Human Genome Epidemiology (HuGE) Review

Associations Between Inflammatory and Immune Response Genes and Adverse Respiratory Outcomes Following Exposure to Outdoor Air Pollution: A HuGE Systematic Review

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Variants of inflammatory and immune response genes have been associated with adverse respiratory outcomes following exposure to air pollution. However, the genes involved and their associations are not well characterized, and there has been no systematic review. Thus, we conducted a review following the guidelines of the Human Genome Epidemiology Network. Six observational studies and 2 intervention studies with 14,903 participants were included (2001–2010). Six studies showed at least 1 significant gene-pollutant interaction. Meta-analysis was not possible due to variations in genes, pollutants, exposure estimates, and reported outcomes. The most commonly studied genes were tumor necrosis factor α (TNFA) (n = 6) and toll-like receptor 4 (TLR4) (n = 3). TNFA –308G>A modified the action of ozone and nitrogen dioxide on lung function, asthma risk, and symptoms; however, the direction of association varied between studies. The TLR4 single-nucleotide polymorphisms rs1927911, rs10759931, and rs6478317 modified the association of particulate matter and nitrogen dioxide with asthma. The transforming growth factor β1 (TGFB1) polymorphism –509C>T also modified the association of pollutants with asthma. This review indicates that genes controlling innate immune recognition of foreign material (TLR4) and the subsequent inflammatory response (TGFB1, TLR4) modify the associations of exposure to air pollution with respiratory function. The associations observed have biological plausibility; however, larger studies with improved reporting are needed to confirm these findings.

Air pollution is of major and increasing concern for public health, reflecting increased industrialization, energy use, and road traffic volumes (1). Numerous adverse health outcomes have been attributed to air pollution, resulting from both long- and short-term exposure—particularly cardiovascular and respiratory problems (2, 3). Several studies have shown an association between air pollution and respiratory indicators, including diagnosis of asthma, wheezing, and measures of pulmonary function in both adults and children (4–8). Despite these general trends, there are interindividual variations in the pulmonary response to air pollution. Such variations

Abbreviations: HuGE, Human Genome Epidemiology; HuGENet, Human Genome Epidemiology Network; MeSH, Medical Subject Headings; PM, particulate matter; SNP, single-nucleotide polymorphism; STREGA, Strengthening the Reporting of Genetic Association Studies; TGF, transforming growth factor; TLR, toll-like receptor; TNF-α, tumor necrosis factor α; TNFR, tumor necrosis factor receptor.

Editor’s note: This article also appears on the website of the Human Genome Epidemiology Network (www.cdc.gov/genomics/hugenet/default.htm).
point to the presence of further factors which influence this interaction (9, 10), one of which may be genetic predisposition to lung injury resulting from exposure to airborne substances (11). This systematic review focuses specifically on the interaction between outdoor air pollutants and the immune system, examining specifically genetic variants that may modify the inflammatory and immune responses.

Respiratory outcome of exposure to air pollution

Studies examining associations between air pollution and respiratory outcomes have used a number of different individual pollutants as indicators of general air pollution levels. Some of the most common pollutants studied are ozone, sulfur dioxide, nitrogen oxides, and particulate matter (PM). These substances have individual as well as shared effects on pulmonary function.

Ozone is formed from the reaction between sunlight and vehicle exhaust products, such as nitrogen oxides, volatile organic compounds, carbon monoxide, and hydrocarbons. Levels of inflammatory mediators, such as interleukins (e.g., interleukin 8), increase within the airways following controlled exposure to ozone in laboratory conditions (12). Furthermore, significant release of tumor necrosis factor α (TNF-α) and interleukins (interleukins 8 and 6), as well as other inflammatory mediators, has been observed in human bronchial epithelial cells when they are exposed to ozone in vitro (13, 14). Ozone can also lead to generation of free radicals and can cause depletion of protective antioxidants such as glutathione, thus contributing to inflammation in the lungs (15, 16). The role of genes governing the antioxidant/xenobiotic response to air pollution was studied in a previous Human Genome Epidemiology (HuGE) review (17).

PM is a mixture of solid and liquid particles in suspension and is classified by its diameter; most frequently studied are particles less than 10 μm in aerodynamic diameter (PM10) and fine particles less than 2.5 μm in aerodynamic diameter (PM2.5). Documented effects of PM on the lungs include the increased presence of inflammatory and immune cells and inflammatory mediators, such as neutrophils, mast cells, and interleukins (18, 19). It has also been suggested that pro-inflammatory cytokine release can be mediated by toll-like receptors (TLRs) on alveolar macrophage cells, implicating and intertwining the inflammatory and innate immune responses (20).

