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Gene by Environment Interaction and Ambient Air Pollution

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Epidemiologic studies have clearly shown that air pollution is associated with a range of respiratory effects. Recent research has identified oxidative stress as a major biologic pathway underlying the toxic effect of air pollutants. Genetic susceptibility is likely to play a role in response to air pollution. Genes involved in oxidative stress and inflammatory pathways are logical candidates for the study of the interaction with air pollutants. In this article we use the example of asthma, a genetically complex disease, to address the issue of gene by environment interaction with air pollution. The majority of studies have focused on the genes *GSTM1*, *GSTP1*, *NQO1*, and *TNF*, but the inconsistency of the results prevents the drawing of firm conclusions. The limited sample size of most studies to date make them underpowered for the study of gene by gene interactions. Large consortia of studies with repeated measurements of environmental exposures and clear phenotypic assessments may help determine special environmental triggers and the window of susceptibility in the development of atopy and asthma. The role of gene by gene interactions and epigenetic mechanisms needs to be considered along with gene by environment interactions.

Keywords: ozone; particulate; gene by environment interaction; oxidative stress; asthma

Epidemiologic studies have clearly shown that air pollution exposure is associated with a range of respiratory effects (1, 2). Recent research has identified oxidative stress as a major pathway underlying the toxic effect of air pollutants that triggers a number of redox-sensitive signaling pathways such as those of inflammatory response and cytokine production (3, 4). Toxicity could arise from an imbalance of biological prooxidant and antioxidant processes linked to increased exposure to oxidants and impairment of antioxidant defenses (5). This has long been recognized in investigations of ozone, one of the most potent oxidants, and more recent studies have focused on this particular mechanistic hypothesis (5). Genetic susceptibility is likely to play a role in the response to air pollution exposure. Genes clearly involved in pathways of biological response to air pollutants, such as the oxidative stress and inflammatory pathways, are logical candidates to study the interaction with air pollutants. Different genes may be involved in different air

pollutant response phenotypes (6). A single polymorphism in a single gene is unlikely to explain variation in all phenotypes, but rather a complex interaction of genes will probably determine the response (6). This is likely due to the relatively small functional effects of individual polymorphisms in complex biological pathways (7).

In this article we take the example of asthma, a genetically complex disease caused by multiple genes and multiple environmental factors that may interact (8), to address the issue of gene by environment interaction with exposure to air pollutants.

SUSCEPTIBILITY TO PULMONARY RESPONSES TO AIR POLLUTANTS RELEVANT TO ASTHMA

The role of genetic background in pulmonary responses to the adverse effect of oxidant exposures was initially suggested by McDonnell (9), who pointed to the role of susceptibility in acute effects of ozone. He reported that the drop in pulmonary function (FEV₁) in response to acute ozone exposure tends to track within an individual when measured on different days. This pattern of greater between-subject than within-subject variability in response to ozone suggests a measure of genetic control. Furthermore, investigators found that O₃-induced inflammation, as indicated by the number of polymorphonuclear leukocytes found in BALF, also varied widely between subjects (10). Because the subjects in these studies were otherwise healthy, nonsmoking young adults, an intrinsic factor, such as genetic variability, was suggested to be an important determinant of variation. Subsequent studies of inbred mouse strains confirmed a genetic component in respiratory responses to ozone. Specific genes identified in mice include tumor necrosis factor- α (*TNF*) and Toll-like receptor 4 (*TLR4*) (11). While animal studies have also been performed on the genetics of response to particulate air pollution, it has been more difficult to find specific genes involved in response variations (12). Particles are a complex mixture and we lack understanding of the specific components responsible for respiratory effects.

ADVERSE EFFECTS OF AIR POLLUTANTS: BIOLOGICAL MECHANISM

Ozone

Numerous studies have shown the association between ozone exposure and pulmonary injury (1). This pollutant is a strong oxidant exerting its biological action either by direct reaction with target molecules or by generating reactive oxygen species (ROS) which result in its biological effects and its toxicity. Ozone activates transcription factors such as nuclear factor- κ B (NF- κ B) and increased expression of a range of proinflammatory cytokines and adhesion genes. Ozone has been shown to

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react readily with ascorbic acid, uric acid, and thiols and to lead to their rapid depletion.

Nitrogen Dioxide

A major source of outdoor NO_2 is related to vehicular traffic. NO_2 contributes also to secondary particles and the formation of O_3 . High concentration of NO_2 and other nitrogen-reactive species have been linked to pulmonary inflammation and injury to the lung epithelium (13). As for ozone, NF- κ B transcription factor is involved in cytokine gene activation and chemokine production (14).

