Glutathione-S-Transferase M1, Obesity, Statins, and Autonomic Effects of Particles

Gene-by-Drug-by-Environment Interaction

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Rationale: **Air pollution by particulate matter (PM) has been associated with cardiovascular deaths, although the mechanism of action is unclear. One proposed pathway is through disturbances of the autonomic control of the heart.**

Objectives: **We tested the hypothesis that such disturbances are mediated by PM increasing oxidative stress by examining the association between PM and the high-frequency (HF) component of heart rate variability as modified by the presence or absence of the allele for glutathione-S-transferase M1 (*GSTM1*) and the use of statins, obesity, high neutrophil counts, higher blood pressure, and older age.**

Methods: **We examined the association between particles less than 2.5 μM in aerodiameter ($PM_{2.5}$) and HF in 497 participants in the Normative Aging Study, using linear regression controlling for covariates.**

Main Results: **A 10-μg/m^3 increase in $PM_{2.5}$ during the 48 h before HF measurement was associated with a 34% decrease in HF, 95% confidence interval (−9%, −52%), in subjects without the allele, but had no effect in subjects with *GSTM1* present. Among *GSTM1*-null subjects, the use of statins eliminated the effect of $PM_{2.5}$. Obesity and high neutrophil counts also worsened the PM effects with or without *GSTM1*.**

Conclusion: **The effects of $PM_{2.5}$ on HF appear to be mediated by reactive oxygen species. This may be a key pathway for the adverse effects of combustion particles.**

Keywords: genetic polymorphisms; heart rate variability; oxidative stress; particles

A large body of evidence has demonstrated that particulate air pollution (PM) is associated with short-term changes in the risk of death (1–6). An early study showed the risk of dead-on-arrival deaths associated with particles was three times that for all deaths (7). This suggests a predominant effect on sudden deaths from arrhythmias and myocardial infarctions. Subsequent studies have confirmed that PM is associated with myocardial infarctions (8, 9), hospital admissions for cardiovascular disease (10, 13), discharges of implantable defibrillators (14), and electrocardiographic disturbances (15).

How airborne particles can be producing these responses in still unclear. However, particles have been linked with changes in intermediate markers, such as clotting factors (16, 17) and increased atherosclerosis (18).

Airborne particles have also been associated with changes in heart rate variability (HRV) (15, 19). The parasympathetic and sympathetic stimulation of the heart produces variations in the time intervals between normal heartbeats; analysis of this variability is therefore an estimate of cardiac autonomic regulation. HRV is a noninvasive measure that independently predicts cardiovascular mortality in patients with and without underlying cardiovascular disease (20, 21). Hence, disturbances in HRV may represent one pathway by which particles might be associated with sudden death.

Overall, studies have generally found significant associations with HRV in elderly subjects, but weaker associations in younger subjects (22), suggesting that age-related decreases in toxic defenses play a role in susceptibility. In a recent review (22), we found the only consistent PM association was with the high-frequency (HF) components of HRV, either HF in the frequency domain, or root mean squared differences between adjacent RR intervals (rMSSD) or proportion of adjacent NN intervals differing by more than 50 ms (PNN50) in the time domain. In contrast, low frequency was not associated with particles in four of five studies. This suggests a paramount effect on the parasympathetic nervous system. This may be because the vagus nerve innervates the lung.

Various mechanisms by which particles exert these effects have been proposed (23). Reactive oxygen species (ROS) have been mentioned as a potential pathway for the adverse effects of particles (24, 25). ROS have established importance in the pathogenesis of cardiovascular diseases (26). Exposure to urban particles increased ROS in a dose-dependent manner in the lung and heart of living animals (27). It is unclear what role ROS may play in explaining the effects of particles on autonomic endpoints, such as defibrillator discharge and HRV. This question may be addressed by examining the effects of particles on HRV in populations with different host defenses to an oxidative stress challenge. Genetic polymorphisms have been linked to important differences in such defenses.

Glutathione pathways play a key role in cellular defenses against ROS (28). Glutathione-S-tranferases (GSTs) are a family of enzymes involved in the metabolism of ROS and xenobiotic compounds.

