Effect of glutathione-S-transferase M1 and P1 genotypes on xenobiotic enhancement of allergic responses: randomised, placebo-controlled crossover study

Frank D Gilliland, Yu-Fen Li, Andrew Saxon, David Diaz-Sanchez

Summary

Background Particulate pollution is associated with the occurrence of asthma and allergy. The model pollutant, diesel exhaust particles, can participate with allergens in starting and exacerbating allergic airway diseases in part by production of reactive oxygen species. Glutathione-S-transferases (GSTs) can metabolise reactive oxygen species and detoxify xenobiotics present in diesel exhaust particles. We tested the hypothesis that null genotypes for GSTM1 and GSTT1, and GSTP1 codon 105 variants (I105 and V105) are key regulators of the adjuvant effects of diesel exhaust particles on allergic responses.

Methods Patients sensitive to the ragweed allergen were challenged intranasally with allergen alone and with allergen plus diesel exhaust particles in a randomised order at separate visits. Nasal allergen-specific IgE, histamine, interleukin 4, and interferon γ concentrations were measured before and 24 h after challenge.

Findings Individuals with GSTM1 null or the GSTP1 I105 wildtype genotypes showed enhanced nasal allergic responses in the presence of diesel exhaust particles. Compared with patients with a functional GSTM1 genotype, GSTM1 null patients had a significantly larger increase in IgE (median 102·5 U/mL [range 1·0–510·5] vs 45·5 U/mL [1·5–60·6], p=0·03) and histamine (13·8 nmol/L [3·1–24·7] vs 7·4 nmol/L [1·2–2·3], p=0·02) after diesel exhaust particles plus allergen challenge. The GSTM1 and GSTP1 genotype was associated with an increase in IgE (120·3 U/mL [6·7–510·5] vs 27·7 U/mL [1·5–60·6], p=0·03) and histamine (13·8 nmol/L [3·1–24·7] vs 5·2 nmol/L [0·2–19·6], p=0·01) after challenge with diesel exhaust particles and allergens. The diesel exhaust particles enhancement was largest in patients with both the GSTM1 null and GSTP1 I/I genotypes.

Interpretation GSTM1 and GSTP1 modify the adjuvant effect of diesel exhaust particles on allergic inflammation.

Introduction Exposure to ambient air pollution is associated with many adverse health effects ranging from increased symptoms of allergic airway disease to increased mortality. Research has focused on the effects of ambient particulate pollution and much evidence indicates that particulate pollution is associated with the occurrence of asthma and allergy. Understanding the effects of diesel exhaust particles on allergic airway diseases has been one focus of research on particulate pollution. Diesel exhaust contains small particles ranging from nanoparticles to coarse particles with mass concentrated in the accumulation mode centred at 0·2 μm in diameter that have high deposition rates in the lung and long residence times in the atmosphere. These primary diesel exhaust particles aggregate into a broad range of sizes and are important contributors to particular matter less than 10 μm in diameter (PM10) and particular matter less than 2·5 μm in diameter (PM2·5). Inhaled diesel exhaust particles can be deposited in the upper and lower respiratory tract and can participate with allergens in starting and exacerbating allergic diseases in the airway. In conjunction with allergen, diesel exhaust particles can act as an adjuvant to enhance IgE antibody responses, T-helper 2 (Th2) cytokine production, and histamine release in vivo. As with other inhaled pollutants, diesel exhaust particles are thought to exert major effects through production of reactive oxygen species. Antioxidants reduce the allergic inflammatory effects of diesel exhaust particles in vitro and in mice. The role of antioxidants in allergic responses to diesel exhaust particles suggests that sensitivity to the effects of diesel exhaust particles is related to variation in antioxidant defences.

Several small molecules and proteins are involved in airway antioxidant defences that might mediate sensitivity to diesel exhaust particles. Glutathione-S-transferases (GSTs) are a large family of proteins that participate in antioxidant defences through several mechanisms including reactive oxygen species metabolism and detoxification of xenobiotics present in diesel exhaust particles. We focused on GSTM1, GSTT1, and GSTP1 genotypes because these genes are expressed in the respiratory tract, are involved in detoxification of chemicals present in diesel exhaust particles, and have common functional variant alleles. These variant alleles result in either total absence or a substantial change in enzyme activity. Furthermore, three members of this superfamily GSTM1, GSTT1, and GSTP1 with common genetic variants are thought to affect allergic airway disease and might explain variation in responses to diesel exhaust particles. To test the hypothesis that common genetic variants null genotypes for GSTM1 and GSTT1, and GSTP1 codon 105 variants (I105 and V05) affect susceptibility to diesel exhaust particles’ enhancement of allergic responses, we used an established human nasal...
Table 1: Participants’ characteristics

<table>
<thead>
<tr>
<th>Genotype</th>
<th>GSTM1</th>
<th>GSTT1</th>
<th>GSTP1*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null</td>
<td>14</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Present</td>
<td>5</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>

*A105G polymorphism codes replacement of I by V.