Nitrogen dioxide represents a significant proportion of primary emissions from motor vehicles, and increasingly there is strong evidence linking it to exacerbations of respiratory and allergic symptoms, as well as premature mortality, in exposed populations (21). Because nitrogen dioxide is usually measured in combination with other particles, the extent to which the health associations are directly attributable to nitrogen dioxide itself is unclear (22). Exposure to high levels of nitrogen dioxide has been linked to epithelial cell death in the lungs (23); however, the mechanism underlying this association is not clear. Sulfur dioxide is mainly formed as a result of fossil fuel combustion—the burning of gas and coal (11, 17). Exposure to sulfur dioxide worsens asthma symptoms and reduces lung function (9). The mechanisms of action are largely unknown, but sulfur dioxide causes rapid bronchoconstriction and is thought to trigger lung inflammation by activating proinflammatory molecules (cytokines and chemokines) in lung tissues.

Innate immunity and inflammation

Exposure to pollutants induces oxidative stress (24) and activates inflammatory pathways. It is thought that TLRs, interleukins, and tumor necrosis factor are key molecules involved in recognition of air pollutants and the subsequent inflammatory response (25). These factors are all involved in the functioning of the innate immune system, which acts to detect and eliminate pathogens. TLRs also play a major role in initiating airway inflammation in response to pollution in animal studies (26, 27). TLR2 and TLR4 are both found on alveolar macrophages, while TLR2 is also found on bronchial epithelial cells. It is possible that air pollutants can act as vectors for the introduction of bacterial wall peptides, such as lipopolysaccharide, into the lungs, thereby initiating an inflammatory response in the respiratory tree (28).

The stimulation of TLRs is one mechanism which triggers the activity of TNF-α, an inflammatory cytokine that exerts its effects by binding to transmembrane receptors—the tumor necrosis factor receptors (TNFR) TNFR1, TNFR (p55TNFR), and TNFR2 (p75TNFR) (27). Signalling of TNFR1 leads to apoptosis and activation of the nuclear factor κB pathway, whereas signalling through TNFR2 mediates cell proliferation and migration (28–30).

Transforming growth factors (TGFs) are a group of regulatory peptides, of which transforming growth factor β1 (TGF-β1) is a subtype. TGF-β1 has both proinflammatory and antiinflammatory properties and exerts its effects by regulating cell proliferation, cell division, and apoptosis. TGF-β1 promotes differentiation of T lymphocytes, promoting release of inflammatory cytokines, particularly T helper 17, and hence increasing the inflammatory response (31).

The immune and inflammatory responses to air pollutants are under a degree of genetic regulation, with the TLR (TLR), TNF-α (TNFA), and TGF-β1 (TGFBI) genes being the most commonly studied. TNFA is located on chromosome 6 (32), TLR4 is found on chromosome 9 (33), and TGFBI is on chromosome 16 (31). Genetic polymorphisms could potentially influence the associations of air pollutants with lung function by altering the protein structure of receptors and mediators in the innate immune response and inflammatory pathways or by affecting the level of gene expression (34). It is therefore plausible that gene variants may modify the associations of air pollution with respiratory outcomes.

In this review, we aimed to identify all studies on associations between innate immune and immune response genes and respiratory outcomes of exposure to outdoor air pollutants, and to present an overview of the genetic associations.

MATERIALS AND METHODS

Inclusion criteria

The inclusion criteria were broad, encompassing all study designs and examining any gene, pollutant, or respiratory outcome (Appendix 1).

Search strategy

We searched 4 online databases from their inception to January 2013 (Medline, 1950–2013; Embase, 1980–2013; The Cochrane Library, 1898–2013; and Web of Science, 1970–2013). Two reviewers (S.V. and R.M.) carried out a preliminary scoping search to gain an understanding of search terms to include and the literature available. From this preliminary search, the National Library of Medicine’s Medical Subject Headings (MeSH) and keywords were used to develop specific searches for each database.

The search strategy, developed with the assistance of an academic librarian (P.F.), reflected the main components of the inclusion criteria. Each component’s main term and any related or synonymous terms were searched using both free text and indexing (MeSH) terms, as follows:

- **Genes or synonyms:** allele, polymorphism, genetic, tumor necrosis factor, toll-like receptor, nuclear factor κB, interleukin.
- **Air pollution or synonyms:** air pollutant, ozone, carbon monoxide, nitric oxide, sulfur dioxide, particulate matter.
- **Lung function or synonyms:** lung function, forced expiratory volume/flow rate, peak expiratory flow, chronic obstructive pulmonary disease, asthma, wheezing, breathing, respiratory, lung disease.
- **Inflammation or synonyms:** inflammatory response.
- **Immunity or synonyms:** immune response.