Particles

Particles are a complex mixture, and several of their components have been linked to toxicity. *In vitro* studies have shown that PM exposure is related to the expression of the nuclear factor NF- κ B-related genes and activation of oxidant-dependent NF- κ B such as TNF- α and interleukin (IL)-8 and -6 (15). The primary determinant of acute inflammatory response appears to be the dose of bioavailable transition metals (such as Fe, Ni, and V), organic compounds (such as polycyclic aromatic hydrocarbons), and biological fractions (such as endotoxins) (15, 16), rather than particulate mass. Diesel exhaust particles (DEP) have a high content of elemental and organic carbon and are thought to be particularly toxic (17). Reactive oxygen species (ROS) formed at the epithelial level after DEP exposure up-regulate IL-10, promoting antigen-presenting cells and allergy to pollen (17).

A hierarchical oxidative stress model has been proposed to explain the dose-dependent response to air pollutant exposure (18). Low exposure would lead to the formation of an ROS-activating antioxidant response followed by the transcription of genes that are significant to detoxification, cytoprotective, and antioxidant responses. These include genes encoding phase II enzymes whose induction serves as a detoxification mechanism (e.g., NAD(P)H:quinone oxidoreductase 1 [NQO1] and glutathione S-transferase [GST]). At higher exposure, the transcription factor NF- κ B and activator protein-1 (AP-1) responses would be activated. This would lead to NF- κ B and mitogen-activated protein kinase signaling, altering the function of mitochondria or NADPH, and to increased expression of proinflammatory cytokines (such as TNF- α and IL-8 and -6) and adhesion genes (15, 17). Enhanced inflammatory response would lead to additional generation of ROS and reactive nitrogen species, together with oxidative DNA damage (18).

EPIDEMIOLOGIC EVIDENCE OF GENE BY AIR POLLUTANT INTERACTION

Study Design

Most of the studies are association studies that evaluate the susceptibility to air pollution exposure, comparing health effects between groups of subjects with different genotypes. The number of different genes studied is relatively small and is focused on common polymorphisms with well-described functional effects in genes thought to be involved in oxidative stress or inflammatory responses.

Election of Candidate Genes and Polymorphisms

Possible candidate genes should ideally be involved in biological pathways related to responses to air pollution (e.g., symptoms, bronchoconstriction, airway inflammation) (6). Many studies have focused on single gene polymorphisms, but it is likely that a hierarchy of genes determines susceptibility rather than one gene driving this process (17).

To the extent that *oxidative stress* is an important pathway for adverse air pollution effects, polymorphisms in genes codifying enzymes involved in oxidative stress response are of primary interest. The following genes have been studied: *GST* (*GSTM1*, *GSTP1*), *CAT*, *SOD*, *GPX1*, *NQO1*, *HMOX1*, *EPHX1*. Figure 1 presents the major enzymatic antioxidant defense system, including enzymes that act against superoxide radicals (SOD), hydrogen peroxide (H_2O_2), and phase II xenobiotic metabolizing enzymes that participate in the detoxification of ROS by catalyzing their conjugation with glutathione (19, 20). Heme oxygenase-1 (HMOX1) enzyme, which can be induced by oxidants and cytokines, can act as a first line of defense against ROS (21). Nicotinamide adenine dinucleotide phosphate quinone (NQO1) plays a detoxifying role, catalyzing quinone to hydroquinone without generating semiquinone, limiting the redox cycling and oxidative stress (22). Other genes encoding enzymes that metabolize xenobiotics or are involved in nitrosative stress could also play a role in the pathogenesis of asthma (23). Epoxide hydrolase 1 (EPHX1) is involved in the detoxification of reactive epoxides from metabolically activated PAHS to generate trans-dihydrodiols metabolized to semiquinones which may produce ROS (24). Arginases encoded by *ARG1* and *ARG2(21)* may contribute to asthma pathogenesis through various mechanisms, including inhibition of nitric oxide (NO) generation, reduced arginine bioavailability, and increased ornithine production. This could lead to airway remodeling caused by altered polyamine and proline synthesis (25).

Figure 1. Oxidant generation by photo-oxidants and the major antioxidant defense systems. $\text{O}_2^{\cdot-}$, superoxide; H_2O_2 , hydrogen peroxide; $\cdot\text{OH}$, hydroxyl radical; NO, nitric oxide; NOS, nitric oxide synthase; ONOO-, peroxy-nitrite; RNS, reactive nitrogen species; SOD, superoxide dismutase; CuZnSOD, copper zinc SOD; MnSOD, manganese SOD; ECSOD, extracellular SOD; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GCL, glutamate cysteine ligase; GST, glutathione-S-transferase; GSSG, oxidized glutathione (glutathione disulfide); GS, glutathione synthase; γ GT, γ -glutamyltranspeptidase; MRP, multidrug resistance protein; TRX, thioredoxin; PRX, peroxiredoxin; GRX, glutaredoxin; CAT, catalase; HMOX1, heme oxygenase-1; NQO1, Nicotinamide adenine dinucleotide (phosphate) reduced:quinone oxidoreductase. Adapted with permission from Reference 49.

Genes identified through murine models of ozone exposure such as *TNF- α* , *Lymphotoxin- α* (*LTA*), and *TLR4* are also of interest (11). Experiments in mice showed that blocking the function of *TNF- α* reduced response to ozone, supporting the importance of this cytokine in mechanisms of injury (11). Polymorphisms in *TLR4* have been shown to affect lung permeability as well as functional response to ozone in mouse models (26, 27).