Table 2: Nasal responses after exposure to allergen plus clean air or allergen plus diesel exhaust particles

<table>
<thead>
<tr>
<th>Clean air and allergen</th>
<th>DEP and allergen</th>
<th>Difference</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE (U/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEP</td>
<td>9-8 (6-4)</td>
<td>121-2 (134-1)</td>
<td>111-4 (129-7)</td>
</tr>
<tr>
<td>Interleukin 4 (U/mL)</td>
<td>0-3 (0-1)</td>
<td>0-6 (0-5)</td>
<td>5-7 (4-9)</td>
</tr>
<tr>
<td>Interferon γ (ng/L)</td>
<td>1-2 (0-6)</td>
<td>0-6 (0-5)</td>
<td>0-6 (0-8)</td>
</tr>
<tr>
<td>Interferon γ/interleukin 4</td>
<td>4-8 (2-7)†</td>
<td>0-6 (1-4)†</td>
<td>0-1 (0-3)†</td>
</tr>
<tr>
<td>Histamine (nmol/L)</td>
<td>3-1 (1-3)</td>
<td>15·0 (7·4)</td>
<td>11·8 (7·0)</td>
</tr>
</tbody>
</table>

DEP=diesel exhaust particles. Values are mean (SD). *Pairwise t tests for means. Value is mean (SD) ratio.

Procedures

We did a single blind, randomised, placebo-controlled crossover study. Nasal washes and provocation challenges were done as described previously. To establish a positive allergen challenge level, we gave participants increasing intranasal doses of short ragweed (Amb aI, Hollister Stier/Baxter, Irwindale, CA) starting at 10 allergic units and increasing in ten-fold steps until a symptom score of 5 (of a possible 12) was achieved. This dose of allergen was used in the subsequent challenges. Participants then underwent two subsequent challenges that were at least 6 weeks apart. At those times, they were challenged intranasally in a randomised, blinded, cross-over fashion with either allergen plus placebo (300 μL saline), or allergen plus 0·3 mg diesel exhaust particles (in 300 μL saline). The diesel exhaust particles used had been generated in 2001 from a light-duty four-cylinder diesel engine (4JB1 type, Isuzu Automobile Company, Japan) using standard diesel fuel. Diesel exhaust particles stocks are stored under nitrogen in the dark and working volumes are stored at −80°C in the dark. This storage prevents oxidation or loss of volatile chemicals.

Nasal washes (5 mL normal saline in each nostril) were done immediately before, and at 10 min, 24 h, and 72 h after the challenge, as described previously. Results are reported for the 10 min post-challenge time point for histamine and the 24-h post-challenge timepoint for other responses since the respective responses have been previously shown to be maximum at these time points.

We collected nasal washes, centrifuged them at 350 g for 10 min at 4°C, and separated the aqueous supernatants from the cell pellets. Total and ragweed-specific IgE in nasal washes were measured by isotype specific ELISAs as described previously. We measured histamine concentrations with a commercial assay (Immunotech, Brea, CA) following the manufacturers’ instructions. The sensitivity of the assay was 0-5 nmol/L. The cytokines interleukin 4 and interferon γ were measured with commercial ELISA kits (BD Pharmingen, MECHANISMS OF DISEASE
and allergen plus diesel exhaust particles for nasal allergen-specific IgE response to allergens plus clean air.

GSTM1 isolation kit (D-5000, GENTRA, Minneapolis, MN). Patients with values below the limit of detection.