Bibliographies of all included studies and recent reviews (11, 25, 35, 36) on this topic were hand-searched. The search strategy was limited to the English language (Appendix 2).

Study selection

Study selection was carried out by 2 reviewers (S.V. and R.M.), with each reviewer independently screening half the total number of titles and abstracts identified. Any article which met all 4 inclusion criteria (detailed in Appendix 1) was included in the review. A total of 150 titles and abstracts were cross-checked by both reviewers, and there was a high level of agreement (κ = 0.82).

Data extraction

A data extraction form based on the Strengthening the Reporting of Genetic Association Studies (STREGA) (37) and Human Genome Epidemiology Network (HuGENet) (38) guidelines was developed, piloted, and subsequently used by 2 reviewers (S.V. and R.M.) independently to extract relevant data from included studies. The form consisted of 5 sections. Section 1 gave details on the study, including its title, author, country, aims, and genes, and the single-nucleotide polymorphism (SNP) being studied. Section 2 gathered information regarding the methods and participants of each study, such as the study design and setting, mean age, ethnicity, and underlying health conditions of participants, also detailing the total number of participants and how they were selected. Section 3 ascertained the variables measured in the study, including the pollutants investigated and the outcomes used. Section 4 collected information with which to assess bias, including the source of the DNA and the method used for genotyping, together with confounding factors and population stratification. Section 5 extracted information on the outcomes of the study. Reported Hardy-Weinberg equilibrium P values were also sought and were recalculated by the reviewers from original data where possible.

Methodological quality assessment

Categories were used to assess quality in accordance with STREGA (37) and HuGENet (38) guidelines. These categories included study size and design, selection of participants, genotyping methods, and Hardy-Weinberg equilibrium. Scores between 0 and 2 were assigned for each category, giving each study a total score out of 20.

The review was additionally registered with the International Prospective Register of Systematic Reviews.

RESULTS

An overview of the process of identifying and screening articles is given in Figure 1. Eight papers (39–46) published between 2001 and 2010 were included in the review, containing reports on 6 different studies. Three of the papers used the same study population, reporting on different pollutants and outcomes (41, 42, 44).

Characteristics of included studies

Of the 8 studies included, 2 were intervention studies (45, 46) and the rest were observational. The intervention studies explored lung function following controlled short-term exposure to a specific pollutant, whereas the observational studies evaluated respiratory outcomes following long-term exposure to pollutants. Five different air pollutants were investigated, and a wide variety of outcomes were assessed. Three studies (41, 42, 46) examined ozone, 3 examined nitrogen oxides (39, 40, 43), and 1 article did not specify the type of air pollution studied (44). TNFA was the main gene of interest, with 6 studies (39, 41–43, 45, 46) evaluating the associations between TNFA polymorphisms and lung function outcomes. The most common individual SNP studied was the −308 SNP of TNFA (rs1800629).

Study size was variable, with the smallest study having only 51 participants (46) and the largest having 3,699 participants (42). The 2 intervention studies were particularly small, whereas all of the observational studies had over 900 participants. All studies were carried out in either Europe or the United States and included both adults and children. Table 1 shows the detailed characteristics of all included studies.

Because of the heterogeneity between the studies in terms of pollutants, genes, and outcomes explored, a meta-analysis was not possible.

Evaluation of study quality

An overview of study quality, with the scoring system used, is presented in Web Table 1 (available at http://aje.oxfordjournals.org/). Methodological quality varied greatly.
with the highest quality score (42, 44) being 18 out of 20 and the poorest-quality study (45) achieving a score of only 4 out of 20. All of the observational studies used appropriate random selection methods to recruit participants. The authors of the 2 intervention studies did not report their recruitment methods adequately. In all studies, DNA was obtained from either blood or buccal cells, and all studies used appropriate methods of genotyping, such as polymerase chain reaction. The proportion of subjects successfully genotyped was adequate in about half of the papers; in 2 studies (43, 45), the genotyping success rate did not meet the specified threshold. Confounding factors were well accounted for, with only 1 article (45) not mentioning the presence of confounding factors.

The difference between numbers of eligible and recruited participants was not adequately reported in most papers. Where it was addressed, there were differences in numbers of eligible and recruited participants, and this difference did not meet the specified threshold. Confounding factors were well accounted for, with only 1 article (45) not mentioning the presence of confounding factors.

Where Hardy-Weinberg equilibrium was assessed, 5 articles (39, 41–43, 46) stated that the alleles were in Hardy-Weinberg equilibrium; however, values were not given. Only 1 study (44) gave values, and when cross-checked by the reviewers, these values were found to be correct. Three studies (39, 40, 43) did not provide sufficient information for the reviewers to calculate Hardy-Weinberg equilibrium. The full details of quality assessment can be found in Web Table 1.