Finally, genes encoding inflammatory cytokines might also have a complex interaction with air pollutants.

Epidemiologic Studies

Human studies on the interaction between specific genetic polymorphisms and exposure to air pollutants are presented in Table 1. A literature search was conducted of articles accessible in PubMed in April 2009. Articles on genetic interactions with occupational exposure are not included; only studies addressing joint or interaction effects on genetic polymorphisms and air pollution were included. The single most commonly examined genetic polymorphism is a highly prevalent deletion polymorphism of the glutathione S-transferase M1 gene (*GSTM1*). Deletion of both copies of the *GSTM1* gene, also referred to as homozygous deletion or the null genotype, abolishes *GSTM1* activity (28). The frequency of the *GSTM1*-null genotype ranges from 25% to 60% depending on the ethnic group. The second most commonly studied gene in relation to ambient air pollution is glutathione S-transferase P1 (*GSTP1*). A functional polymorphism (*Ile105Val*) occurs at relatively high frequency (28).

We conducted a study in Mexico with children who are highly exposed to ozone, and observed an association between polymorphisms in the oxidative stress genes, *NQO1* and *GTSM1*, and the development of asthma (22). We observed that subjects with homozygous deletion of *GSTM1* who were also carriers of the serine allele for *NQO1* were at significantly lower risk of asthma compared with Pro/Pro homozygotes. While no formal test of interaction with ozone was done in this study, results suggest a protective effect of the *NQO1* Ser allele (with reduced enzymatic activity) in *GSTM1*-null children with high ozone exposure (22). In a clinical trial of antioxidant vitamins (vitamin C and vitamin E) in children with asthma in Mexico City, we showed that children with the *GSTM1*-null genotype were more susceptible to the effect of ozone exposure (change in lung function in repeated measurements over 3 months) unless protected by antioxidant supplementation with vitamins C and E. An increase in breathing difficulty was also seen in children with asthma oxidative stress risk genotypes (*GSTM1* and *GSTP1 Val/Val*) (29). We further evaluated the potential protective effect of antioxidant intake and its modulating effect on the interaction between *GSTM1* and ozone exposure in a larger sample. As in our first panel study, we observed that children with *GSTM1*-null genotype were more susceptible to ozone, including larger lung function decrements and lower pH in exhaled breath, than were their counterparts. Children with higher dietary intake had higher lung function at baseline. Susceptible children with high vitamin C intake had a lesser decline in lung function related to ozone than did children with low vitamin C intake (I. Romieu, personal communication).

Several analyses from the Children's Health Study (CHS) in Los Angeles, California have evaluated gene by air pollution interaction in relation to lifetime asthma and asthma incidence. Genetic polymorphism in *HMOX1* ("short allele") was associated with a reduced risk of new-onset asthma in non-Hispanic whites, and this protective effect was greater in children residing in low-ozone communities. In contrast, a variant of the *CAT-262 T* allele (CT or TT) in Hispanic children was associated with

an increased risk of asthma. These results emphasize the importance of evaluating the ethnic-specific effect of polymorphism in assessing gene-disease associations and the potential role of population admixture (30). In this cohort, the risk of new onset of asthma was associated with the number of sports played outdoors as a surrogate measure of ozone exposure, and a significant interaction between participation in team sports and *GSTP1 ile105Val* polymorphism was observed among children with *ile* homozygote. This effect was even stronger among those with *ile* homozygote residing in high-ozone communities, although no test of interaction could be conducted because of small numbers (24). In this same cohort, the authors observed a significant interaction between polymorphisms in the arginases encoding gene *ARG1*, a previously identified candidate gene for asthma (24), and ambient ozone and atopy (21).

Exposure to particles has also been studied using distance to high-traffic roads as a surrogate measure of vehicular exhaust. A significant interaction was observed between *EPHX1* and distance to traffic. High metabolic activity of *EPHX1* was associated with higher risk of lifetime asthma and late onset of asthma. This effect was greater among children with the *GSTP1 Val* homozygote genotype (24).

Polymorphism in the gene coding for proinflammatory cytokine *TNF- α* has been shown to interact with ozone exposure. In an experimental controlled study, subjects with *TNF-308G/G* genotype had a greater fall in FEV₁ associated with ozone exposure compared with subjects with *308G/A* or *A/A* genotypes (6). In the CHS, the *TNF-308G/G* genotype reduced the risk of wheezing, an effect that was greater in communities with low ozone concentrations. This protective effect was reduced in *GSTM1*-null and *GSTP1 Ile/Ile* genotypes (25). Recently, among children from a large Swedish birth cohort, interaction between several *GSTP1* SNPs and traffic NO_x exposure during the first year of life were observed for allergic sensitization to any allergen. Children with *Ile105Val* or *Val105Val* genotypes were at increased risk of sensitization when exposed to elevated levels of traffic NO_x. In children with *TNF-308 GA/AA* genotypes, the *GSTP1*-NO_x interaction effects were even more pronounced (31).

