



Original Contribution

Functional Variants in the Catalase and Myeloperoxidase Genes, Ambient Air Pollution, and Respiratory-related School Absences: An Example of Epistasis in Gene-Environment Interactions

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The individual effect of functional single nucleotide polymorphisms within the catalase and myeloperoxidase genes (*CAT* and *MPO*) has been studied in relation to asthma; however, their interrelationship with ambient air pollution exposures has yet to be determined. The authors investigated the interrelationships between variants in *CAT* and *MPO*, ambient air pollutants, and acute respiratory illness. Health information, air pollution, and incident respiratory-related school absences were ascertained in January–June 1996 for 1,136 Hispanic and non-Hispanic white US elementary schoolchildren as part of the prospective Children's Health Study. Functional and tagging single nucleotide polymorphisms for the *CAT* and *MPO* loci were genotyped. The authors found epistasis between functional polymorphisms in the *CAT/MPO* loci, which differed by levels of oxidant-stress-producing air pollutants. Risk of respiratory-related school absences was elevated for children with the *CAT* (G/G) and *MPO* (G/A or A/A) genes (relative risk = 1.35, 95% confidence interval: 1.03, 1.77; *P*-interaction = 0.005). The epistatic effect of *CAT* and *MPO* variants was most evident in communities exhibiting high ambient ozone levels (*P*-interaction = 0.03). The association of respiratory-illness absences with functional variants in *CAT* and *MPO* that differ by air pollution levels illustrates the need to consider genetic epistasis in assessing gene-environment interactions.

air pollution; catalase; epistasis, genetic; peroxidase; respiratory tract infections

Abbreviations: *CAT*, catalase; *MPO*, myeloperoxidase; PM₁₀, particulate matter less than 10 μm in aerodynamic diameter; SNP, single nucleotide polymorphism.

Catalase (*CAT*) and myeloperoxidase (*MPO*) are enzymes that play a role in the etiology of respiratory conditions related to oxidative stress (1–8). As part of the oxidative stress defense pathway in the airways, both *CAT* and *MPO* react with a potent prooxidant (hydrogen peroxide) to form water + oxygen and hypochlorous acid + hydroxide radical, respectively, under conditions of elevated oxidative stress (Figure 1) such as occurs during respiratory infections and exposure to ambient air pollutants. We hypothesized that variation in the level or function of these enzymes would modulate respiratory illness risk, especially under high levels of oxidative stress. There is a paucity of studies that have investigated this hypothesis.

Enzyme activity is recognized as being modulated by a single nucleotide polymorphism (SNP) in the promoter region of the *CAT* and *MPO* genes. Functional studies show that in the *CAT* gene locus, the minor allele (A) (rs1001179: G-330A) increases gene transcription, resulting in increased blood *CAT* levels (4). For *MPO*, the minor allele (A) (rs2333227: G-463A) has lower enzyme activity as measured in bronchoalveolar lavage fluid (9). Because these DNA promoter variants affect levels of 2 enzymes that have the same substrate and function in a common biologic pathway, we hypothesized that this biologic interaction would be reflected in chronically increased levels of highly reactive oxidants in children with combinations of *MPO* and *CAT* variants and result in a greater risk of respiratory illness.