Genetic polymorphisms of the GSTs are common, and have been shown to modify the response to air pollutants (29). The *GSTM1* gene is deleted in approximately half of the white population (the polymorphic "null" genotype), and lack of the *GSTM1* protein has been associated an enhanced nasal allergic

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response to diesel exhaust particles (30). Children who are *GSTM1*-null and are exposed to environmental tobacco smoke had elevated odds of developing asthma (31), and the *GSTM1*-null genotype interacts with tobacco smoke in increasing the risk of coronary disease (32).

Drugs that modify oxidant defenses may also influence susceptibility to particle-induced ROS. Statins are a widely prescribed class of drugs originally developed for their lipid-lowering properties, but they have been shown to have substantial antiinflammatory and antioxidant activity as well (33). In addition to lowering C-reactive protein concentrations (34), statins have been shown to decrease superoxide production (35), increase release of nitric oxide (NO) (36), which is an antioxidant as well as a vasodilator, and reduce markers of oxidative stress in $APOE^{-1/-1}$ mice (37).

We examined the association of fine-particulate air pollution of less than 2.5 μM in aerodynamic diameter ($PM_{2.5}$) on the HF HRV of elderly subjects living in the Boston metropolitan area, and how that association varied by *GSTM1* genotype or statin use. In addition, because statins have important effects beyond their antioxidant properties, we examined whether obesity or elevated neutrophil count, which are associated with inflammation and oxidative stress, also modified the association. Finally, we examined two more generic markers of susceptibility, older age or higher blood pressure, as modifiers of the PM effect. This study was reviewed and approved by the institutional review boards of all of the participating institutions.

METHODS

Study Population

The Normative Aging Study is a longitudinal study established by the Veterans Administration in 1961, enrolling 2,280 men from the greater Boston area who were free of known chronic medical conditions (38). Beginning in 2000, during each participant's regularly scheduled evaluation, HRV was measured. Further details have been described previously (22). That study examined a range of air pollutants and measures of HRV, but did not look at genetic or other factors related to oxidative stress. It found the most consistent associations with $PM_{2.5}$, and with HRV measures indicative of a parasympathetic effect (HF, rMSSD).

HRV Measurement

HRV was measured for 7 min in a sitting position using a two-channel (five-lead) ECG monitor (Trillium 3000; Forest Medical, East Syracuse, NY). Only normal-to-normal (NN) beat intervals were included in the analysis. We used the best 4-consecutive-min intervals for the HRV calculations, and computed the HF (0.15–0.4 Hz) component of HRV using software complying with guidelines (39). Subjects with irregular ECG patterns that interfere with HRV estimation were excluded.

GSTM1 Genotyping. The assay consists of polymerase chain reaction amplification of exons 4 and 5 of the *GSTM1* allele. Because this polymorphism is a gene deletion, polymerase chain reaction product indicates the presence of one or more copies of the gene. Further details are in the online supplement.

Air Pollution and Weather Data

Continuous $PM_{2.5}$ was measured at a monitoring site 1 km from the exam site, using the Tapered Element Oscillating Microbalance (TEOM, model 1400A; Rupprecht & Pataschnick, Albany, NY), with a season-specific correction to compensate for the loss of semivolatile mass (40). Weather measurements were obtained from the airport weather station.

To control for outdoor weather, we used apparent temperature, defined as a person's perceived air temperature, given the humidity (41). We used the average of $PM_{2.5}$ concentrations in the 48 h before examination as our exposure index, because that exposure period has been most consistently associated with sudden death (4, 5).

Statistical Methods

HRV was $\log_{10}$-transformed to improve normality and stabilize the variance. The following variables were chosen *a priori* and included in the linear regression analysis: age, cigarette smoking, body mass index, diastolic blood pressure, fasting blood glucose, alcohol consumption (⩾ 2 drinks/d), use of β-blockers, angiotensin-converting enzyme inhibitors, and/or calcium channel blockers, season, room temperature, and average apparent temperature 48 h before the HRV measurement. We used a spline with 3 degrees of freedom to account for potential nonlinearity in the relationship between apparent temperature and HRV. After 14 subjects with missing values of covariates were excluded, 497 subjects were available for the analyses.

Stratified regression models examined subjects with and without the *GSTM1* gene, with and without statin use, and by the four possible combinations of genotype and statin use. Stratified analyses were also done, in turn, by the four possible combinations of *GSTM1* gene and by whether or not the subjects were in the most adverse quartile of neutrophil count, blood pressure, or age, or whether they had a body mass index above 30.