In summary, *GSTM1* appears to play an important role, although in some studies interaction is observed only in a subgroup. Regarding *GSTP1* and *TNF-308*, results are inconsistent with regard to polymorphism with higher susceptibility to ozone exposure.

Limitations of Existing Studies

One of the main limitations in the existing studies is the lack of power to detect significant interactions (32). Both genetic polymorphisms and ambient pollutants produce weak independent effects, and given the complexity in response pathways, strong interaction effects with any single gene are unlikely (33). In addition, it is important to consider the number of cases (of asthma or the relevant subphenotype) in any given study rather than the total sample size because it is the most important determinant of statistical power (32). Another issue is the problem of multiple comparisons, which is not accounted for in most studies. Publication bias is also a serious problem, given that negative studies are less likely to be published. Replication of positive findings remains an important issue in genetic association studies. Finally, in many studies there is a lack of consideration for population admixture (33). Polymorphisms alleles tend to vary between ethnic groups, and if disease is more frequent in one ethnic group, this might produce a "false" gene-disease association. In addition, adjustment for ethnicity is

TABLE 1. STUDIES OF INTERACTIONS BETWEEN SPECIFIC GENETIC POLYMORPHISMS AND AMBIENT AIR POLLUTANTS AMONG CHILDREN

First Author, Year	Study Type, Population	Outcome(s)	Case N, or Total N if No Cases*	Exposure Metric	% Exposed	Gene	Polymorphism	P for Interaction†	Interaction Findings
David 2003 (22)	Case triad Children, Mexico	Asthma	218	Lifetime residence in Mexico City	100	NQO1 GSTM1 GSTM1	Pro187Ser Deletion Deletion	Not given	↓ Asthma risk for NQO1 variant only among GSTM1 null
Romieu 2004 (29)	Randomized controlled trial of antioxidants	PFTs-PEF ₂₅₋₇₅	158 total	Ozone:50 ppb difference in 1 hour maximum	NA			P = 0.14	↑ Drop in FEF ₂₅₋₇₅ cozone in G STM1 null
Lee 2004 (45)	Children with asthma, Mexico	Repeated measures							Antioxidant supplements negate FEF ₂₅₋₇₅ drop Antioxidant benefit mostly in GSTM1 null
	Cross-sectional	Asthma	61	Outdoor air pollution (low, medium, high)	70	GSTP1	Ile105Val	P < 0.035	Ile/Ile → ↑ wheeze, but not in low pollution areas
Li 2006 (46)	School children, Taiwan	Ever asthma	403	Ozone annual average of 1 hr averages > 50 ppb	59	TNF	TNF-308	P < 0.04	↓ Wheeze with TNF-308G/G only in low ozone
	Children	Ever wheezing	886			GSTM1	Deletion	No 3-way	↓ Wheeze with TNF-308G/G in low ozone only with GSTM1 null
	Multiethnic	Current wheezing	493			GSTP1	Ile105Val		↓ Wheeze with TNF-308G/G in low ozone only with GSTP1Ile/Ile
		Meds for wheezing	296						No ozone interaction with GSTM1 or GSTP1 alone
Romieu 2006 (34)	Panel study	Breathing difficulties	151 total	Ozone 20 ppb difference in 1 hour maximum	NR	GSTM1	Deletion	P < 0.05	↑ Ozone effects in GSTM1 null/GSTP1Val/Val
	Children with asthma, Mexico	Bronchodilator use				GSTP1	Ile105Val		
Salam 2007 (24)	Cohort, California, United States	Lifetime asthma	422	Close (< 75 m) to major road	23	EPHX1	Tyr113His	P < 0.05	↑ OR for EPHX high activity greater in exposed
	Children	Late-onset asthma	276			EPHX1	His139Arg	P < 0.05	↑ OR for EPHX high activity+P1Val/Val in exposed
	Multiethnic	Early persistent asthma	217			GSTP1	Ile105Val		P < 0.05 for some outcomes
		Current asthma	308			GSTM1	deletion		
						GSTT1	deletion		
Islam 2008 (30)	Cohort, California, United States	New-onset asthma	103	Long-term ozone > median Also examined PM ₁₀ , PM _{2.5} and NO ₂	50	HMOX1	[G]n repeat	P < 0.003	OR for HMOX1 s-allele stronger in low ozone
	Children					CAT	–262C>T		
	Whites (non-Hispanic)					CAT	–844C>T		
						MNSOD	Ala-9Val		
Chen 2007 (47)	Cohort, California, United States	PFTs:	210 total	Ozone	100	GSTM1	Deletion	P < 0.05	Ozone related ↓ in FEF ₂₅₋₇₅ ↑ in GSTM1null/NQO1Pro in females
	College students	FEV ₁ , FVC, FEF ₂₅₋₇₅		(lifetime in high ozone areas of California)		NQO1	Pro187Ser	P < 0.15	Ozone-related ↓ in FEF ₂₅₋₇₅ ↑ in GSTP1Val in males
Hong 2007 (48)	No respiratory illness Panel	PFTs: PEF	43 total	PM ₁₀ , PM _{2.5}	100	GSTP1 GSTM1	Ile105Val Deletion	Not given	No effect of GSTM1 null or GSTT1 null on PEF
	Children Korea			Pb, Mn in PM		GSTT1	Deletion		
Salam 2007 (23)	Cohort, California, United States	Life time asthma	330	Distance to freeway <500m	1.5	TGF-β1	C-509T TT	Not given	Higher risk of asthma in children living closer to freeway