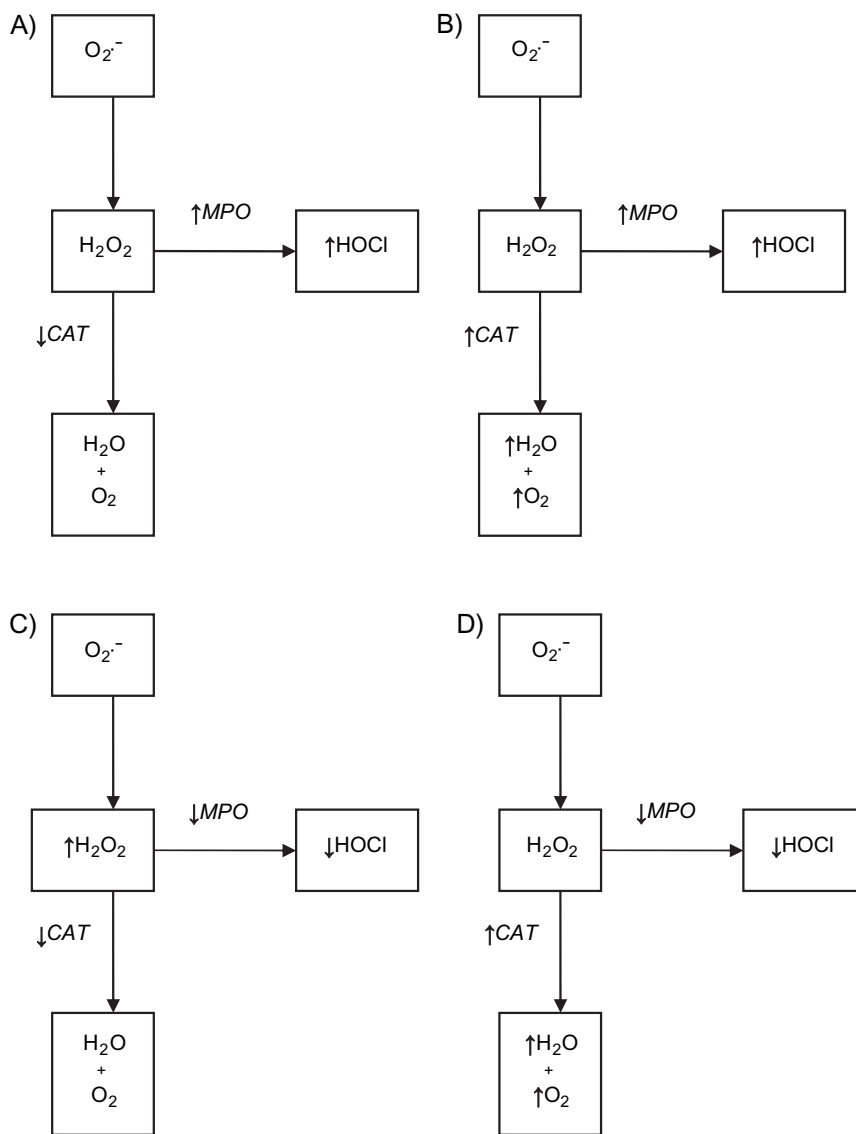


Figure 1. The role of catalase (CAT) and myeloperoxidase (MPO) in the oxidative stress pathway. H_2O , water; H_2O_2 , hydrogen peroxide; HOCl, hypochlorous acid; O_2 , oxygen; O_2^- , superoxide. O_2^- , H_2O_2 , and HOCl are prooxidant compounds that increase oxidative stress. The variant allele in the *CAT* gene (A) increases catalase enzyme activity, whereas the variant allele in the *MPO* gene (A) decreases myeloperoxidase enzyme activity. The 4 parts illustrate the effect of *CAT* and *MPO* interaction on net oxidative balance: A) *CAT* (G/G) – *MPO* (G/G): increased oxidative stress (increased HOCl). B) *CAT* (G/A or A/A) – *MPO* (G/G): balanced oxidative state (increased HOCl/increased $H_2O + O_2$). C) *CAT* (G/G) – *MPO* (G/A or A/A): increased oxidative stress (increased H_2O_2). D) *CAT* (G/A or A/A) – *MPO* (G/A or A/A): decreased oxidative stress (increased $H_2O + O_2$).

Furthermore, this risk would be expected to be highest under exposures that increase oxidative stress, such as ambient air pollutants. In addition to these 2 functional SNPs, other variations in the DNA coding sequence at these loci have the potential to affect risk. Therefore, we also investigated whether additional SNPs that account for the majority of variation across each locus add information to the functional SNP associations.

The Air Pollution and Absence Study was a substudy of the prospective Children's Health Study (10). It offered us an opportunity to investigate the effects of air pollution,

genetic variation, and respiratory-related absences in elementary schoolchildren residing in 12 southern California communities.

MATERIALS AND METHODS

The Air Pollution and Absence Study was a population-based, prospective cohort study conducted as part of the Children's Health Study (10, 11). The cohort included 1,935 fourth-grade students from 27 elementary schools across 12 southern California communities and focused on

absence data collected between January and June 1996. Parents or guardians of study participants completed written informed consent and baseline questionnaires pertaining to their child's sociodemographics, medical history, exposure history, and household characteristics. Of the 1,935 children, approximately 70% ($n = 1,351$) provided a buccal cell sample. This analysis was restricted a priori to Hispanic and non-Hispanic white children ($n = 1,136$). The University of Southern California's institutional review board for human studies approved the study protocols.