RESULTS

Table 1 shows the demographic and clinical characteristics and HRV measurements of the subjects, as well as environmental variables. The study participants were all male, and their average age was 72.7 yr (SD, 6.6 yr). The correlation between temperature and particle concentrations was modest (0.35).

In a model including all subjects, and the covariates listed above, a 10-μg/m^3 increase in $PM_{2.5}$ was associated with a 27% decrease in HF (95% confidence interval, −8%, −42%). Note that the interquartile range for $PM_{2.5}$ in these data was 7 μg/m^3. When stratified by *GSTM1* status, no relationship of $PM_{2.5}$ and HF was seen in persons with the gene, whereas a significant association (34% decrease; 95% confidence interval, −9%, −52%)

TABLE 1. DESCRIPTIVE STATISTICS OF THE VARIABLES (MEAN [SD] OR NUMBER [%])

Variable	All Subjects (*n* = 497)
Age, yr	72.7 (6.6)
Body mass index, kg/m^2	28.3 (4.1)
Diastolic blood pressure, mm Hg	75.7 (9.4)
Heart rate, beat/min	70.7 (6.7)
Fasting blood glucose, mg/dl	108.0 (29.0)
Cholesterol, mg/dl	197.0 (37.6)
HDL, mg/dl	49.7 (13.5)
Smoking status, n (%)	
Never smoker	160 (32.2)
Former smoker	311 (62.6)
Current smoker	26 (5.2)
Alcohol intake (⩾ 2/day), n (%)	96 (19.3)
Hypertension, n (%)	335 (67.4)
Use of β-blocker, n (%)	163 (32.8)
Use of Ca-channel blocker, n (%)	70 (14.1)
Use of ACE inhibitor, n (%)	100 (20.1)
Use of statins, n (%)	179 (36)
Neutrophil count (% of cells)	62 (8.8)
Heart rate variability	
Log_{10} HF, ms^2	1.9 (0.66)
Environmental variables	
$PM_{2.5}$, μg/m^3	11.4 (8.0)
Apparent temperature, °C	11.4 (9.9)
Room temperature, °C	24.5 (1.4)

Definition of abbreviations: ACE = angiotensin-converting enzyme; HDL = high-density lipoprotein; HF = high frequency; $PM_{2.5}$ = particulate matter less than 2.5 μM in aerodiameter.

Values are listed as mean (SD) or numbers (%).

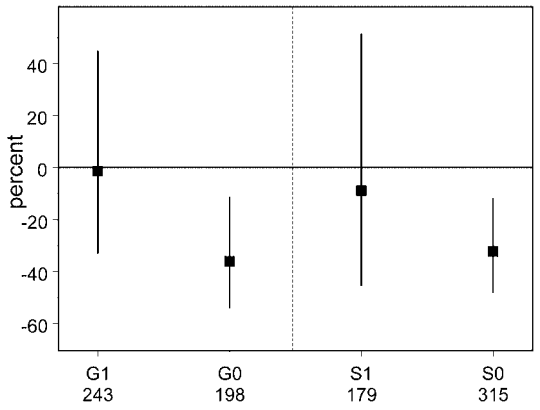

Figure 1. The estimated covariate-adjusted percentage decrease in the high-frequency (HF) component of heart rate variability (and 95% confidence interval) that is associated with a 10-μg/m³ increase in concentrations of particles less than 2.5 μM in aerodiameter ($PM_{2.5}$) is shown for persons with (G0) and without (G1) the deletion of the *GSTM1* gene (*left side*), and for persons with (S1) and without (S0) statin use (*right side*). The covariates controlled were age, cigarette smoking, body mass index, diastolic blood pressure, fasting blood glucose, alcohol consumption, use of β-blockers, use of angiotensin-converting enzyme inhibitors, use of calcium channel blockers, season, room temperature, and outdoor temperature. The numbers *below* the labels are the number of subjects in each analysis.

was seen in subjects with the *GSTM1* null deletion (Figure 1). Similarly, when stratified by statin use, only subjects not taking statins evidenced diminished HF in the presence of elevated $PM_{2.5}$ (Figure 1). When the data were analyzed by strata of both *GSTM1* status and statin use, *GSTM1*-null subjects who were taking statins were protected against the effect of $PM_{2.5}$, which was only present in subjects with the gene deletion who were not taking statins (Figure 2, Table 2).