Gene-pollution interactions

In 6 studies (39, 40, 42–44, 46), at least 1 significant gene-pollutant interaction was reported. Table 2 provides details on all of the gene-pollutant interactions for which information was obtained. Of the 2 studies reporting on SNPs within the TLR4 gene (39, 40), only 2 SNPs (rs10759931, rs1927911) were found to interact with air pollution. Both SNPs were identified in the same study (n = 916), which explored the association between long-term exposure to PM and nitrogen dioxide (40).

Four studies on TNFA (39, 42, 43, 46) found some evidence of interaction between 2 SNPs and pollutants. The
Table 1. Characteristics of Studies Included in a Systematic Review of Associations Between Inflammatory and Immune Response Genes and Adverse Respiratory Outcomes Following Exposure to Outdoor Air Pollution, 2001–2010

<table>
<thead>
<tr>
<th>First Author, Year</th>
<th>No. of Participants</th>
<th>Study Design</th>
<th>Study Name</th>
<th>Population Type</th>
<th>Setting</th>
<th>Location</th>
<th>Outcome</th>
<th>Outcome Measure</th>
<th>Pollutant</th>
<th>Measurement of Pollutant Exposure</th>
<th>Gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kerkhof, 2010 (40)</td>
<td>916</td>
<td>Cohort</td>
<td>PIAMA</td>
<td>Children</td>
<td>Prenatal clinics</td>
<td>The Netherlands</td>
<td>1) Physician-diagnosed asthma 1) Physician-diagnosed asthma—physician-diagnosed asthma that was present during the last 12 months Asthma symptoms—at least 1 attack of wheeze or dyspnea and/or the prescription of inhaled steroids in the last 12 months</td>
<td>PM2.5</td>
<td>Long-term average exposure (1 year), measured at monitoring sites and modeled to home addresses using land-use regression</td>
<td>TLR2 TLR4</td>
<td></td>
</tr>
<tr>
<td>Lee, 2009 (41)</td>
<td>3,593</td>
<td>Nested case-control</td>
<td>CHSa</td>
<td>Children</td>
<td>Schools</td>
<td>California (United States)</td>
<td>Bronchitic symptoms</td>
<td>1 or more of the following: bronchitis, chronic cough, or chronic phlegm</td>
<td>Ozone</td>
<td>Long-term average exposure (2 years), classified as high (above 50 ppb) or low (below 50 ppb), measured at monitoring stations</td>
<td>TNFA</td>
</tr>
<tr>
<td>Li, 2006 (42)</td>
<td>3,699</td>
<td>Cohort</td>
<td>CHSa</td>
<td>Children</td>
<td>Schools</td>
<td>California (United States)</td>
<td>1) Ever asthma 1) Ever asthma: reported lifetime history of a physician’s diagnosis of asthma 2) Ever wheezing: lifetime 3) Current wheezing: past-12-months symptoms 4) Medication for wheezing</td>
<td>Ozone</td>
<td>Long-term exposure (for 1 year), classified as high (above 50 ppb) or low (below 50 ppb), measured at monitoring stations</td>
<td>TNFA</td>
<td></td>
</tr>
<tr>
<td>Melen, 2008 (43)</td>
<td>982</td>
<td>Case-cohort</td>
<td>BAMSE</td>
<td>Children</td>
<td>Stockholm, Sweden</td>
<td>1) Transient asthma 1) Transient: 3 or more episodes between ages 3 months and 2 years 2) Late-onset asthma 2) Late-onset: no episode between ages 3 months and 2 years but 1 or more episodes in the preceding 12 months 3) Persistent wheezing 3) Persistent: 1 or more episodes between ages 3 months and 2 years and 1 or more episodes in the preceding 12 months 4) Peak expiratory flow 4) Peak expiratory flow</td>
<td>Nitrogen oxides</td>
<td>Long-term exposure modeled using dispersion modelling and emission databases mapped to home address</td>
<td>TNFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salam, 2007 (44)</td>
<td>3,023</td>
<td>Cohort</td>
<td>CHSa</td>
<td>Children</td>
<td>Schools</td>
<td>California (United States)</td>
<td>1) Lifetime asthma 1) Lifetime: physician-diagnosed asthma 2) Early persistent asthma 2) Early persistent: diagnosis before age 3 years and at least 1 episode of wheeze, or use of asthma medication</td>
<td>Traffic pollution</td>
<td>Long-term exposure, measured as distance to nearest freeway from the subject’s home</td>
<td>TGFB1</td>
<td></td>
</tr>
</tbody>
</table>
Table 1. Continued