Respiratory-illness incidence

Incident absences were ascertained by using an active surveillance system augmented by telephone interviews with parents or guardians to collect additional information. An incident absence was defined as one that followed attendance on the preceding school day and was regarded as an independent event regardless of whether it occurred for the same subject. Participating schools provided daily absence summary information for study children within 4 weeks of each absence, and each reported absence was categorized as an illness-related or non-illness-related absence. Telephone interviews with parents were conducted within 4 weeks of a reported illness-related school absence or absence that could not be categorized based on existing data; non-illness-related absences were not further characterized (12).

Illness-related school absences were classified into respiratory, gastrointestinal, and other types of absences on the basis of symptoms information collected by telephone interviews with parents. Respiratory absences were defined as absences associated with one or more of the following symptoms: runny nose/sneezing, sore throat, cough (any), earache, wheezing, or asthma attack (12).

Ambient air pollution exposure

Ambient ozone, particulate matter less than 10 μm in aerodynamic diameter (PM_{10}), and nitrogen dioxide were measured continuously, with hourly averaging at central-site monitors in each of the 12 communities (10). We calculated the daily 1-hour maximum ozone, the 24-hour ozone average, and the 10 AM–6 PM ozone average, as well as the 24-hour averages of PM_{10} and nitrogen dioxide. We focused on the 10 AM–6 PM average of ozone because it is an index of exposure during the temporal peak of ozone and outdoor activity. The 24-hour averages of PM_{10} and nitrogen dioxide were used because they lack the temporal peak exhibited by ozone. To assess effects of long-term average levels of ozone, PM_{10} , and nitrogen dioxide on acute effects, we divided communities into high and low groups for each pollutant based on their ranking regarding median levels (Web Table 1; this information is described in the first of 4 supplementary tables, each referred to as "Web table" in the text and posted on the *Journal's* website (<http://aje.oupjournals.org/>)).

Laboratory methods

Genomic DNA was extracted from buccal mucosal cells by using the PUREGENE DNA purification kit (Gentra

Systems, Minneapolis, Minnesota). Genotyping for *CAT* G-330A (rs1001179) and *MPO* G-463A (rs2333227) was performed by using the TaqMan allelic discrimination assay (Applied Biosystems, Foster City, California). The DNA fragment containing each SNP was amplified by using the primers and probes shown in Web Table 2. The TaqMan genotyping reaction was amplified on a GeneAmp PCR System 9600 (50°C for 2 minutes and 95°C for 10 minutes, followed by 35 cycles of 92°C for 15 seconds and 60°C for 1 minute), and fluorescence was detected on an ABI PRISM 7700 Sequence Detection System (Applied Biosystems). In each run, 10% of the samples were randomly selected and used for quality control. The results from the TaqMan PCR assay were validated by using polymerase chain reaction/restriction fragment length polymorphism methods and automatic sequencing (BigDye version 3.1, 377XL DNA sequencer; Applied Biosystems).

SNP selection and genotyping

For each gene, we identified a set of SNPs from dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP/>) and other sequencing databases (i.e., the Environmental Genome Project (<http://www.niehs.nih.gov/research/supported/programs/egp/>)) with an SNP density of 1–3 SNPs/kb over a region 20 kb upstream and 10 kb downstream of each gene. SNPs were selected on the basis of validation status, Illumina design score (Illumina Inc., San Diego, California), and functional potential of the SNPs.

