Mechanisms of disease

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 (14·0 nmol/L [–0·2–24·7] *vs* 7·4 nmol/L [1·2–12·3], p=0·02) after diesel exhaust particles plus allergen challenge. The I105 *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.

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Introduction

Exposure to ambient air pollution is associated with many adverse health effects ranging from increased symptoms of allergic airway disease to increased mortality.[1–3] 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.[4–9]

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.[10] 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 (PM_{10}) and particular matter less than 2·5 μm in diameter ($PM_{2·5}$). 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.[5,6,11–16] 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.[9,17–19] 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.[20] 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.[21] 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.[22–24]

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

GLOSSARY

DIESEL EXHAUST PARTICLES
Diesel exhaust particles are respirable particles produced during compression ignition of diesel fuel. The particles are composed of elemental and organic carbon compounds as well as trace amount of other elements with toxic properties including transition metals.

GSTM1
Glutathione-S-transferases are involved in phase 2 xenobiotic and reactive oxygen species metabolism and have coordinate regulation based on antioxidant response element in their promoter region. GSTM1 is a member of the M family of glutathione-S-transferases. The gene is located at 1p13.3 and has a common allele that results in no protein product.

GSTP1
Glutathione-S-transferase P1 is a member of the P family of glutathione-S-transferases. GSTP1 is located at 11q13.3 and has a common single nucleotide polymorphism at codon (A105G) that results in an aminoacid change in the protein from isoleucine to valine. This aminoacid substitution has pleotropic effects on the enzyme function.

GSTT1
Glutathione-S-transferase T1 is a member of the γ family of glutathione-S-transferases. GSTT1 is located at 22q11.23 and, like GSTM1 has a common allele that results in no protein product.

REACTIVE OXYGEN SPECIES
Important reactive oxygen species for biological systems include superoxide, hydrogen peroxide, and hydroxyl radical. At low levels, these species may function in cell signalling process. At higher levels, reactive oxygen species damage cellular macromolecules and participate in apoptotic processes.

provocation model. Nasal allergic responses were measured after challenge with allergen and allergen plus diesel exhaust particles to determine whether functional variants in genes involved in antioxidant defences can account for the variation between individuals in their responses to diesel exhaust particles.

Methods

Participants

We recruited 19 non-smoking volunteers (seven males and 12 females) in Los Angeles, CA, USA. All had a positive epicutaneous skin test (>4 mm wheal with surrounding erythema) to short ragweed (an allergen not present in the Los Angeles region) and an allergy history consistent with allergic rhinitis. Based on interview responses, none of the volunteers had any atypical exposure to pollutants and no air pollution alerts in the Los Angeles area were reported during the study periods. None of the participants reported a respiratory infection in the previous 4 weeks and none had used topical or systemic steroids in the 3 months before the study or oral antihistamines for the previous week. None had ever received allergy immunotherapy. All participants were asked to fill out symptom score cards 2 days before and throughout the study period. All studies were approved by the human subject protection committee of the University of California at Los Angeles and all participants gave written informed consent.

	Participants (n=19)
Sex	
Women	12
Men	7
Age (years)	
20–25	7
26–30	8
30–34	4
Ethnic origin	
White	8
Hispanic	5
African American	1
Asian	5
Genotype	
GSTM1	
Null	14
Present	5
GSTT1	
Null	9
Present	10
*GSTP1**	
I/I	13
I/V	6
V/V	0

*A105G polymorphism codes replacement of I by V.