(continued)

TABLE 1. (CONTINUED)

First Author, Year	Study Type, Population	Outcome(s)	Case N, or Total N if No Cases*	Exposure Metric	% Exposed	Gene	Polymorphism	P for Interaction†	Interaction Findings
Islam 2008 (30)	Children Cohort, California, United States	Early persistent asthma New asthma onset	141 150	Distance to freeway <500m Ozone Number of sport team participation	1.5 58	TGF-β1 GSTP1	C-509T TT Ile105Val	Not given	Number of sports in high ozone communities ↑ new onset of asthma in GSTP1 Ile/Ile
Salam 2009 (21)	Children Cohort, California, United States	Lifetime asthma	450	Ozone level in communities (low and high) PM ₁₀	52	GSTM1 ARG1 ARG2	Deletion ARG1h4 haplotype	P < 0.05	Protective effect on asthma with larger effect in high ozone communities. Higher protective effect in atopic children
Melen 2008 (31)	Children Birth cohort Sweden	Sensitization Asthma Persistent wheezing	266 136 211	NO ₂ during 1 year of life (difference between 5th and 95th = 44 µg/m ³)	100	ADRB2 TNF GSTP1	Glu27Gln TNF-308 Ile105Val Ala114Val	P > 0.001	Higher risk of sensitization with higher NO ₂ in GSTP1 105 Ile/Val and Val/Val and 114 Ala/Val and Val/Val. Interaction (↑ sensitization) between GSTP1 105 Ile/Val and Val/Val and TNF-308 GA/AA

Definition of abbreviations: EBC = exhaled breath condensate; FEF = forced expiratory flow; FEF₂₅₋₇₅ = forced midexpiratory flow rate or the average expiratory flow over the middle half of forced vital capacity; IgE = immunoglobulin E; IL = interleukin; LTB-4 = leukotriene B-4; MEF = maximal expiratory flow; Mn = manganese; NA = not applicable (for this type of study); NS = not statistically significant; PFTs = pulmonary function tests; PM_{2.5} = particulate matter < 2.5 µm; PM₁₀ = particulate matter < 10 µm; Pb = lead; PMN-ROS = reactive oxygen species generation from neutrophils (PMNs); TBARS = thiobarbituric reactive substances.

* Where information was available, only subjects with genotype and exposure data are included in the case or total N. Where the study examined a case group, the number of that case type is N. For studies of continuous outcomes the total N is given. Where additional case groups were analyzed and too numerous to list, numbers are given for case groups where interactions were reported by the authors.

† NS indicates that the P value for interaction was given but did not reach statistical significance at a level of 0.05.

We present the relevant number of cases whenever a dichotomous outcome has been studied for interaction rather than the total sample size. If the interaction was found only in one subgroup, we present only the number of cases in this subgroup. Some portions of this table have been reprinted with permission from reference 32.

not sufficient because exposure dose to pollutants is also likely to vary between ethnic groups, and therefore specific test of effect modification by ethnicity should be evaluated (32).

IMPLICATION OF GENETIC STUDIES OF RESPONSE TO AIR POLLUTION AND SUGGESTION FOR FUTURE STUDIES

Understanding genetic variation in response to air pollution would help us understand underlying biology, variability, and pathogenesis and identify at-risk individuals, potential preventive measures, and treatment (6). However, the power limitation in detecting interaction between air pollution and genetic polymorphism in the existing literature impairs a clear interpretation of the findings. To improve power, future studies could improve either exposure assessment or asthma phenotypes, or both. For example, there is a large indoor/outdoor ratio in O₃ concentrations and these can be heterogeneous across homes; personal O₃ exposure may therefore be poorly estimated using outdoor ambient levels. Particles might have different effects based on their composition, and it might be useful to measure the redox activity of particles such as OH radical formation and antioxidant depletion rates. Repeated exposure assessment overtime in long-term studies could improve precision. Asthma is a heterogeneous disease, which makes it difficult to identify the specific gene by environment interaction. Homogenous phenotypes could be defined, including repeated measurements of lung function and markers of atopy and inflammatory response (such as IL-8, exhaled NO). We took this approach in a study of genetic modification of acute ozone effect (34).

Population admixture needs to be taken into account. Spurious associations may be the result of differences in population genetic structure. A variety of statistical methods have been developed to control for type I errors in case-control studies (35), as in family-based designs (36). Most of these methods are applicable to cross-sectional studies, but there are some contributions related to longitudinal data analysis (37).