The selected SNPs were first genotyped by using the Illumina GoldenGate Genotyping Assay (Illumina Inc.) in a sample of 71 Hispanic white and 71 non-Hispanic white participants in the Multi-Ethnic Cohort Study (13). These data were used to define ethnic-specific allele frequencies and patterns of linkage disequilibrium. The squared correlation between the true haplotypes (R_h^2) and their estimates were then calculated; calculation of R_h^2 is described in detail by Stram et al. (14). Haplotype-tagging SNPs were then chosen by using TagSNPs, a software program (written by D. O. Stram of the Keck School of Medicine at the University of Southern California) that implements an expectation maximization algorithm approach by finding the minimum set of SNPs (within a block) that would have R_h^2 values of ≥ 0.85 for all haplotypes with an estimated frequency of $\geq 5\%$ in either ethnic group. Functional SNPs were forced into the TagSNP selection process irrespective of minor allele frequency. We also examined the pairwise correlation between SNPs and selected additional tagSNPs to provide redundant coverage in the event of assay failure. This SNP list was refined to provide adequate performance on the Illumina BeadArray platform (Illumina Inc.), a high-throughput assay. We excluded poor-performing, monomorphic, and low-allelic-frequency SNPs. We also excluded samples with SNP call rates of $< 90\%$.

Statistical analysis

A Poisson regression model was fitted to estimate relative risks and 95% confidence intervals for associations of genotypes with respiratory-related school absences (15, 16).

An offset term, comprising the log-expected value of the dependent variable, was included in the model to normalize the fitted cell means on a per-subject basis. The deviance divided by its degrees of freedom was used as a correction factor on the standard errors to account for overdispersion in all models (17). Inclusion of potential confounders in the models was based on a review of the literature and changes in univariate genetic effect estimates of at least 10% in multivariate analyses. On the basis of these criteria and the study design, the following variables were selected as potential confounders: community, race, gender, age, asthma status, family income, health insurance status, secondhand smoke, in utero smoke, body mass index, and cat and dog ownership. For the single-SNP analyses, the additive genetic model was utilized. Because of low numbers of subjects with homozygous variant genotypes, we used the dominant genetic model for all joint SNP models.

To address potential confounding by population stratification, 4 coefficients of ancestry variables were also included in the model (18, 19). These variables were constructed from 4 principal components derived from a set of 233 unlinked ancestry informative markers selected to differentiate 4 parental populations (African, European, American Indian, and East Asian). Controlling for these ancestry variables provided adjustment for ancestral history beyond adjustment for typical self-reported racial and ethnic categories.

The interactions between SNPs, and the interactions between SNPs and air pollutants, were evaluated by adding the corresponding product term to the model and using a likelihood ratio test to evaluate its significance. All tests assumed a 2-sided alternative hypothesis and a 0.05 significance level. All analyses were conducted by using SAS/STAT software, version 9.1 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

The demographic characteristics of the Hispanic and non-Hispanic white subjects in the Air Pollution and Absence Study are described in Table 1. All study participants were in the fourth grade at the time of data collection, and nearly all were between 9 and 10 years of age. Boys and girls participated in the study equally. Approximately 15% of children had physician-diagnosed asthma in their lifetime, which corresponds to the national asthma prevalence. More than 80% were in the normal range for body mass index, and more than 85% had health insurance. Approximately 17% of children were exposed to secondhand smoke in the home, and 17% were exposed in utero to maternal smoking.

The genotype distribution for each SNP did not deviate significantly from that expected under Hardy-Weinberg equilibrium. SNP2 in the *CAT* locus and SNP1 in the *MPO* locus are both functional SNPs located in the promoter region (G-330A and G-463A, respectively). There were no statistically significant associations between individual SNPs and respiratory-related school absences, adjusted for community of residence, age, gender, income, health insurance, secondhand smoke, in utero smoke, body

Table 1. Selected Participant Characteristics of Genotyped Hispanic and Non-Hispanic White Subjects, Air Pollution and Absence Study, United States, January–June 1996

Variable	Frequency	%
Age, years		
9	374	32.9
10	733	64.5
>10	29	2.6
Asthma		
No	944	84.7
Yes	171	15.3
Body mass index, percentile		
<5	40	3.6
5–85	746	67.7
>85–95	164	14.9
>95	152	13.8
Cat ownership		
No	658	57.9
Yes	478	42.1
Dog ownership		
No	459	40.4
Yes	677	59.6
Gender		
Female	577	50.8
Male	559	49.2
Health insurance		
No	155	13.8
Yes	967	86.2
Income, \$		
<50,000	413	41.5
≥50,000	582	58.5
In utero smoke		
No	919	82.9
Yes	190	17.1
Secondhand smoke		
No	916	82.8
Yes	191	17.3

mass index, race/ethnicity, cat and dog ownership, and asthma status (Table 2).