<table>
<thead>
<tr>
<th>First Author, No. of Participants</th>
<th>Study Design</th>
<th>Study Type</th>
<th>Outcome Measure</th>
<th>Pollutant Exposure</th>
<th>Pollutant</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winterton, 62 Intervention Adults</td>
<td>University/State clinics</td>
<td>Lung function</td>
<td>1) FEV1, Sulfur dioxide 0.5 ppm exposure (0.5 ppm)</td>
<td>Short-term fixed exposure</td>
<td>Sulfur dioxide</td>
<td>IL4, CC16</td>
</tr>
<tr>
<td>Yang, 51 Intervention Adults</td>
<td>Germany</td>
<td>Lung function</td>
<td>1) FEV1, Ozone, 2) Vital capacity</td>
<td>Short-term controlled exposure (200–400 ppb ozone)</td>
<td>Ozone</td>
<td>TNFA, CC16</td>
</tr>
</tbody>
</table>

Abbreviations: BAMSE, Barn [Children], Allergy, Milieu, Stockholm, and Epidemiological Survey; CC16, Clara cell 16; CHS, Children’s Health Study; ECRHS II, European Community Respiratory Health Survey II; FEF25–75, forced expiratory flow at 25% and 75% of vital capacity; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; IL, interleukin; PEFR, peak expiratory flow rate; PIAMA, Prevention and Incidence of Asthma and Mite Allergy; PM2.5, particulate matter less than 2.5 μm in aerodynamic diameter; TGF, transforming growth factor; TLR, toll-like receptor; TNF, tumor necrosis factor.

Of the 2 studies that did not show significant interactions with TNFA, 1 was an intervention study comprising 62 participants (45). This study, which had low statistical power, found no significant results for any of the genes explored. TLR2 was studied in 1 paper (40), with no significant interactions being found. One small intervention study (45) examined associations between polymorphisms in the interleukin 4 (IL4), Clara cell 16 (CC16), and tumor necrosis factor β (TNFB) genes, again with no significant results. The TGFBI –509C>T polymorphism was evaluated in 1 observational study consisting of 3,023 participants (44), and a significant interaction was obtained. Since no other studies explored this gene, the results obtained could not be confirmed.

**DISCUSSION**

This review found some evidence for an association between polymorphisms in inflammatory and immune response genes and adverse respiratory outcomes from exposure to outdoor air pollution. Of the 8 studies included, 6 (39, 40, 42–44, 46) reported at least 1 association between a genetic polymorphism and respiratory response: 2 polymorphisms in TNFA, 3 in TLR4, and 1 in TGFBI. Despite the biological plausibility of the associations, conclusions drawn from this review must be interpreted with caution, given the small sizes of some studies and the large number of polymorphisms assessed.

Given the importance of the inflammatory and immune responses to pollutants and the many reviews and editorials addressing this topic (3, 4, 7, 9, 11), relatively few primary studies have been conducted. This is in contrast to the number of studies that have examined associations between antioxidant/xenobiotic genes and response to pollution (17). The main antioxidant/xenobiotic genes studied include glutathione S-transferase genes, NAD(P)H dehydrogenase (quinone) 1 (NQO1), and heme oxygenase 1 (HMOX1). However, the novelty of this area can be noted from the recent dates of the studies included and the increasing investment in them in terms of sample size.

**Limitations of the included studies**

One key limitation of this review arises from the broad scope of the literature search and the subsequent diversity of the articles. In the small number of studies reviewed, many polymorphisms were evaluated; however, there was little overlap of specific polymorphisms between studies. It might be more advantageous if studies were to include the same gene variants, since repetition of results is important in confirming gene-environment interactions, which guards
Table 2. Selected Genes, Polymorphisms, and Allele Frequencies and Their Associations With Respiratory Response to Air Pollution in a Systematic Review of 8 Studies, 2001–2010

