthma before age 12 (n=6) or history of secondhand tobacco smoke exposure (n=34) (11).

Statistical Analysis

Genotypes for antioxidant enzyme polymorphisms were coded as follows: GSTM1 wild type (+) = 0 or null = 1 and GSTP1 Ile105 wild type (homozygous AA) = 0 or Val105 variant

(heterozygous AG or homozygous GG) = 1. The GSTM1/NQO1 combination genotype included only GSTM1 null subjects and used the following coding: NQO1 wild type Pro187 (homozygous CC) = 0 or Ser187 variant (heterozygous CT or homozygous TT) = 1. Because it is well established that lung function differs between males and females (35), sex-specific multivariable linear regression was used to model lung function variables. Except for FEV₁, natural logarithmic transformations of lung function variables were used. The initial model for each lung function variable included the subject's height, weight, race/ethnicity, and each genotype and was based on the optimal model among several tested as previously described (11, 31). Final models included height, weight, race/ethnicity, genotype, and lifetime exposures to O₃, PM₁₀, and NO₂ as previously described (11, 31). The combined genotype, GSTM1*NQO1, was treated as single term, based on reported interactions between the null allele of GSTM1 and the serine polymorphism for NQO1. The FEF₂₅₋₇₅/FVC ratio was treated as an interaction term for reasons previously discussed (11). Finally, we did not use measurement error correction procedures for the O₃ effects, since we have shown previously (11) that the coefficients for O₃ and the O₃-(FEF₂₅₋₇₅/FVC) interaction were not affected by such corrections.

Data were analyzed with SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Allele and genotype frequencies differed significantly among ethnicities (Table 2). For GSTM1, frequency of GSTM1 null status was significantly different for both Hispanics (60%) and Caucasians (57%) compared to Asians (44%). For NQO1, the NQO1 T allele (Ser187) was significantly lower in Caucasians (18%) than Asians (40%) and Hispanics (35%). For GSTP1, the G allele (Val105) was significantly more common in Hispanics (48%) than Caucasians

(20%) and Asians (29%).

Effect of Genotype on Lung Function

The effects of the three polymorphisms on lung function can be seen in Table 3 (see online supplement for complete model parameter estimates). In males, GSTM1 null homozygosity was associated significantly with decreased FEF₂₅₋₇₅ (-98 ml/s; 95% CI: -15, -181ml/s). However, when the GSTM1 null genotype occurred in combination with NQO1 wild type (homozygous Pro187), this association was no longer significant. Similarly, GSTM1 null was associated significantly with decreased FEF₇₅ (-133 ml/s; 95% CI: -27, -240 ml/s) while GSTM1 null/NQO1 wild type-combination genotype was not. For FEV₁, no association between either the GSTM1 null or GSTM1 null/NQO1 wild type-combination genotype was found in males (data not shown).

In contrast to males, there were no significant changes in FEF₂₅₋₇₅ and FEF₇₅ for the GSTM1 null variant alone in females. However, a decrease in FEF₂₅₋₇₅ (-136 ml/s; 95%CI: -29, -243 ml/s) and FEF₇₅ (-125 ml/s; 95%CI: -2, -253 ml/s) was associated significantly with the GSTM1 null/NQO1 wild-type combination genotype. For FEV₁, there was no association with either GSTM1 null or GSTM1 null/NQO1 wild type-combination genotype in females (data not shown).

Finally, effects of GSTP1 Val105 in male and females subjects also differed. The variant allele was marginally associated with changes in both FEF₂₅₋₇₅ and FEF₇₅ in both sexes. However, for males the variant allele was non-significantly associated with decreases in these flow measures, while for females the trend increases. No associations were found between GSTP1 val105 and FEV₁ in either males or females (data not shown).

Effect of Genotype and Lifetime O₃ Exposure on Lung Function

To explore the effect of genotype on risk of lung function changes due to chronic exposure to O₃, our final models included lifetime exposure to O₃, PM₁₀, and NO₂, and an interaction term for O₃*(FEF₂₅₋₇₅/FVC), in addition to the variables included in the initial genotype-only models. When this approach was used in sex-specific models for GSTM1 null and GSTM1 null/NQO1 wild type-combination genotypes, the only significant association with risk of O₃-related decreased lung function was observed in females for the combination genotype and FEF₂₅₋₇₅ (Table 4).