Finally, the consideration of gene by gene interactions, or epistasis, is also important given that a complex gene interaction is likely to explain variation in phenotypes. This adds a level of complexity and the need for still larger sample sizes. A large consortia of studies are being formed to examine gene by environment interaction and to test for higher-order interaction, including other susceptibility factors such as diet. Genome-wide association (GWA) data will enable the study of these interactions.

Published studies have focused on interactions between environmental exposure and DNA sequence variation. Environmental exposure may also interact with genetic predisposition via epigenetic mechanisms. Epigenetics refers to the study of processes that alter gene activity without changing the DNA sequence, and epigenetic changes to DNA can be inherited (38). One mechanism is DNA methylation, the covalent addition of a methyl group to a cytosine residue in a CpG site (i.e., where a cytosine lies next to a guanine in the DNA sequence). The methylation states of CpG sites may affect gene activity and expression (39). While epigenetic alterations are believed to predominantly occur prenatally and shortly after birth, recent evidence suggests that they can occur during later periods, influencing gene expression in different ways throughout the lifespan, and that epigenetic drift is associated with aging (40). In addition, loss of genomic imprinting such as DNA methylation appears to be heritable during cell division (41). While DNA sequence remains essentially the same, the epigenetic state varies among tissues and during a lifetime, and epigenetic mechanisms can remember these changes in the normal pro-

gramming and reprogramming of gene activity (38). A recent animal study has shown that exposure to diesel exhaust induces hypermethylation of IFN- γ promoter and hypomethylation of IL-4 in CD4⁺ T cells among mice sensitized to the fungus allergen *Aspergillus fumigatus*. Altered methylation of promoters of both genes was correlated significantly with changes in IgE levels (42). Two studies have recently linked ambient exposure to air pollution to methylation in human adults. In a study of 78 gas station attendants, 77 traffic police officers, and 58 office workers in Milan, Italy, ambient benzene concentrations were associated with reductions in global methylation (43). In the Boston area Normative Aging Study, DNA methylation of the long interspersed nucleotide element (LINE-1) and Alu repetitive elements was determined in 1,097 blood samples from 718 elderly participants. Repetitive element DNA methylation of LINE-1 decreased after recent exposure to black carbon and PM_{2.5} traffic particles. While there is no clear understanding of the potential health impact of such changes in methylation, this work suggests that epigenetic mechanisms may contribute to biologic effects of air pollutants (44).

CONCLUSIONS

The majority of human genetic association studies of air pollutants have examined the effect of ozone exposure, and only two reports have explored the effect of traffic exposure. The greatest effect has been on lung function, with some studies looking at the lifetime risk of asthma and asthma incidence. Polymorphisms in oxidative stress genes (*GSTM1*, *GSTP1*, *NQO1*) have been the most studied, but the inconsistency of results is a limitation to drawing firm conclusions. The limited sample size of the study does not allow for the study of multiple genes and high-order interactions. The traditional view that inter-individual risk for asthma, as for other complex diseases, is determined solely by interactions between genetic polymorphisms and environmental exposures needs to be reconciled with new findings suggesting that epigenetic mechanisms may also contribute to modifying the effect of deleterious genes or be influenced by the environment (39).

Longitudinal birth cohort studies enrolling pregnant women with repeated measurements of environmental exposure, collection of biological samples over time, and comprehensive clinical outcome assessments have great potential. They would help determine special environmental triggers and critical windows of susceptibility in the development of atopy and asthma, including the role of gene by environment interaction and epigenetics. Consortia of such studies are under way and should help understand the role of the relationship between the genome and the environment and may provide new clues to modifying these effects for disease prevention and therapy (38). While genetic and dietary susceptibility are clearly important and more studies are needed to identify specific interactions, high priority should be given to reducing air pollution exposure in general.