Table 1: **Participants' characteristics**

Procedures

We did a single blind, randomised, placebo-controlled crossover study. Nasal washes and provocation challenges were done as described previously.[5,25,26] To establish a positive allergen challenge level, we gave participants increasing intranasal doses of short ragweed (*Amb a*I, 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 points) 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.[5,25,26] 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.[5,27] 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,

	Clean air and allergen	DEP and allergen	Difference	p*
IgE (U/mL)	9·8 (6·4)	121·2 (134·1)	111·4 (129·7)	0·002
Interleukin 4 (U/mL)	0·3 (0·1)	6·0 (5·0)	5·7 (4·9)	<0·0001
Interferon γ (ng/L)	1·2 (0·6)	0·6 (0·5)	–0·6 (0·8)	0·002
Interferon γ/ interleukin 4	4·8 (2·7)†	0·6 (1·4)†	0·1 (0·3)†	<0·0001
Histamine (nmol/L)	3·1 (1·3)	15·0 (7·4)	11·8 (7·0)	<0·0001

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

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

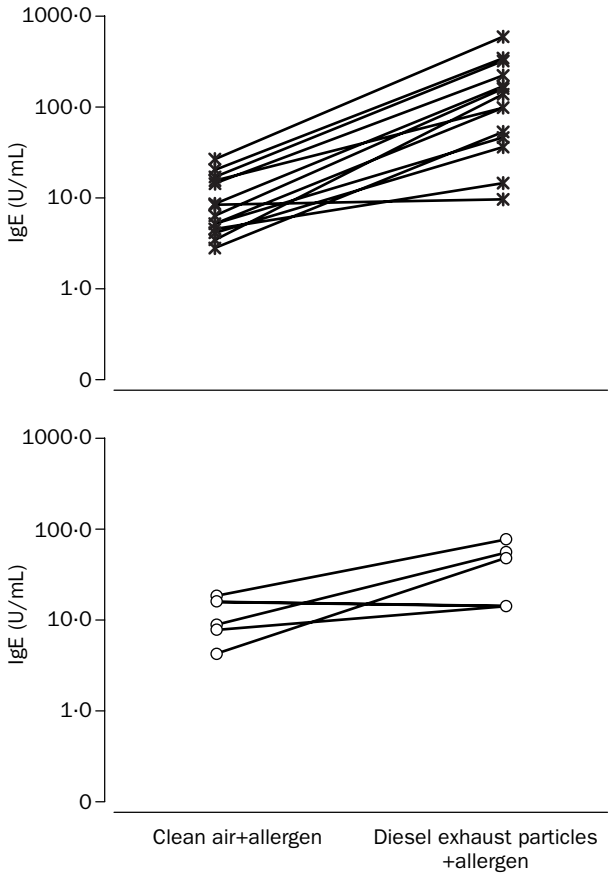
	GSTM1			*GSTT1*			*GSTP1*		
	Null (n=14)	Present (n=5)	p	Null (n=9)	Present (n=10)	p	I/I (n=13)	I/V (n=6)	p
IgE									
Clean air and allergen	6·9 (2·6–24·3)	8·9 (4·3–18·8)	0·40	7·9 (3·8–24·3)	7·8 (2·6–18·7)	0·57	7·8 (3·2–24·3)	8·4 (2·6–18·8)	1·00
DEP and allergen	106·6 (8·8–534·8)	49·8 (14·2–79·4)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

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.



Nasal allergen-specific IgE response to allergens plus clean air and allergen plus diesel exhaust particles for *GSTM1* absent (upper) and present (lower) genotypes
Y axis is log scale of median IgE concentrations.

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

GSTM1	*GSTP1*	n	IgE difference	Histamine difference
Present	I/I	2	26·1 (6·7–45·5)	7·73 (3·13–12·32)
Present	I/V	3	48·9 (–1·5–60·6)	7·44 (1·22–7·48)
Null	I/I	11*	137·0 (29·9–510·5)	14·33 (8·14–24·67)
Null	I/V	3	9·1 (1·0–46·2)	2·98 (–0·22–19·59)

Values are median (range). *p=0·0034 for IgE and p=0·0073 for histamine calculated by the Wilcoxon test comparing *GSTM1* null/*GSTP1* I/I with the other three genotype groups combined.