The GSTP1 variant allele, however, was associated with greater risk of O₃-related decreases in FEF₂₅₋₇₅ (p<0.11) and FEF₇₅ in males (p<0.04) (Table 4) after adjustment for lifetime O₃ exposure and its interaction with airway size. The magnitude of the effect can be estimated from the final model based on the male-specific 25th percentile FEF₂₅₋₇₅/FVC ratio and mean lifetime O₃ exposure difference between subjects from LA and SF (17ppb). For males who are homozygous GSTP1 Iso105 (wild-type), the 17 ppb lifetime O₃ exposure difference results in a 20 ml/s (95% CI: -18, -22 ml/s) decrease in FEF₇₅. For males carrying the Val105 variant GSTP1 allele, the 17 ppb lifetime O₃ exposure difference results in a 28 ml/s (95% CI: -26, -30 ml/s) decrease in FEF₇₅. The magnitude of the combined effect of the GSTP1 variant allele and lifetime O₃ exposure is almost 50% less for males with the median FEF₂₅₋₇₅/FVC ratio (i.e., larger airway size).

For females, the GSTP1 val105 variant allele did not have a statistically significant effect on lifetime O₃-related decreases in lung function.

DISCUSSION

In a previous study, we showed that estimated lifetime exposure to ambient O₃ in a cohort of adolescents was associated with reduced levels of lung function measures that reflect the function of small airways (11). In this study, we found that, without consideration of the effect of O₃, the male subjects of our cohort with the GSTM1 null genotype had lower lung function measures that reflect small airways compared to those without this genotype. We did not find this same gene effect for female subjects of our cohort. However, when lifetime exposure to O₃ was included in the models, we found no deleterious role for GSTM1 null on lung function in either sex, although the GSTM1 null/NQO1 wild type-combination genotype was associated with increased risk of O₃-related decreases in FEF₂₅₋₇₅ in females.

A novel finding of our study is that the GSTP1 Val105 variant genotype was a risk factor for decreased lung function in association with lifetime exposure to O₃ in males. On the other hand, our data suggest that this genotype may have a protective effect in females. This sex difference in effect of the GSTP1 Val105 variant genotype may help explain the finding of greater male sensitivity to O₃-induced lung function changes that we reported in our earlier paper (11). Although we can only speculate about the possible mechanism for such sex-specific modification, GSTP1 is known to have sex-specific patterns of expression (36, 37).

Previously, we have shown that the deleterious effect of O₃ on lung function was dependant on intrinsic airway size as measured by the FEF₂₅₋₇₅/FVC ratio (33, 34), with more deleterious effect from O₃ on lung function in subjects with smaller airway size (11). Here, in a model that includes antioxidant enzyme genotypes as well as the FEF₂₅₋₇₅/FVC ratio, our results show that the deleterious effect of O₃ on lung function remains dependent on airway size.

Gilliland and colleagues studied a large group of subjects from the Children's Health Study (CHS) in southern California and found that non-Hispanic white children with GSTM1 null genotype had a lower rate of lung function growth (27). The results from male subjects of our cohort are consistent with those of this CHS study. The analysis presented by the CHS investigators, however, was not stratified by sex. Thus, the role of sex in modifying the effects of enzyme genotypes on growth of lung function bears further investigation.

Several studies have suggested an association between O₃-induced airway oxidative injury and certain antioxidant enzyme genetic polymorphisms in non-asthmatic subjects, specifically with the GSTM1 null alone and the GSTM1 null/NQO1 wild type-combination genotypes. In a small field study, Bergamaschi *et al.* showed an association between the O₃ level in ambient air and decrements in lung function and changes in plasma CC16 only in individuals with the GSTM1 null/NQO1 wild type-combination genotype (21). Later, in a controlled exposure study, the same group of investigators showed a differential change in some biomarkers of oxidative stress after O₃ exposure between subjects with the GSTM1 null/NQO1 wild type combination genotype and those with other genotypes (38). The results of our chronic exposure study also suggest that women with the combined GSTM1 null/NQO1 wild type-combination genotype have increased susceptibility to O₃-related remodeling of small airways.

The lack of concordant findings with regard to our male subjects may be due to multiple differences between the Italian studies and the current study. Bergamaschi *et al.* and Corradi *et al.* studied the effects of acute O₃ exposure while our study is of the effect of chronic lifetime exposure. Our sample size is also much larger, potentially allowing us to uncover sex-specific differences in gene-environment interaction. In addition, the racial/ethnic composition of the

study populations is likely quite different since our population included Asian and Hispanic subjects, who were probably not represented in the Italian studies.