The lower limit of detection for each assay was used for instructions. For the purposes of statistical analyses, the San Diego, CA) following the manufacturers’ instructions. We obtained buccal cells from participants as a source of genomic DNA. Details of buccal cell processing and genotyping assays have been described previously.28 Genomic DNA. Details of buccal cell processing and genotyping assays have been described previously.28

Table 3: Effects of GSTM1, GSTT1, and GSTP1 genotype on nasal IgE (U/mL) and histamine (nmol/L) when exposed to allergen plus clean air or allergen plus diesel exhaust particles (DEP)

<table>
<thead>
<tr>
<th></th>
<th>GSTM1 Null (n=14)</th>
<th>Present (n=5)</th>
<th>p</th>
<th>GSTT1 Null (n=9)</th>
<th>Present (n=10)</th>
<th>p</th>
<th>GSTP1 I/I (n=13)</th>
<th>I/V (n=6)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE</td>
<td>6·9 (2·6–24·3)</td>
<td>8·9 (4·3–18·8)</td>
<td>0·40</td>
<td>7·9 (3·8–24·3)</td>
<td>7·8 (2·6–18·7)</td>
<td>0·57</td>
<td>7·8 (3·2–24·3)</td>
<td>8·4 (2·6–18·8)</td>
<td>1·00</td>
</tr>
<tr>
<td>Clean air and allergen</td>
<td>106·6 (8·8–534·8)</td>
<td>49·8 (14·2–79·4)</td>
<td>0·15</td>
<td>89·5 (13·3–534·5)</td>
<td>49·3 (8·8–312·5)</td>
<td>0·35</td>
<td>123·5 (14·5–534·8)</td>
<td>31·5 (8·8–79·4)</td>
<td>0·02</td>
</tr>
<tr>
<td>Difference</td>
<td>102·5 (1·0–510·5)</td>
<td>45·5 (–1·5–60·6)</td>
<td>0·03</td>
<td>84·7 (9·1–510·5)</td>
<td>45·9 (–1·5–293·8)</td>
<td>0·35</td>
<td>120·3 (6·7–510·5)</td>
<td>27·7 (–1·5–60·6)</td>
<td>0·03</td>
</tr>
<tr>
<td>histamine</td>
<td>2·9 (1·3–5·9)</td>
<td>2·8 (1·9–6·7)</td>
<td>0·96</td>
<td>2·8 (2·2–4·3)</td>
<td>2·9 (1·3–6·7)</td>
<td>0·65</td>
<td>2·9 (1·3–6·7)</td>
<td>3·0 (1·9–6·0)</td>
<td>0·63</td>
</tr>
<tr>
<td>Clean air and allergen</td>
<td>16·9 (2·9–27·6)</td>
<td>9·8 (3·1–19·0)</td>
<td>0·08</td>
<td>15·7 (7·3–25·8)</td>
<td>16·4 (2·9–27·6)</td>
<td>1·00</td>
<td>17·2 (6·2–27·6)</td>
<td>8·5 (2·9–25·5)</td>
<td>0·04</td>
</tr>
<tr>
<td>Difference</td>
<td>14·0 (–0·2–24·7)</td>
<td>7·4 (1·2–12·3)</td>
<td>0·02</td>
<td>12·9 (3·0–21·8)</td>
<td>12·7 (–0·2–24·7)</td>
<td>0·97</td>
<td>13·8 (3·1–24·7)</td>
<td>5·2 (–0·2–19·6)</td>
<td>0·01</td>
</tr>
</tbody>
</table>

Values are median (range). p values calculated with Wilcoxon rank sums test.

Table 3: Effects of GSTM1, GSTT1, and GSTP1 genotype on nasal IgE (U/mL) and histamine (nmol/L) when exposed to allergen plus clean air or allergen plus diesel exhaust particles (DEP)

San Diego, CA) following the manufacturers’ instructions. For the purposes of statistical analyses, the lower limit of detection for each assay was used for patients with values below the limit of detection.

We obtained buccal cells from participants as a source of genomic DNA. Details of buccal cell processing and genotyping assays have been described previously.28 Briefly, DNA was extracted with a PUREGENE DNA isolation kit (D-5000, GENTRA, Minneapolis, MN). GSTM1, GSTT1, and GSTP1 genotypes were determined by real-time PCR with a TaqMan 7700 (Applied Biosystems, Foster City, CA). The presence or absence of a fluorescent amplification signal was used as an indication of whether the GSTM1 and GSTT1 alleles were present or absent in a genomic DNA sample. Samples showing no signal or late cycle number for start of amplification were repeated and further analysed with primers and probes for the actin gene to verify the presence of amplifiable DNA. We analysed the single nucleotide polymorphism at codon 105 in the GSTP1 gene with allele-specific probes.