Table 4: **IgE (U/mL) and histamine (nmol/L) differences by joint *GSTM1* and *GSTP1* genotype**

interleukin 4 responses while decreasing production of interferon γ.[5,13] 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 [1·0–510·5] *vs* 45·5 U/mL [–1·5–60·6], 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 [6·9–510·5] *vs* 27·7 U/mL [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/I 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* I/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 most populations. For example, the null allele variant of *GSTM1* occurs in about 50% of individuals.[29] 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[22,24,30] 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.[22] 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.[20] GSTs might also affect synthesis of eiconosoids such as leucotrienes 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

	GSTM1			*GSTT1*			*GSTP1*		
	Null (n=14)	Present (n=5)	p	Null (n=9)	Present (n=10)	p	I/I (n=13)	V/V (n=6)	p
Interferon γ (ng/L)	–0·5 (–1·9 to 0·2)	–0·8 (–1·8 to 0·5)	0·75	–0·9 (–1·9 to 0·2)	–0·2 (–1·8 to 0·5)	0·18	–0·8 (–1·9 to 0·5)	–0·6 (–1·7 to 0·3)	1·00
Interleukin 4 (U/mL)	5·6 (0·0 to 15·0)	2·8 (0·0 to 8·1)	0·15	5·1 (0·0 to 9·2)	4·7 (0·0 to 15·0)	0·97	5·1 (0·0 to 15·0)	3·3 (0·7 to 14·4)	0·51
Interferon γ/interleukin 4	0·02 (0·0 to 0·44)	0·03 (0·0 to 1·32)	0·49	0·02 (0·0 to 0·44)	0·05 (0·0 to 1·32)	0·65	0·02 (0·0 to 1·32)	0·03 (0·0 to 0·28)	0·69

Values are median (range).

Table 5: **Effects of *GSTM1*, *GSTT1*, and *GSTP1* genotype comparing differences and ratio of responses to allergen plus diesel exhaust with allergen plus clean air**

reactive oxygen species derived from diesel exhaust. Studies in mice[31] have shown that an antioxidant will block production of interleukin 4 and IgE that is enhanced by diesel exhaust particles. 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.[32] 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 to 40% of the PM_{10} found in the air in the Los Angeles basin. Results of studies in people and animals[32,33] 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.[5,13,34] 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 while decreasing oxygen scavenging ability.[18] Pretreatment of these mice with antioxidants will decrease eosinophilia induced by diesel exhaust particles, mucus hyper-secretion, airway hyper-responsiveness, and IgE responses to bystander antigen.[18,19] Additionally, Nightingale and colleagues[35] showed that exposure of healthy volunteers to resuspended diesel exhaust particles (200 μg/m^3) in an inhalation chamber results in an increase in sputum inflammatory cells along with an increase in exhaled carbon monoxide concentrations, an indicator of oxidant stress.

Diesel exhaust particles consist of a carbon core surrounded by chemicals including quinones and polyaromatic hydrocarbons, which can be metabolised to produce oxy-polyaromatic hydrocarbons.[36] These chemicals can induce reactive oxygen species leading to activation of intracellular signalling pathways and induction of gene transcription.[9] Generation of hydroxyl radicals induces a decrease in glutathione (GSH) concentrations, which in turn will increase transcription of GST genes. Our study suggests that the GST member with the most pronounced effect on diesel exhaust particles sensitivity is *GSTM1*, a class μ GST isoenzyme.

GSTM1 is present in lung and nasal tissue although its expression is highest in the liver.[37] Different members of the GST family use distinct but overlapping substrates. It is notable that *GSTM1* is involved mainly with detoxification of oxy-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. *GSTP1* 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.[38–41] 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 *GSTP1* 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 sensitised individuals living in urban environments. Because the *GSTM1* and *GSTP1* 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 *GSTP1* 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*—whose 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.[42] In addition, exposure to diesel exhaust particles will change intracellular glutathione concentrations in alveolar macrophages and lymphocytes.[43] Furthermore, the *GSTP1* gene product might provide more than 90% of the

RELEVANCE OF THIS PAPER TO PRACTICE

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 relations 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 metabolised reactive oxygen species 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,[44] 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

F Gilliland supervised genotyping, the statistical analyses, and manuscript preparation. D Diaz Sanchez supervised participant recruitment, nasal lavage procedures, and lavage assays, 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

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