We found evidence that the biologic relation of *CAT* and *MPO* in the oxidative stress pathway resulted in an epistatic effect of the known functional *CAT* and *MPO* SNPs on respiratory-related school absences (Table 3). The results showed an increased risk of respiratory-related school absences with the following genotype combination: *CAT* (G/G), *MPO* (G/A or A/A) (relative risk = 1.35, 95% confidence interval: 1.03, 1.77). Subjects with this SNP combination had the highest rate of respiratory absence, equaling approximately 10 per 1,000 subject-days (Web Figure 1; this supplementary figure is also posted on the *Journal's* website (<http://aje.oupjournals.org/>)). Although not statistically

Table 2. Relative Risks and 95% Confidence Intervals of *CAT* and *MPO* SNPs and Respiratory-related School Absences, Air Pollution and Absence Study, United States, January–June 1996^a

Gene ^b	MAF	HWE <i>P</i> value	RR	95% CI	Location
<i>CAT</i>					
SNP1 (rs208682)	0.20	0.24	1.10	0.90, 1.33	Upstream
SNP2 (rs1001179)	0.19	0.19	0.88	0.71, 1.08	Upstream
SNP3 (rs1049982)	0.40	0.30	1.06	0.91, 1.25	Exon1
SNP4 (rs494024)	0.34	0.27	0.91	0.77, 1.07	Intron1
SNP5 (rs2284367)	0.26	0.42	1.15	0.97, 1.37	Intron9
<i>MPO</i>					
SNP1 (rs2333227)	0.20	0.23	1.05	0.88, 1.26	Upstream
SNP2 (rs7208693)	0.08	0.87	1.14	0.88, 1.49	Exon2
SNP3 (rs11649901)	0.33	0.99	1.06	0.89, 1.25	Intron8
SNP4 (rs2071409)	0.17	0.13	1.00	0.80, 1.24	Intron11

Abbreviations: *CAT*, catalase gene; CI, confidence interval; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; *MPO*, myeloperoxidase gene; RR, relative risk; SNP, single nucleotide polymorphism.

^a Individual SNP models were adjusted for community, age, gender, income, health insurance, secondhand smoke, in utero smoke, body mass index, race, cat ownership, dog ownership, and asthma status.

^b *CAT* SNP2 is a known functional SNP: G-330A; *MPO* SNP1 is a known functional SNP: G-463A.

significant, the SNP combination with at least one variant in *CAT* and *MPO* resulted in the lowest risk of respiratory-related school absences (relative risk = 0.81, 95% confidence interval: 0.55, 1.19), which contributed to the highly significant epistatic effect (*P*-interaction = 0.005) (Table 3). These subjects had the lowest rate of respiratory absence (Web Figure 1): 7 per 1,000 subject-days.

Consistent with our hypothesis, the epistatic interaction of *CAT* and *MPO* was apparent among children exposed to high levels of ambient air pollutants (Table 4). Among participants exposed to high levels of nitrogen dioxide and ozone, there was strong evidence of *CAT/MPO* epistasis (*P*-interaction = 0.002 and *P*-interaction = 0.0004, respectively), whereas none was evident in low-pollution communities. In high-ozone communities, the *CAT/MPO* genotypes that resulted in decreased oxidative stress (i.e., *CAT*: G/A or A/A and *MPO*: G/A or A/A) were associated with a decreased risk of respiratory-related school absences compared with the *CAT/MPO* wild-type genotype (relative risk = 0.42, 95% confidence interval: 0.20, 0.89). Among subjects with high-risk genotypes (*CAT*: G/G, and *MPO*: G/A or A/A), we found a trend toward an increased risk of school absences in communities with high levels of nitrogen dioxide, ozone, and PM₁₀. Furthermore, relative risk

Table 3. Joint Effect of *CAT* and *MPO* Genotypes on Respiratory-related School Absences, Air Pollution and Absence Study, United States, January–June 1996^a

<i>CAT</i> ^b	<i>MPO</i> ^c	No.	RR	95% CI
rs1001179	rs2333227			
G/G	G/G	478	1.00	
G/A or A/A	G/G	256	1.15	0.87, 1.53
G/G	G/A or A/A	262	1.35	1.03, 1.77
G/A or A/A	G/A or A/A	140	0.81	0.55, 1.19
<i>P</i> -interaction			0.005	

Abbreviations: *CAT*, catalase gene; CI, confidence interval; *MPO*, myeloperoxidase gene; RR, relative risk.