The two other potential effect modifiers that we hypothesized were more strongly related to oxidative stress showed similar patterns of effect modification (Table 2). For subjects with obesity or higher neutrophil counts, there was some effect of particles, even with the *GSTM1* gene, and an enhancement of the $PM_{2.5}$ effect for persons both without the gene and with the other oxidative stress–related effect modifier. In contrast, older age did not modify the $PM_{2.5}$ effect in subjects with or without the gene. Higher blood pressure was intermediate, showing some indication of a $PM_{2.5}$ effect in subjects with the gene, and a modest enhancement of the effect in subjects without the gene.

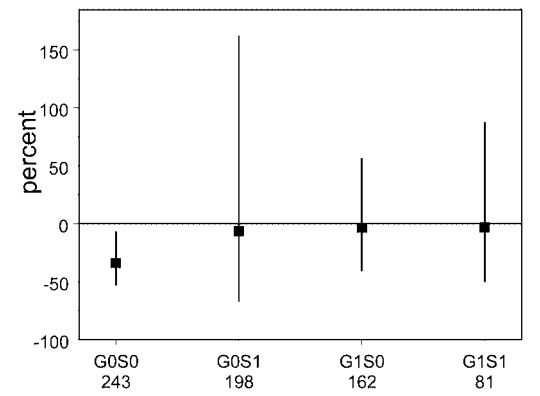

Figure 2. The estimated covariate-adjusted percentage decrease in the HF component of heart rate variability (and 95% confidence interval) associated with a 10-μg/m³ increase in $PM_{2.5}$ concentrations is shown for persons who are (S1) and are not (S0) taking a statin, with (G0) or without (G1) the deletion of *GSTM1*. The covariates controlled are as in Figure 1. The number of subjects in each category is listed *below* the category label.

DISCUSSION

We found that the association between $PM_{2.5}$ and reduced HF is only evident in persons missing the allele for *GSTM1* or in persons likely to have greater than average baseline systemic inflammation and oxidative stress, such as in obese individuals. Furthermore, among *GSTM1*-null subjects, statins were protective against the effects of $PM_{2.5}$. In nonobese subjects, we saw effect modification by *GSTM1*, but the response was almost doubled in obese subjects who were *GSTM1* null. Being in the upper quartile of neutrophil counts also substantially increased the $PM_{2.5}$ effect in subjects who were *GSTM1* null.

These results suggest that the confluence of two factors resulting in increased levels of or impaired defenses against oxidative stress results in even greater response to particles.

Although *GSTM1*, obesity, increased neutrophils, and statins involve several physiologic pathways, the striking observation that statins counter the susceptibility to $PM_{2.5}$-associated reductions in HF conferred by the *GSTM1* deletion, and that the genotype interacts with obesity and increased neutrophil counts, suggests there exists a common mechanism of action. It seems likely that ROS (a common mechanism of action of these modifiers) plays an important role in this response. Obesity is known to increase systemic inflammation and oxidative stress, and increased neutrophil count is also a marker of systemic inflammation. That subjects with those conditions had a response to $PM_{2.5}$ even in the presence of *GSTM1*, but an enhanced response in its absence, also suggests a central role of inflammation and oxidative stress in the autonomic effects of $PM_{2.5}$.

TABLE 2. EFFECT OF A 10-μg/m³ INCREASE IN $PM_{2.5}$ ON HIGH FREQUENCY BY STRATA OF *GSTM1* AND OTHER POTENTIAL EFFECT MODIFIERS