<table>
<thead>
<tr>
<th>First Author, Year (Reference No.)</th>
<th>Gene</th>
<th>Polymorphism</th>
<th>Allele Frequency</th>
<th>Quality Score</th>
<th>Results¹</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castro-Giner, 2009 (39)</td>
<td>TLR4</td>
<td>rs2844484</td>
<td>C: 0.59; T: 0.41</td>
<td>16</td>
<td>No significant modification of asthma prevalence in the presence of nitrogen dioxide for any of the SNPs evaluated.</td>
<td>No evidence of interaction for TLR4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>The CC genotype increased the odds of asthma as nitrogen dioxide levels increased by 10 ( \mu g/m^3 ) (OR = 2.02, 95% CI: 1.30, 3.27).</td>
<td>Possible interaction between this TNFA SNP and asthma</td>
</tr>
<tr>
<td>Kerkhof, 2010 (40)</td>
<td>TLR2</td>
<td>rs10759931</td>
<td>G: 0.59; A: 0.41</td>
<td>12</td>
<td>No significant modification of the prevalence of asthma or asthma symptoms in the presence of high levels of particulate matter and nitrogen dioxide.</td>
<td>No evidence of interaction for TLR2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1927911</td>
<td>C: 0.76; T: 0.24</td>
<td></td>
<td>The TT genotype increased the odds of asthma in the presence of high levels of particulate matter (OR = 4.43, 95% CI: 1.68, 11.73).</td>
<td>Possible interaction between these TLR4 SNPs and asthma</td>
</tr>
<tr>
<td>Lee, 2009 (41)</td>
<td>TNFA</td>
<td>Not stated</td>
<td></td>
<td>15</td>
<td>No significant modification in bronchitis with exposure to high levels of ozone.</td>
<td>No evidence of interaction for TNFA</td>
</tr>
<tr>
<td>Li, 2006 (42)</td>
<td>TNFA</td>
<td>rs1800629</td>
<td>Not stated</td>
<td>18</td>
<td>The presence of the A allele was protective, as measured by asthma symptoms, in the presence of high levels of ozone (OR = 0.65, 95% CI: 0.46, 0.90).</td>
<td>Evidence of interaction between this TNFA SNP and asthma symptoms—protective role of A allele</td>
</tr>
<tr>
<td>Melen, 2008 (43)</td>
<td>TNFA</td>
<td>rs1800629</td>
<td>G: 0.84; A: 0.16</td>
<td>10</td>
<td>Interaction between SNP and wheezing: ( P = 0.09 ).</td>
<td>Weak interaction between SNP and wheezing in the presence of nitrogen dioxide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs3093664</td>
<td>A: 0.92; G: 0.08</td>
<td></td>
<td>Interaction between SNP and wheezing: ( P = 0.05 ).</td>
<td>Evidence of interaction with this TNFA SNP</td>
</tr>
<tr>
<td>Salam, 2007 (44)</td>
<td>TGFB1</td>
<td>rs1800469</td>
<td>C: 0.64; T: 0.36</td>
<td>18</td>
<td>The TT genotype increased the odds of lifetime asthma with exposure to generalized traffic pollution (OR = 2.71, 95% CI: 1.10, 6.69).</td>
<td>Evidence of interaction between this TGFB1 SNP and risk of asthma in the presence of traffic pollution</td>
</tr>
<tr>
<td>Winterton, 2001 (45)</td>
<td>IL4</td>
<td>CC16 TNFA TNFB</td>
<td></td>
<td>4</td>
<td>No significant change in FEV(_1), after exposure to sulfur dioxide in a controlled environment.</td>
<td>No significant modifications in lung function for any of the genes</td>
</tr>
<tr>
<td>Yang, 2005 (46)</td>
<td>TNFA</td>
<td>rs1800629</td>
<td>G: 0.80; A: 0.20</td>
<td>9</td>
<td>The GG genotype decreased lung function as measured by FEV(_1), (mean change as percentage of baseline = (-6.2), 95% CI: (-11.5, -0.8)) and vital capacity (mean change as percentage of baseline = (-4.6), 95% CI: (-8.7, -0.5)) in the presence of ozone in a controlled environment.</td>
<td>Possible reduction in lung function for this TNFA SNP</td>
</tr>
</tbody>
</table>

Abbreviations: CC16, Clara cell 16; CI, confidence interval; FEV\(_1\), forced expiratory volume in 1 second; IL, interleukin; OR, odds ratio; SNP, single-nucleotide polymorphism; TGF, transforming growth factor; TLR, toll-like receptor; TNF, tumor necrosis factor.

¹ Significant effect sizes are given where reported.
against the possibility of false-positive results. An alternative and equally effective approach would be to use a haplotype tagging strategy where possible to ensure that previously tested gene variants are included in future studies. Within individual studies in this review, numerous interactions were often evaluated without consideration of the effects of multiple testing, which increased the risk of false-positive results. There is also the possibility of selective reporting, such that authors may have tested many more interactions but reported on only a sample of them, including those illustrating positive associations. Because of the small number of studies, we were not able to assess publication bias formally. Power calculations were not done or not reported, despite the importance of sample size in evaluation of gene-environment interactions. Similarly, often a clinically significant effect size for interaction was not outlined, and effect sizes that were deemed significant seemed to have been arbitrarily set.