^a The model was adjusted for community, age, gender, income, health insurance, secondhand smoke, in utero smoke, body mass index, race, cat ownership, dog ownership, and asthma status.

^b *CAT* (rs1001179): G-330A.

^c *MPO* (rs2333227): G-463A.

estimates of the effect of *CAT* and *MPO* on respiratory-related absences significantly varied by ozone exposure levels (*P*-interaction = 0.03). There were no statistically significant associations in the joint-effect analyses of *CAT/MPO* variants in areas of low pollution, although there was a suggestion of a *CAT/MPO* interaction in communities with low levels of PM₁₀ (*P*-interaction = 0.03).

To examine the added effects of additional variants at each locus, we assessed the joint effects of the functional SNPs with multiple tagging SNPs in the *CAT* (5 SNPs) and *MPO* (4 SNPs) loci. We did so by performing interaction tests between the known functional SNPs and all other SNPs utilizing Bonferonni correction for multiple testing. We found that no additional interaction achieved statistical significance, suggesting that the known functional *CAT/MPO* SNPs adequately explained the extent of the *CAT/MPO* associations with respiratory-related school absences (Web Table 3).

DISCUSSION

This study provides evidence illustrating the importance of considering genetic epistasis when examining gene-environment associations. We found that 2 functional SNPs within the promoter region of the *CAT* and *MPO* loci—genes involved in prooxidant defense by acting on hydrogen peroxide—were jointly associated with acute respiratory illness as measured by respiratory-related school absences. Consistent with our hypothesis, the epistatic effect of *CAT/MPO* variants was most apparent among subjects exposed to high levels of ambient air pollutants. The common functional *CAT* G-330A and *MPO* G-463A polymorphisms, although nonsignificant when considered individually, provide an example of examining interactions between genes known to be involved in clearance of hydrogen peroxide in the lungs, which may be modified by air pollution exposure. Other SNPs within the *CAT* and *MPO* loci did not add to the information on susceptibility provided by these 2 functional *CAT* and *MPO* SNPs.

Table 4. Joint Effect of *CAT* and *MPO* Genotypes on Respiratory-related School Absences in High- and Low-Pollution Exposure Groups, Air Pollution and Absence Study, United States, January–June 1996^a

<i>CAT</i> ^b	<i>MPO</i> ^c	Nitrogen dioxide				Ozone ^d				PM ₁₀				
		Low		High		Low		High		Low		High		
		RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI	
rs1001179	rs2333227													
G/G	G/G	100		1.00		1.00		1.00		1.00		1.00		1.00
G/A or A/A	G/G	1.38	0.91, 2.11	1.09	0.75, 1.60	1.07	0.73, 1.56	1.23	0.80, 1.88	1.41	0.91, 2.18	1.05	0.73, 1.50	
G/G	G/A or A/A	1.18	0.77, 1.82	1.53	1.09, 2.14	1.21	0.83, 1.75	1.45	0.99, 2.14	1.39	0.92, 2.09	1.35	0.96, 1.90	
G/A or A/A	G/A or A/A	1.04	0.62, 1.75	0.63	0.37, 1.10	1.04	0.66, 1.65	0.42	0.20, 0.89	0.94	0.56, 1.60	0.66	0.38, 1.15	
<i>P</i> -interaction		0.16		0.002		0.39		0.0004		0.03		0.02		

Abbreviations: *CAT*, catalase gene; CI, confidence interval; *MPO*, myeloperoxidase gene; PM₁₀, particulate matter less than 10 μm in aerodynamic diameter; RR, relative risk.

^a The models were adjusted for community, age, gender, income, health insurance, secondhand smoke, in utero smoke, body mass index, race, cat ownership, dog ownership, and asthma status.

^b *CAT* (rs1001179): G-330A.

^c *MPO* (rs2333227): G-463A.

^d The relative risk estimates of *CAT* and *MPO* on respiratory-related absences were statistically significantly different by ozone (*P*-interaction = 0.03).