Statistical analysis

First, we assessed the distributions of allergen-specific IgE, interleukin 4, interferon γ, the ratio of interleukin 4 to interferon γ, and histamine, and found that each was skewed and did not follow a normal distribution. Therefore, we compared median concentrations and median differences of allergen-specific IgE, interleukin 4, interferon γ, and histamine after allergen challenge and after allergen plus diesel exhaust particles challenge. We also used the median values to assess the ratio of interleukin 4 to interferon γ. We tested the hypothesis with non-parametric Wilcoxon signed-rank tests for difference in the median values. We also provide means and t tests for differences in mean concentrations for completeness. The effect of GSTM1, GSTT1, and GSTP1 genotypes on allergen-specific IgE, histamine, interleukin 4, and interferon γ concentrations after allergen alone or diesel exhaust particles plus allergen were assessed by comparisons of median responses between different genotypes and statistical testing was again done with Wilcoxon signed-rank tests for median differences. All analyses were done with SAS software version 8.0 and all reported p values are based on a two-sided alternative hypothesis. p values were judged significant if they were less than 0·05.

Role of the funding source

The sponsors had no role in study design, data collection, data analysis, data interpretation, or writing and submission of this report.

Results

Table 1 shows the participants’ characteristics. GSTM1 and GSTT1 null genotypes were present in 74% (14 of 19) and 47% (nine of 19) of patients, respectively. Most (68%, 13 of 19) patients were homozygous for the GSTP1 I105 wild-type allele and none was homozygous for the GSTP1 V105 variant allele. We selected the patients on the basis of their nasal allergy status, which probably explains the genotype distribution that differs from that seen in general population studies.

We have previously reported that diesel exhaust particles enhance allergen-driven, IgE, histamine, and...
interleukin 4 responses while decreasing production of interferon γ. Diesel exhaust particles greatly increased the allergic response after nasal challenge. On exposure to diesel exhaust particles plus allergen, nasal allergen-specific IgE concentrations increased more than ten-fold compared with allergen alone (table 2) for all participants. Histamine concentrations were also about five-fold higher after diesel exhaust particles plus allergen than after allergen alone (table 2). Compared with allergen challenge alone, exposure to diesel exhaust particles plus allergen increased interleukin 4 and decreased interferon γ concentrations consistent with an enhancement of the allergic response (table 2).

Table 3 and the figure show that individuals with either a null GSTM1 or homozygous GSTP1 I105 genotypes had much higher nasal IgE responses to diesel exhaust particles than to the allergen alone. Compared with participants with GSTM1 present genotype, those with GSTM1 null had a significantly larger increase in antiragweed IgE (median 102·5 U/mL [I–Q 1·0–510·5] vs 45·5 U/mL [I–Q 1·9–60·6], p=0·03) after diesel exhaust particles plus allergen challenge. Compared with participants with a GSTP1 V105 variant, those with the homozygous wild-type I105 GSTP1 genotype had a significantly larger increase in allergic specific IgE after diesel exhaust particles plus allergen challenge (median 120·3 U/mL [I–Q 6·9–510·5] vs 27·7 U/mL [I–Q 1·5–60·6], p=0·03). By contrast, GSTT1 genotype was not associated with diesel exhaust particles-enhanced IgE responses. None of the GSTs modified the allergic response to allergen challenge alone.

Parallel effects of the GSTM1 null and I105 GSTP1 genotypes were seen with histamine release enhanced by diesel exhaust particles (table 3). In participants with a null GSTM1, histamine concentrations were significantly higher after diesel exhaust particles plus allergen challenge than in those with the functional GSTM1 genotype (table 3). Similarly, those with the GSTP1 I105 variant had higher histamine concentrations after diesel exhaust particles plus allergen challenge than did those with the homozygous genotype (table 3).

The joint GSTM1 and GSTP1 genotype seems to be an important determinant of response to diesel exhaust particles (table 4). Of the 14 participants who were allergic and had the GSTM1 null genotype, 11 had the normal GSTP1 I/V genotype. Those with this joint genotype had significantly higher allergic responses to diesel exhaust particles than the other genotypes combined. Our sample size does not allow a full assessment of gene-gene-environment interaction for the GSTs in this study.

Interleukin 4 responses to diesel exhaust particles did not significantly differ between the GSTM1 null and GSTP1 I/I genotype and the GSTM1 present and the GSTP1 V/V genotype, respectively (table 5). The concentrations of interferon γ and the ratio of interleukin 4 to interferon γ did not show consistent patterns by genotype.