The total number of participants included in this review was 14,903; however, the range of individual study sizes was very wide—from 51 participants to 3,699. For detection of gene-gene interactions, it has been suggested that 10,000 participants are needed as a minimum (47). Additionally, it would have been advantageous to separate studies involving adults and children; however, because of the small number of studies and the heterogeneity present, this was not possible in our overview.

There are a number of areas in which measurement error could have taken place in these studies, including pollutant exposure measurement, outcome definition and measurement, and genotyping. Pollutant exposure was evaluated using a variety of methods to estimate overall levels. The degree of personal exposure is difficult to delineate because of factors such as individual outdoor time expenditure, which often is not adjusted for. The difficulty in measuring pollutant levels accurately is considerable; in addition, the pollutants most frequently measured (ozone, PM, nitrogen dioxide) represent only a few of the many different gaseous and particulate components of outdoor air pollution.

Population stratification was not addressed in the majority of the studies reviewed, suggesting that spurious associations may have arisen due to variations in genotype frequencies across an unexpectedly heterogeneous study population. Population stratification may be particularly important in assessing genetic modification of the associations of pollution with respiratory health, since ethnic minorities may be economically disadvantaged and thus may live in parts of a city where pollution levels are high.

Successful genotyping rates were generally reported to be high; however, in general, the issue of Hardy-Weinberg equilibrium was not adequately addressed. Deviation from Hardy-Weinberg equilibrium for genetic markers is rare in outbred populations in the absence of consanguinity, and where it is present it often indicates failure of the genotyping process. Other reasons for deviation include missing genotyping data and the effect of population stratification (38). Thus, it is essential that all investigators conducting studies of this nature report the statistic used and present the data from which it was calculated.

A further clinically important area for consideration is the role of interacting genes. The influence of air pollution at an individual level is likely to reflect not a direct association of a single SNP with a pollutant but rather a complex interaction between the different genetic components affecting the consequent complex inflammatory and immune pathways of respiratory disease (11). Thus, for example, persons with TLR4 variants likely to be stimulated by pollutants who also have high-activity TNFA variants may be particularly susceptible to respiratory effects of air pollution. A putative overview of the interacting effects of genes and pollutants is shown in Web Figure 1. Clearly, individual studies designed to evaluate these effects will need to be very large; or alternatively, large-scale meta-analyses, ideally with individual patient data, will need to be undertaken.

**Future work**

There is a high level of interest in genetic susceptibility to the adverse effects of air pollution, and since previous studies have mainly focused on antioxidant genes, this review suggests that further research should be carried out on the inflammatory and immunological aspects of the response to pollution. Additionally, for polymorphisms which have been identified thus far, larger studies need to be carried out exploring the same SNPs in order to quantify these effects and to exclude the possibility that the interactions may have occurred by chance.

To improve the quality of studies, ideally observational studies should be performed over long durations of time. It is essential to carry out power calculations to determine the sample size required to show genetic associations and interactions and to ensure that individual studies are of adequate size (47–49). A consortium approach allowing assessment of associations across existing cohorts would be useful. An alternative to increasing sample sizes to detect gene-environment interactions would be to improve the measurement of the exposure and outcome. This approach would allow smaller studies to detect clinically important interactions (50).

There was considerable heterogeneity in how individual exposure was assigned between the selected studies, with the use of dispersion (39, 43) and land-use regression models (40) as well as traffic proximity measures—distance to the nearest busy road (43, 44), for example. Actual pollutant measurements from background monitoring stations were also employed (41, 42), but the articles seldom adequately addressed how representative of the areas under consideration these sites were. There is clearly a need for better standardization of exposure models across population-based studies, requiring a clear appreciation of their relative strengths and weaknesses, inherent uncertainty, and evidence of validation against actual measured pollutant concentrations (51). Where available, high-resolution dispersion models should be preferred, but even here there is debate about how representative exposure assessments can be due to their failure to capture the true exposure of a mobile individual. Personal monitoring offers a partial solution to this problem (52), though it is clearly not amenable to large population studies, and the development of hybrid exposure models that probabilistically capture population time-activity patterns across time-resolved dispersion models may represent the best way forward (53). Although more accurate assessment of pollutant exposure...
may be more costly, the benefits of investing in such research will reduce exposure misclassification and far outweigh the negatives.

New advances in genomics, such as exome and whole-genome sequencing, will bring increasingly fine detail to genetic assessment. However, this will not lead to a gain in knowledge about susceptibility to the adverse effects of pollution without commensurate improvement in quantification of environmental exposure. Similarly, standardization of the phenotype and outcome measures is also necessary to make the best use of the increasingly detailed genetic data (54). This would facilitate replication of findings and allow both conventional and individual patient data meta-analysis of individual studies from which more precise estimates of the strength of gene-environment interactions could be derived.