Although both *CAT* and *MPO* act upon the same substrate, no studies to our knowledge have examined the joint effect of these 2 genes on respiratory illness. Mak et al. (5) found that the *CAT* -330 variant allele (*A*) was associated with a decreased risk of asthma for nonsmokers (odds ratio for subjects possessing at least one *A* allele vs. the wild type = 0.35, 95% confidence interval: 0.15, 0.83) and an insignificant decreased risk for ever smokers (odds ratio = 0.28, 95% confidence interval: 0.05, 1.61). However, the *CAT* genotypes were not associated with erythrocyte *CAT* enzyme levels. Ghosh et al. (20) performed in vitro studies examining key markers of inflammation due to asthma. The authors found reduced *CAT* enzyme activity among asthmatics and concluded that *CAT* inactivity amplifies oxidative stress, contributing to the inflammation inherent in asthmatic airways (20). These reports support a role for the functional *CAT* variant in diseases associated with increased oxidative stress.

Two recent studies have examined the role of the *MPO* enzyme among asthmatic patients, with conflicting results (21, 22). Tauber et al. (21) found that serum *MPO* levels were not associated with childhood asthma, whereas Monteseirín et al. (22) found that patients with allergic asthma, compared with nonallergic controls, had a propensity to release more *MPO*. Although we are not aware of studies that have examined the association of the functional *MPO* G-463A SNP with asthma or acute respiratory disease, the inconsistent findings for patients with high levels of oxidant stress may reflect the fact that the *CAT* variant genotypes were not considered.

Both *CAT* and *MPO* are important enzymes in the lungs that act upon hydrogen peroxide, a potent oxidant (Figure 1). The lack of both *CAT* and *MPO* enzymes may result in respiratory illness that may be due to an accumulation of prooxidants in the lungs, resulting in an acute inflammatory response or exacerbation of an acute respiratory response. The known functional SNP in *CAT* (rs1001179) produces a

G-330A polymorphism in the promoter region of the gene. The *A* allele is thought to increase *CAT* enzyme activity, resulting in increased conversion of hydrogen peroxide to water and oxygen (4); this conversion is thought to work optimally during times of severe oxidative stress, whereas glutathione peroxidase is the major antioxidative enzyme in the detoxification of hydrogen peroxide to water and oxygen under normal circumstances (4). This mechanism could underlie our results that show the largest protective effect of *CAT/MPO* in areas of high pollution, although more work is needed to elucidate these findings. The *MPO* gene has a functional SNP also located in the promoter region (rs2333227: G-463A) whereby the minor allele (*A*) is thought to decrease enzyme activity, resulting in slower conversion of hydrogen peroxide to hypochlorous acid (9). *MPO* is a potent bactericide but is also known to increase production of heme oxygenase, which may lead to acute or chronic respiratory illness (23).

Our results should be interpreted in light of some study limitations. Of the total number of eligible white children, approximately 30% did not provide buccal cell samples for this study, which may give rise to selection bias. However, comparing those children who were and were not genotyped did not reveal marked differences in demographic factors or absence rates, making selection bias an unlikely explanation for our findings. Furthermore, other intrinsic and extrinsic cofactors may potentially confound the relation between *CAT/MPO* and respiratory illness. Some that have been described in the literature include personal and secondhand smoke (24, 25), pet and plant allergens (26), and asthma and family history of asthma (27, 28). Statistical adjustments for community, secondhand smoke, pets, and asthma status were utilized in all models. Adjustments for family history of asthma and allergens such as pests, mold, and mildew resulted in a negligible change in effect estimates and were thus excluded from the final models.

In association studies involving multiethnic populations, confounding due to population stratification could bias the results. To address this potential bias, we included only white (Hispanic and non-Hispanic) subjects in our analyses, examined risk estimates stratified by ethnicity, and performed sensitivity analyses adjusted for an index of population stratification (q factor) (18, 19). The results did not vary appreciably by ethnic stratification and q-factor adjustment.

In conclusion, the association of respiratory illness absences with functional variants in *CAT* and *MPO* that vary by air pollution levels illustrates the need to consider genetic epistasis when assessing gene-environment interactions. In the current approaches to candidate loci and genome-wide association studies, it may be essential to consider epistasis among genes, as defined by this well-studied biologic pathway, when examining susceptible subgroups for the diverse effects of air pollution.

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