The genetic background of subjects from different self-identified racial/ethnic groups, which includes their genotypes for other antioxidant enzymes, likely plays an important role in determining their responses to O₃ exposure. Population stratification, which can cause spurious associations in candidate gene association studies, exists when the total population has been formed by admixture of two or more ancestral populations and when admixture proportions vary among individuals. If the risk of the outcome varies with ancestry proportions, then admixture will confound associations of the outcome with genotypes at any locus where allele frequencies vary between ancestral populations. Because genotype frequencies for our three candidate genes varied across racial/ethnic groups (Table 2), we adjusted for race/ethnicity in our regression models. However, inclusion of race/ethnicity in the models had little impact on the results (data not shown). Exclusion of the 16 subjects that did not self-identify as Asian, Caucasian, or Hispanic also did not significantly change the results of the analyses.

We acknowledge several limitations of our study. First, while larger than many of the other studies that have assessed the effects of the GSTM1 null, GSTM1 null-NQO1 wild-type combination, and GSTP1 Val105 genotypes on response to oxidant pollutants, our study population is too small to definitely assess gene-environment interactions, especially if sex-specific modification is present. Second, other genes that we did not study likely play a role in determining susceptibility to chronic exposure to O₃. Finally, although we attempted to control for population stratification by including self-identified race/ethnicity in our regression models, the use of genetic markers might have improved our ability to do so.

In conclusion, we found the GSTP1 Val105 variant genotype to increase risk of the deleterious effects of chronic exposure to O₃ on measures of lung function that reflect small airway remodeling in a group of healthy adolescent males, but this genotype may have a protective effect in their female counterparts. Unlike previous reports from smaller studies of acute exposures, we did not find the GSTM1 null genotype in either sex or the GSTM1 null/NQO1 wild type-combination genotype in men to be associated with decreased lung function due to chronic exposure to O₃. However, we did find that the GSTM1 null/NQO1 wild type-combination genotype to increase risk of O₃-related loss of FEF₂₅₋₇₅ in women. Our results suggest that the effects of antioxidant enzyme gene polymorphisms on risk of decreased lung function related to chronic exposure to O₃ may be modified by sex-specific factors.

ACKNOWLEDGEMENTS

We greatly appreciate the expert assistance of Drs. Kenneth Beckman and Maria Bastaki with genotyping, the dedication of research assistants Lucas Carlton, Jessie Murphy, and Sarah Deamer, and the participation of the University of California students who made the study possible.

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Table 1. Description of Cohort Study Subjects

	Males, N= 90	Females, N=120
Age (years); %		
<18	46	57
19	49	41
20+	5	2
Ethnicity^a; %		
Asian/Pacific Islander	54	60
Caucasian	39	28
Hispanic	7	12
Residence^b; %		
San Francisco Bay Area	41	47
Los Angeles	56	48
Both	3	5
Estimated Lifetime Exposure^c to:		
median (range; IQR)		
O ₃ (monthly 8-hr average, ppb)	37 [14-59; 28-46]	33 [26-42; 9-57]
PM ₁₀ ^d (4-hr average, mcg/m ³)		
prior to year 1987	73 [33-115; 53-94]	69 [23-91; 52-92]
1987 and later	36 [11-61; 26-44]	29 [9-50; 26-43]
NO ₂ (average, ppb)	29 [9-48; 22-41]	26 [5-47; 21-40]

a. Self-reported ethnicity.

b. Lifetime residence before enrollment at the University of California Berkeley. Los Angeles: between the latitudes 32-35° and longitudes 115.5-120.75°; San Francisco: between latitudes 37-38.5° and longitudes 121.67-123°; Both: spent equal time in both Los Angeles and San Francisco (11).

c. Values are medians [range; interquartile range] of estimated lifetime exposure to air pollutants.