The candidate-gene approach adopted so far in studies of susceptibility to pulmonary effects of air pollution clearly has limitations. Selection of candidates a priori based on postulated biological mechanisms may produce an incomplete picture of the full range of genes involved in determining the pulmonary response. For example, TNFA has been examined for asthma associations in 79 studies catalogued in the HuGE database (www.hugeneighbor.net), with varying results. However, no significant associations have been found in genome-wide studies, which are non-hypothesis-directed. This suggests that TNFA may be less important in asthma than originally thought.

The same might also apply to the role of TNFA genetic variations in predisposition to adverse outcomes from pollution exposure. While the biological argument may seem strong, there may be a range of other genes with unknown mechanisms of action that are more important. The same reasoning might also apply to TGFBI. Although the activation of TLRs by bacterial material carried on PM may seem plausible, there have been no genome-wide investigations implicating TLR4 in any of the bacterial disease studies in the HuGE database.

Thus, there is clearly a place for non-hypothesis-driven studies of genetic associations with pulmonary response to air pollution. These studies may then direct further candidate gene studies as well as laboratory studies, which will advance biological understanding of the underlying mechanisms. This iterative approach from candidate genes to genome-wide studies and then back to candidate-gene and mechanistic studies has been fruitful in other clinical areas (55).

In addition to the effects of DNA sequence variation on gene-environment interactions, the role of epigenetic mechanisms is increasingly being researched. Animal studies have shown potential associations between pollution and DNA methylation; however, investigations in humans remain in the early stages (56, 57). The role of methylation in health outcomes is not yet understood, but animal studies suggest that epigenetic mechanisms may merit further investigation (58). Further to studies on epigenetics, investigations into the transcriptome (mRNA expression) and microRNAome (microRNA expression) may also be important in improving our understanding of the adverse effects of air pollution (59).

Future studies could relate the proportions of pollutants to an overall level rather than examining the influence of a single pollutant. This approach could potentially help to identify the pollutants that are most important in causing respiratory disease. However, since levels of individual pollutants are highly correlated, this approach would require very large numbers of participants in order to precisely measure associations with individual pollutants.

Future research is essential to quantify the strength of the interactions found and to understand the mechanisms behind them. The trends that were observed have biological plausibility; however, larger studies adhering more closely to STREGA guidelines (37) and having sufficient power to detect gene-pollutant and gene-gene interactions need to be carried out. The identification of genetic determinants which alter the association between respiratory outcomes and air pollutants remains crucial to public health, as this could lead to targeted preventive measures in susceptible persons and might inform policy for limiting pollution exposure to achieve maximum health benefit (60). Better understanding of the mechanisms involved in the adverse effects of air pollution will allow us to deal with this modern phenomenon more effectively, especially in those particularly susceptible—furthering better diagnosis, new treatments, and improved prognosis.

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APPENDIX 1

Gene—Any gene (excluding antioxidants) that may influence the immune system or inflammatory response.

Pollutant—Any outdoor air pollutant (excluding occupational exposure) quantified using any temporal measure (long-term/short-term) and any technique (e.g., land-use regression, dispersion modeling).

Outcome—Lung function, respiratory symptoms, or incidence of any respiratory disease.

Type of study—Any study design for which investigators report on the association between genes and the outcome of air pollution exposure effects on human respiratory function.

APPENDIX 2

Search Strategy

The Medline search strategy used for this review was split into 4 blocks based on 4 components, as follows: gene* or allele or polymorph* or TNF or TLR or NFKB or interleukin or genes [MeSH] or alleles [MeSH] or genetic polymorphism [MeSH] or genetics [MeSH] or tumor necrosis factor [MeSH] or toll-like receptors [MeSH] or NF-kappa B [MeSH] or interleukin [MeSH] AND asthma* or wheez* or respiratory* or COPD or FEV1 or pulmonary function or asthma [MeSH] or respiration [MeSH] or forced expired [MeSH] or peak expiratory [MeSH] or chronic obstructive pulmonary disease [MeSH] or lung diseases [MeSH] or pulmonary function [MeSH] OR ozone or carbon monoxide or CO2 or nitric oxide or sulfur dioxide or sulfur dioxide or SO2 or air pollut* or particulate matter or ozone [MeSH] or carbon dioxide [MeSH] or carbon monoxide [MeSH] or nitric oxide [MeSH] or sulfur dioxide [MeSH] or air pollutants [MeSH] or particulate matter [MeSH] AND inflammation* or immune* or immune response or inflammation [MeSH] or immunity [MeSH].

The Embase, Cochrane Library, and Web of Science databases were searched using the same strategy, adjusting where necessary.