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References

1. Brunekreef B, Holgate ST. Air pollution and health. *Lancet* 2002;360:1233–1242.
2. Rojas-Martinez R, Perez-Padilla R, Olaiz-Fernandez G, Mendoza-Alvarado L, Moreno-Macias H, Fortoul T, McDonnell W, Loomis D, Romieu I. Lung function growth in children with long-term exposure to air pollutants in Mexico City. *Am J Respir Crit Care Med* 2007;176:377–384.
3. Mudway IS, Kelly FJ. Ozone and the lung: a sensitive issue. *Mol Aspects Med* 2000;21:1–48.
4. Nel A. Atmosphere. Air pollution-related illness: effects of particles. *Science* 2005;308:804–806.
5. Bowler RP, Crapo JD. Oxidative stress in allergic respiratory diseases. *J Allergy Clin Immunol* 2002;110:349–356.
6. Yang IA, Fong KM, Zimmerman PV, Holgate ST, Holloway JW. Genetic susceptibility to the respiratory effects of air pollution. *Thorax* 2008;63:555–563.
7. Todd JA. Statistical false positive or true disease pathway? *Nat Genet* 2006;38:731–733.
8. Postma DS, Weiss ST. Genetics of asthma and chronic obstructive pulmonary disease. New York: Informa Healthcare; 2007.
9. McDonnell WF. Intersubject variability in human acute ozone responsiveness. *Pharmacogenetics* 1991;1:110–113.
10. Aris RM, Christian D, Hearne PQ, Kerr K, Finkbeiner WE, Balmes JR. Ozone-induced airway inflammation in human subjects as determined by airway lavage and biopsy. *Am Rev Respir Dis* 1993;148:1363–1372.
11. Kleeberger SR, Levitt RC, Zhang LY, Longphre M, Harkema J, Jedlicka A, Eleff SM, DiSilvestre D, Holroyd KJ. Linkage analysis of susceptibility to ozone-induced lung inflammation in inbred mice. *Nat Genet* 1997;17:475–478.
12. Ohtsuka Y, Brunson KJ, Jedlicka AE, Mitzner W, Clarke RW, Zhang LY, Eleff SM, Kleeberger SR. Genetic linkage analysis of susceptibility to particle exposure in mice. *Am J Respir Cell Mol Biol* 2000;22:574–581.
13. Persinger RL, Poynter ME, Ckless K, Janssen-Heininger YM. Molecular mechanisms of nitrogen dioxide induced epithelial injury in the lung. *Mol Cell Biochem* 2002;234–235:71–80.
14. Janssen-Heininger YMW, Persinger RL, Korn SH, Pantano C, McElhinney B, Reynaert NL, Langen RCJ, Ckless K, Shrivastava P, Poynter ME. Reactive nitrogen species and cell signaling: implications for death or survival of lung epithelium. *Am J Respir Crit Care Med* 2002;166:S9–S16.
15. Shukla A, Timblin C, Berube K, Gordon T, McKinney W, Driscoll K, Vacek P, Mossman BT. Inhaled particulate matter causes expression of nuclear factor (NF)- κ B-related genes and oxidant-dependent NF- κ B activation *in vitro*. *Am J Respir Cell Mol Biol* 2000;23:182–187.
16. Gonzalez-Flecha B. Oxidant mechanisms in response to ambient air particles. *Mol Aspects Med* 2004;25:169–182.
17. Saxon A, Diaz-Sanchez D. Air pollution and allergy: you are what you breathe. *Nat Immunol* 2005;6:223–226.
18. Xiao GG, Wang M, Li N, Loo JA, Nel AE. Use of proteomics to demonstrate a hierarchical oxidative stress response to diesel exhaust particle chemicals in a macrophage cell line. *J Biol Chem* 2003;278:50781–50790.
19. Hayes JD, Strange RC. Glutathione S-transferase polymorphism and their biological consequences. *Pharmacology* 2000;61:154–166.
20. Rushmore TH, Pickette CB. Glutathione S-transferase, structure, regulation, and therapeutic implications. *J Biol Chem* 1993;268:11475–11478.
21. Salam MT, Islam T, Gauderman WJ, Gilliland FD. Roles of arginase variants, atopy, and ozone in childhood asthma. *J Allergy Clin Immunol* 2009;123:596–602.
22. David GL, Romieu I, Sienra-Monge JJ, Collins WJ, Ramirez-Aguilar M, del Rio-Navarro BE, Reyes-Ruiz NI, Morris RW, Marzec JM, London SJ. Nicotinamide adenine dinucleotide (phosphate) reduced: quinone oxidoreductase and glutathione S-transferase m1 polymorphisms and childhood asthma. *Am J Respir Crit Care Med* 2003;168:1199–1204.
23. Salam MT, Gauderman WJ, McConnell R, Lin PC, Gilliland FD. Transforming growth factor- β 1 C-509T polymorphism, oxidant stress, and early-onset childhood asthma. *Am J Respir Crit Care Med* 2007;176:1192–1199.
24. Salam MT, Lin PC, Avol EL, Gauderman WJ, Gilliland FD. Microsomal epoxide hydrolase, glutathione S-transferase p1, traffic and childhood asthma. *Thorax* 2007;62:1050–1057.
25. Li H, Romieu I, Sienra-Monge JJ, Ramirez-Aguilar M, Del Rio-Navarro BE, Kistner EO, Gjessing HK, Lara-Sanchez C, Chiu GY, London SJ. Genetic polymorphisms in arginase i and ii and childhood asthma and atopy. *J Allergy Clin Immunol* 2006;117:119–126.
26. Hollingsworth JW, Kleeberger SR, Foster WM. Ozone and pulmonary innate immunity. *Proc Am Thorac Soc* 2007;4:240–246.
27. Kleeberger SR. Genetic aspects of susceptibility to air pollution. *Eur Respir J* 2003;40:52S–56S.
28. Strange RC, Spiteri MA, Ramachandran S, Fryer AA. Glutathione-S-transferase family of enzymes. *Mutat Res* 2001;482:21–26.