**Discussion**

Our results show that susceptibility to an adverse health effect of diesel exhaust particles, a model oxidant pollutant, can be controlled by functional variation in natural antioxidant defenses. Several polymorphic genes including those for GSTs have been associated with atopy (allergy, asthma, and atopic dermatitis). Here, we provide evidence that the GSTM1 and GSTP1 genotypes play an important part in susceptibility to the adjuvant effects of oxidant pollutants such as diesel exhaust particles, but are not associated with the magnitude of allergic response to allergen per se. The importance of these results is heightened by the high frequency of polymorphisms of these genes in many populations. For example, the null allele variant of GSTM1 occurs in about 50% of individuals. To assess the extent to which the reported variability reported in responses to diesel exhaust particles is explained by these polymorphisms, we examined the joint GSTM1 null and GSTP1 I/I genotype, which show the largest enhancement of allergic responses from diesel exhaust particles. GSTM1 null genotype frequency is about 50% and the GSTP1 I/I genotype frequency is roughly 40%. Because the genes are on different chromosomes, they assort independently. On the basis of this information, we estimate that 15–20% of the general population are at the highest risk for a large enhancement of allergic responses to diesel exhaust particles. Among individuals who are allergic, the proportion of the population at risk for diesel exhaust particles enhancement could be larger than the proportion among the non-allergic population.

Results of epidemiological studies have shown that the GSTP1 and GSTM1 polymorphisms are associated with airway hyper-responsiveness and asthma, especially in those whose asthma is related to xenobiotic exposure. Furthermore, the frequency of the GSTP1 V105/V105 genotype is reduced in patients who are atopic compared with those who are not. Airway inflammation is thought to result in formation of reactive oxygen species and small molecular and enzymatic antioxidants can mitigate the formation and effects of reactive oxygen species. GSTs might also affect synthesis of eicosanoids such as leukotrienes that modulate allergic responses. Here, we suggest an additional role for GSTs wherein members of the GST family can play a key part in controlling the response to diesel exhaust particles by detoxifying...
reactive oxygen species derived from diesel exhaust. Studies in mice have shown that an antioxidant will block production of interleukin 4 and IgE that is enhanced by diesel exhaust. Many in-vitro studies also suggest that the effect of diesel exhaust particles on interleukin 4 is due to generation of oxidative stress. We have previously shown that the earliest detectable source of interleukin 4 after nasal challenge with diesel exhaust particles plus allergen derives from CD117 positive cells. Our failure to find significant differences in interleukin 4 and interferon γ is probably due to a type 2 error. However, it is not surprising that a small increase in interleukin 4 and a small reduction in interferon γ result in a large change in IgE. Formation of allergic antibodies is a complex process that involves many cytokines and processes. Enhancement of interleukin 4 by diesel exhaust particles probably leads to a cascade effect resulting in promotion of a Th-2 environment including further production of interleukin 4, interleukin 13, and interleukin 6. In addition, diesel exhaust particles can increase antigen presentation and T-cell responses. The combination of these factors leads to a more robust increase in IgE production than does each factor alone.

Particles in diesel exhaust have been used as a model particulate pollutant. Diesel exhaust particles make up 40% of the PM2.5 found in the air in the Los Angeles basin. Results of studies in people and animals have shown that diesel exhaust particles can participate in both starting and enhancing allergic immune responses. In this study, we exposed participants to an amount equivalent to 40 h of exposure of people living in Los Angeles. We have previously shown that this amount of diesel exhaust particles can act as an adjuvant when given with allergen to augment IgE, Th-2 cytokine, and chemokine production while increasing symptom severity and histamine release. Evidence for involvement of reactive oxygen species generation in diesel exhaust particles' health effects has come from both human and animal exposure models. In mice models of asthma, diesel exhaust particles can increase cytochrome P450 reductase activity in the lung. Pretreatment of these mice with antioxidants will decrease eosinophilia whereas control mice will increase oxygen scavenging ability. Pretreatment with diesel exhaust particles plus allergen derives from GST family use distinct but overlapping substrates. It is notable that GSTM1 is involved mainly with detoxification of oxygen-polyaromatic hydrocarbons. It is not surprising that no association was found between GSTT1 genotypes and diesel exhaust particles susceptibility since the main substrates for GSTT1, eg, ethylene oxide, are not found in diesel exhaust particles. GSTPI detoxifies lipid peroxidation products and DNA oxidation products. GSTM1 might be involved in restricting initial generation of the reactive oxygen species response by diesel exhaust particles chemicals whereas GSTP1 plays a part in a later stage when inflammation and oxidant damage is taking place. Inhalation challenge studies are useful models and have been used extensively to study the potential of environmental agents to change the immune response under controlled conditions. This system has been used to investigate airway responses to diesel exhaust particles, ozone, second-hand smoke, sulphur dioxide, and nitrous oxide, among others. A common feature of these challenges is observation of both interindividual variability and intraindividual consistency such that a non-sensitive individual will always have a weak or small response to the pollutant.