Category	Change (%)	95% Confidence Interval	n
GSTM1 null, no statin	−34.0	−53.0, −7.20	162
GSTM1 null, statin	−6.4	−66.5, 161.9	81
GSTM1 present, no statin	−3.6	−40.5, 56.2	117
GSTM1 present, statin	−3.2	−50.0, 87.2	81
GSTM1 null, high neutrophils*	−55.7	−88.0, 63.1	64
GSTM1 null, normal neutrophils†	−36.1	−55.2, −8.7	179
GSTM1 present, high neutrophils*	−49.6	−86.4, 86.1	51
GSTM1 present, normal neutrophils†	17.6	−20.0, 73.0	147
GSTM1 null, obese‡	−57.3	−88.0, 52.0	61
GSTM1 null, not obese§	−31.0	−50.6, −3.6	182
GSTM1 present, obese‡	−34.2	−77.9, 96.5	54
GSTM1 present, not obese§	7.5	−29.7, 64.3	144
GSTM1 null, older*	−37.0	−64.9, 13.0	63
GSTM1 null, younger†	−33.1	−55.3, 0.3	180
GSTM1 present, older*	−7.6	−57.1, 98.8	49
GSTM1 present, younger†	−0.3	−41.6, 70.1	149
GSTM1 null, higher blood pressure*	−47.3	−81.0, 45.7	59
GSTM1 null, lower blood pressure†	−34.9	−54.7, −6.5	184
GSTM1 present, higher blood pressure*	−21.1	−76.4, 164.4	48
GSTM1 present, lower blood pressure†	−8.7	−40.0, 38.9	150

* Upper 25th percentile of the distribution in the study population.
† Lower 75% of the distribution in the study population.
‡ Obesity: body mass index of 30 kg/m² or greater.
§ Body mass index < 30 kg/m².

Particles increase ROS production, perhaps in a catalytic fashion via redox cycling (24, 25, 27). A recent follow-up to the study that showed particles induced ROS in the lung and heart (27) found that administration of N-acetyl cysteine, a glutathione precursor, blunted that effect (42). Those results suggest an important role of the glutathione pathway in the defense against urban particles.

Particles induce proinflammatory mediators such as cytokines in the lungs (24, 43), and increase extracellular calcium influx, possibly through activation of calcium channels in the plasma membrane (44). Recently, particle exposure has been shown to increase circulating levels of asymmetric dimethylarginine, an endogenous inhibitor of NO synthase that is associated with impaired vascular function and increased risk for cardiovascular events (45). This suggests that NO concentrations may be impaired after particle exposure. This fits in well with the observation that statins, which blocked the effects of $PM_{2.5}$ in this study, enhance NO release. In general, all three PM-associated impairments have been linked with an increase in sympathetic and a reduction in vagal tone (46–48).

Nevertheless, we cannot rule out the importance of other pathways in the modification of the $PM_{2.5}$ effects. Statins are associated with lower risk of arrhythmic events (49) and increased HF component of HRV (50). The mechanisms of this antiarrhythmic properties are unclear, but may include enhanced NO synthase (51), decreased endothelin-1 (52), or other pathways not yet understood. Similarly, obesity affects many metabolic pathways, and does not merely increase inflammation.

We have also demonstrated that questions of mechanism of action of environmental agents, often considered the domain of toxicology, can also be addressed in humans using gene by environment, gene by drug by environment, and gene by phenotype by environment interactions. Although there are limitations to this approach, the ability to study the species of interest in the exposure range of interest makes it a valuable tool for examining mechanisms of environmental toxins.

There are a number of limitations to this analysis. First, we have used $PM_{2.5}$ concentrations at a single monitoring site as a surrogate for recent exposure to $PM_{2.5}$. A recent study comparing personal exposures to monitoring at the same site, in several panels of subjects, reported a high longitudinal correlation between the monitor's readings and personal exposure (53). In addition, $PM_{2.5}$ concentrations have been shown to be spatially homogeneous over the Boston area, suggesting that this is a reasonable approximation, and the error is likely to be nondifferential (53). Other genes affect responses to ROS, and our findings suggest that these also may play a role in individual response to air pollution–induced morbidity and/or mortality. We believe our current findings provide further evidence that ROS are an important pathway for particle toxicity.

Conflict of Interest Statement: J.S. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.K.P. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.S.O. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. P.S.V. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. D.S. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.W. received a grant for $900,065, Asthma Policy Modeling Study, from AstraZeneca from 1997–2003. He has been a coinvestigator on a grant from Boehringer Ingelheim, which began in 2003, to investigate a chronic obstructive pulmonary disease natural history model. He has received no funds for his involvement in this project. He has been an advisor to the TENOR study for Genentech and has received $5,000 for 2003–2004. He received a grant from Glaxo-Wellcome for $500,000 for genomic equipment from 2000–2003. He was a consultant for Roche Pharmaceuticals in 2000 and received no financial renumeration for this consultancy. K.K. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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