29. Romieu I, Sienra-Monge JJ, Ramírez-Aguilar M, Moreno-Macías H, Reyes-Ruiz NI, Río-Navarro BE, Hernández-Avila M, London SJ. Genetic polymorphism of *gstm1* and antioxidant supplementation influence lung function in relation to ozone exposure in asthmatic children in Mexico City. *Thorax* 2004;59:8–10.
30. Islam T, McConnell R, Gauderman WJ, Avol E, Peters JM, Gilliland FD. Ozone, oxidant defense genes, and risk of asthma during adolescence. *Am J Respir Crit Care Med* 2008;177:388–395.
31. Melén E, Nyberg F, Lindgren CM, Berglind N, Zucchelli M, Nordling E, Hallberg J, Svartengren M, Morgenstern R, Kere J, *et al.* Interactions between glutathione S-transferase p1, tumor necrosis factor, and traffic-related air pollution for development of childhood allergic disease. *Environ Health Perspect* 2008;116:1077–1084.
32. London SJ, Romieu I. Gene by environment interaction in asthma. *Annu Rev Public Health* 2009;30:55–80.
33. Cardon LR, Bell JI. Association study designs for complex diseases. *Nat Rev Genet* 2001;2:91–99.
34. Romieu I, Ramírez-Aguilar M, Sienra-Monge JJ, Moreno-Macias H, del Río-Navarro BE, David G, Marzec J, Hernandez-Avila M, London S. *Gstm1* and *gstp1* and respiratory health in asthmatic children exposed to ozone. *Eur Respir J* 2006;28:953–959.
35. Tian C, Gregersen PK, Seldin MF. Accounting for ancestry: population substructure and genome-wide association studies. *Hum Mol Genet* 2008;17:R143–R150.
36. Rabinowitz D, Laird N. A unified approach to adjusting association tests for population admixture with arbitrary pedigree structure and arbitrary missing marker information. *Hum Hered* 2000;50:211–223.
37. Gauderman WJ, Macgregor S, Briollais L, Scurrah K, Tobin M, Park T, Wang D, Rao S, John S, Bull S. Longitudinal data analysis in pedigree studies. *Genet Epidemiol* 2003;27:S18–S58.
38. Feinberg AP. Epigenetics at the epicenter of modern medicine. *JAMA* 2008;299:1345–1350.
39. Miller RL, Ho SM. Environmental epigenetics and asthma: current concepts and call for studies. *Am J Respir Crit Care Med* 2008;177:567–573.
40. Martin GM. Epigenetic drift in ageing identical twins. *Proc Natl Acad Sci USA* 2005;102:10413–10414.
41. Cruz-Correa M, Zhao R, Oviedo M, Bernabe RD, Lacourt M, Cardona A, Lopez-Enriquez R, Wexner S, Cuffari C, Hyland L, *et al.* Temporal stability and age-related prevalence of loss of imprinting of the insulin-like growth factor-2 gene. *Epigenetics* 2009;4:114–118.
42. Liu J, Ballaney M, Al-alem U, Quan C, Jin X, Perera F, Chen LC, Miller RL. Combined inhaled diesel exhaust particles and allergen exposure alter methylation of T helper genes and IgE production *in vivo*. *Toxicol Sci* 2008;102:76–81.
43. Bollati V, Baccarelli A, Hou L, Bonzini M, Fustinoni S, Cavallo D, Byun HM, Jiang J, Marinelli B, Pesatori AC, *et al.* Changes in DNA methylation patterns in subjects exposed to low-dose benzene. *Cancer Res* 2007;67:876–880.
44. Baccarelli A, Wright RO, Bollati V, Tarantini L, Litonjua AA, Suh HH, Zanobetti A, Sparrow D, Vokonas PS, Schwartz J. Rapid DNA methylation changes after exposure to traffic particles. *Am J Respir Crit Care Med* 2009;179:572–578.
45. Lee YL, Lin YC, Lee YC, Wang JY, Hsiue TR, Guo YL. Glutathione S-transferase p1 gene polymorphism and air pollution as interactive risk factors for childhood asthma. *Clin Exp Allergy* 2004;34:1707–1713.
46. Li YF, Gauderman WJ, Avol E, Dubeau L, Gilliland FD. Associations of tumor necrosis factor g-308a with childhood asthma and wheezing. *Am J Respir Crit Care Med* 2006;173:970–976.
47. Chen C, Arjomandi M, Tager IB, Holland N, Balmes JR. Effects of antioxidant enzyme polymorphisms on ozone-induced lung function changes. *Eur Respir J* 2007;30:677–683.
48. Hong YC, Hwang SS, Kim JH, Lee KH, Lee HJ, Lee KH, Yu SD, Kim DS. Metals in particulate pollutants affect peak expiratory flow of schoolchildren. *Environ Health Perspect* 2007;115:430–434.
49. Kinnula VL. Focus on antioxidant enzymes and antioxidant strategies in smoking related airway disease. *Thorax* 2005;60:693–700.