d. PM₁₀ = particulate matter $\leq 10 \mu\text{m}$ in diameter

Table 2. Genotype and Allele Frequencies by Race/ethnicity

	API	Cauc	Hisp	Total
GSTM1 - % (n)				
null	56 (65)	43 (30)	40 (8)	50 (103)
present [§]	44 (52)	57 (39)*	60 (12)*	50 (103)
NQO1 - % (n)				
CC	40 (47)	66 (45)	50 (10)	50 (102)
CT	40 (47)	31 (21)	30 (6)	36 (74)
TT	20 (24)	3 (2)	20 (4)	15 (30)
Alleles %				
C	60	82	65	67
T	40	18*	35	33
GSTP1 - % (n)				
AA	63 (74)	51 (35)	30 (6)	56 (115)
AG	34 (40)	41 (28)	45 (9)	37 (77)
GG	3 (4)	9 (6)	25 (5)	7 (15)
Alleles %				
A	80	71	53	74
G	20	29	48*	26
GSTM1 null/NQO1 - % (n)				
CT or TT	64 (41)	41 (12)	63 (5)	59 (58)
CC	36 (23)	59 (17)	38 (3)	41 (43)

API = Asian/Pacific Islander, Cauc = Caucasians, Hisp = Hispanics

*p<0.05 (logistic regression), API as referent group

[§]homozygous (2 alleles) or heterozygous (1 allele)

Table 3. Genotype-only Model of Lung Function

		Parameter Estimates (Standard Error)				
		Males		Females		
		<i>GSTP1</i> Val105	<i>GSTM1</i> -null	<i>GSTM1</i> -null/ <i>NQO1</i> Pro187	<i>GSTM1</i> -null	<i>GSTM1</i> -null/ <i>NQO1</i> Pro187
FEF₂₅₋₇₅	genotype	-0.023 (0.042)	-0.098 (0.042)*	-0.023 (0.057)	-0.012 (0.041)	-0.136 (0.055)*
	adjusted R ²	0.16	0.20	0.25	0.07	0.20
FEF₇₅	genotype	-0.058 (0.054)	-0.133 (0.055)*	-0.049 (0.064)	-0.01 (0.051)	-0.125 (0.065)*
	adjusted R ²	0.17	0.21	0.36	0.09	0.17

Each model includes height (or height²) and weight as determined previously (11) and race/ethnicity. The parameter estimate for the “genotype” variable can be interpreted as the unit change in the lung function measure for subjects carrying the variant allele of the genotype. For example, the *GSTP1* parameter estimate for FEF₂₅₋₇₅ can be interpreted as a decrease in 23 ml/s for male subjects carrying at least one copy of the variant allele (Val105) compared to those who are homozygous for the wild-type allele (Ile105).

*p<0.05.

Table 4. Genotype and Ozone Exposure Model of Lung Function (adjusted for airway size and race/ethnicity)

		Parameter Estimates (Standard Error)				
		Males		Females		
		<i>GSTPI</i> Val105	<i>GSTMI</i> -null	<i>GSTMI</i> -null/ <i>NQO1</i> Pro187	<i>GSTPI</i> Val105	<i>GSTMI</i> -null/ <i>NQO1</i> Pro187
FEF₂₅₋₇₅	O ₃	-0.023 (0.002)	-0.024 (0.003)	-0.023 (0.002)	-0.019 (0.002)	-0.018 (0.004)
	O ₃ *(fef/fvc)	0.022 (0.001)	0.022 (0.002)	0.021 (0.002)	0.020 (0.001)	0.019 (0.002)
	genotype	-0.036 (0.022) [§]	-0.008 (0.026)	-0.011 (0.024)	-0.001 (0.023)	-0.075 (0.035)*
	adjusted R ²	0.77	0.76	0.73	0.73	0.69
FEF₇₅	O ₃	-0.033 (0.003)	-0.031 (0.003)	-0.021 (0.005)	-0.025 (0.003)	-0.024 (0.005)
	O ₃ *(fef/fvc)	0.027 (0.002)	0.026 (0.002)	0.022 (0.003)	0.023 (0.002)	0.023 (0.002)
	genotype	-0.081 (0.031)*	-0.029 (0.034)	-0.013 (0.043)	0.005 (0.030)	-0.054 (0.041)
	adjusted R ²	0.74	0.72	0.74	0.70	0.71

Units for parameter estimates -- for O₃: ppb; for interaction: ppb *sec⁻¹; for genotype: unit change in lung function measure for subjects carrying variant allele of each polymorphism. Each model includes height (or height²), weight, and lifetime total exposure to PM₁₀ and NO₂, as determined previously (11) and race/ethnicity. Only O₃-specific and genotype parameter estimates are shown, full model can be found in the online supplement.

*p<0.05

§p<0.15