In view of the substantial effects of GSTM1 and GSTPI variants on enhancement of allergic responses by diesel exhaust particles, our results suggest that these genes have an important role in modification of the airway response to diesel exhaust particles. These results, therefore, have obvious clinical and public-health relevance especially for those living in urban environments. Because the GSTM1 and GSTPI variants we investigated are common, the number of susceptible individuals with symptomatic allergic airway disease in the setting of diesel exhaust particles or other xenobiotic exposures would be expected to be large. It is unlikely that GSTM1 and GSTPI are the only loci that confer protection from airborne pollutants such as diesel exhaust particles, or that they are unique in their action. Other antioxidant genes with functional polymorphisms exist—such as MnSOD and NQO1—which products can also detoxify quinones. Furthermore, small molecule antioxidants and dietary intake might contribute to antioxidant defenses directly or by increasing expression of antioxidant genes. Although our model is one of inflammation in the upper airway, there is evidence to suggest that the results also apply to the lower airway. Diesel exhaust particles can enhance airway hyper-reactivity in mice. This hyper-reactivity is associated with increased production of reactive oxygen species in the alveolar spaces. In addition, exposure to diesel exhaust particles will change intracellular glutathione concentrations in alveolar macrophages and lymphocytes. Furthermore, the GSTP1 gene product might provide more than 90% of the oxidation products and DNA oxidation products.

### MECHANISMS OF DISEASE

**BACKGROUND**

Pollution can trigger asthma in some individuals, but the genetic factors that underlie this process are complex. One possible factor in sensitivity to pollution might be how well an individual can neutralise the toxic effects of environmental pollution.

This paper looked at genes in a pathway that neutralises reactive oxygen species generated from diesel exhaust particles. It examined relationships between genotypes that are more or less effective in neutralising such species, with response to inhalation of a known allergen, with or without the diesel particles. In two of three genes studied, individuals that carried the genotypes that most efficiently oxidise peroxidase had lower IgE and histamine responses to allergen and diesel exhaust particles.

**IMPLICATIONS**

These findings highlight how genetic and environmental factors interact to produce a complex disease. They suggest a direct way that pollution could be triggering allergic symptoms, at least in some individuals, and perhaps a possible route of intervention.
glutathione-S-transferase activity in the lung, suggesting that lower airway and upper airway effects of diesel exhaust particles are mostly modulated by GSTP1.

In conclusion, our findings suggest that common polymorphisms in GSTM1 and GSTP1 identify a large genetically susceptible population for enhanced adjuvant and other adverse health effects of diesel exhaust particles exposure. Larger scale studies will be needed to identify other less common polymorphisms, and to provide additional power to detect smaller, but meaningful relations between genotypes and increases in interleukin 4 and reduction in interferon γ by diesel exhaust particles. Furthermore, in view of the intense debate on the effects of particulate matter on cardiovascular events, investigating the role of these genes in the relation between diesel exhaust and other particulate matter components with acute cardiopulmonary morbidity and mortality is warranted. Our results point to the potential for new insights from prospective epidemiological studies investigating responses to air pollutants in different genotypes.

Contributors
Frank Gilliland supervised genotyping, the statistical analyses, and manuscript preparation. Diaz-Sanchez supervised participant recruitment, nasal lavage procedures, and assay protocols, and contributed to the manuscript preparation. A Saxon contributed to the study design, analysis, and manuscript preparation. Y-F Li did the statistical analysis and contributed to the manuscript preparation.

Conflict of interest statement
None declared.

Acknowledgments
We thank Dorothy Starnes for providing technical support in the preparation of this manuscript. This study was supported by the National Heart, Lung and Blood Institute (HL-61768) the National Institute of Environmental Health Sciences, and the Hastings Foundation. Environmental Protection Agency (R82670801), the UCLA Allergy, Heart, Lung and Blood Institute (HL-61768) the National Institute of Environmental Health Sciences, and the Hastings Foundation.

References
14 Delfino RJ. Epidemiologic evidence for asthma and exposure to air toxics: linkages between occupational, indoor, and community air pollution research. Environ Health Perspect 2002; 110 (suppl) 573–89.


