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Genetic aspects of susceptibility to air pollution

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ABSTRACT: Inter-individual variation in human responses to air pollutants suggests that some subpopulations are at increased risk, and it is increasingly clear that genetic background is an important susceptibility factor.

Genetically standardised animal models provide useful investigative tools. Linkage analyses using inbred mice identified chromosomal segments (quantitative trait loci (QTL)), with genes controlling susceptibility to the lung inflammatory (chromosome 17), injury (chromosome 11), and hyperpermeability (chromosome 4) responses to ozone (O_3) exposure. An immune dysfunction response induced by exposure to sulphate-associated particles is linked to the identical chromosome 17 and 11 QTLs described for O_3 susceptibility, thus similar genetic mechanisms may be controlling pulmonary responses to these pollutants. Candidate genes within the QTLs on chromosomes 4 and 17 include the toll-like receptor 4 and the pro-inflammatory cytokine, tumour necrosis factor-α, respectively. Functional analyses strongly support a role for these candidate genes in determining susceptibility to O_3 and particulates. Because striking linkage homology exists between the human and mouse genomes, candidate susceptibility genes identified in the mouse are likely to aid research aimed at understanding human genetic factors that contribute to differential susceptibility.

To date, no studies have examined the interaction between age and genetic background in the development of air pollution-induced lung disease. However, investigations have suggested an influence of age on genetic susceptibility to lung cancer and other diseases, which indicate that an interaction between age and genetic background may be important in air pollution disease pathogenesis.

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Air pollution continues to be an important public health and economic concern in industrialised cities throughout the world. Numerous epidemiological associations of adverse health outcomes with air pollution episodes support these concerns. Inter-individual variation in human responses to air pollutants indicates that not all individuals exposed to pollutants respond similarly. That is, some subpopulations are at increased risk to the detrimental effects of pollutant exposure. The United States Clean Air Act specifies that primary National Ambient Air Quality Standards for criteria pollutants be set low enough to protect the health of all susceptible groups within the population. Exceptions include those requiring life-support systems, intensive care unit patients, and newborn infants in nurseries. However, identification of those factors that determine susceptibility continues to be elusive.

Broadly, inter-individual variation in biological responses to environmental stimuli is a consequence of internal and external factors. External factors include physical forces (*e.g.* temperature, altitude), socioeconomic status, and previous exposure. Internal factors include sex, age, diet, and predisposing disease (*e.g.* asthma). Genetic background has become increasingly recognised as another important internal or host factor in environmental disease predisposition. In human populations, gene polymorphisms have been associated with susceptibility to environmental agents including pesticides and infectious agents [1–3].

It is also clear that multiple internal and external factors contribute to individual responses to air pollutants. A number of populations have been identified that are particularly susceptible to the toxic effects of airborne oxidants and particulates, including the elderly and individuals with cardiopulmonary disease [4, 5]. Evidence also exists for genetic determinants of susceptibility to airborne pollutants, including ozone (O_3) and particulates (see below). The primary objective of this manuscript is to discuss briefly studies that have identified a genetic basis for susceptibility to air pollution in animal models and human subjects, and the approaches used to identify susceptibility genes.

Research strategies employed to identify candidate genes

Two broad research strategies have been utilised to identify genes (quantitative trait loci (QTLs)) that determine susceptibility. The first is a genome scan or positional cloning (formerly known as reverse genetics). This strategy attempts to associate expression of genes or markers (*e.g.* microsatellite markers, see below) with phenotype(s) in segregant populations. A genome-wide screen is designed to identify linkage to any chromosomal intervals within the entire genome that may contain genes that are polymorphic between two strains of mice and may account for the differential response phenotype under study. That is, no *a priori* hypothesis about the role of a specific gene or genes is tested. This is ideally suited for genetically well-controlled models, particularly inbred mice, but is applicable to human populations as well. The second research strategy is the candidate gene approach (formerly

"forward genetics"), in which genes are chosen *a priori* as likely mechanisms that determine the phenotype of interest. With the candidate gene approach, linkage is then assessed between the phenotype of interest and markers flanking the candidate genes or the candidate genes themselves. This strategy may implicate certain genes in the expressed phenotype. However, without a genome scan, the candidate gene approach may exclude other important loci that determine a quantitative trait, as well as the interaction between them. These strategies, alone or in combination, have been utilised in inbred mice to identify genetic mechanisms of a number of diseases. These include models of Huntingtons disease [6, 7], Duchenne muscular dystrophy [8], amyotrophic lateral sclerosis [9], insulin-dependent diabetes mellitus [10], Alzheimer′s disease [11], von Willebrand disease [12], chronic granulomatous disease [13], and Niemann-Pick C1 disease [14]. The considerable resources and energy applied to mapping of the mouse genome as an integral component of the Human Genome Project underlie the importance of this animal model for human diseases [15]. One of the unifying concepts for genetic studies in mice is the linkage relationship of homologous loci from human and mouse. Specifically, highly significant homologies in gene order and chromosomal structure have been maintained between mice and humans since their divergence [16]. Therefore, identification of the chromosomal location of a susceptibility gene in the mouse provides the basis for potentially localising a homologous gene in the human [17, 18].

Candidate genes for susceptibility to air pollutants

Identification of a QTL is a multi-step process that may be broadly grouped into three research objectives. The first objective is to determine whether the response phenotype is genetically determined and quantitative. After determining that the quantitative phenotype has a genetic basis, then the susceptibility QTL(s) are sought. Finally, significant informative QTLs are searched for candidate genes that may explain differential susceptibility/responsiveness in the model. It is beyond the scope of this manuscript to explain all of the details of linkage mapping; therefore, the reader is referred to excellent reviews of this topic by BROMAN [19], MOORE and NAGLE [20], and SILVER [21]. Selected investigations that have led to the identification of susceptibility QTLs for O_3- and particle-induced pulmonary inflammation and injury are described below. This discussion is not exhaustive, and it is meant only to illustrate the approach to identify the genetic basis of susceptibility to air pollutants as well as candidate genes that may be important determinants of susceptibility.

Genetic determinants of susceptibility to ozone-induced lung inflammation and injury

O_3 exposure induces multiple pulmonary and extrapulmonary responses in humans and animal models. O_3 elicits inflammation, hyperreactivity and epithelial damage of the airways, as well as altered ventilation and decrements in pulmonary function [4, 22]. O_3 has also been demonstrated to either suppress or enhance immune responsiveness [23]. Significant inter-strain variation in the magnitude of these responses has also been demonstrated in rats and mice, and has thus provided strong evidence of a genetic component to O_3 responsiveness [24–26].

The inter-strain variation in susceptibility among inbred mice led the author′s laboratory to conduct studies to identify the chromosomal location of the O_3 susceptibility genes using susceptible C57BL/6J (B6) and resistant C3H/HeJ (C3) strains. A genome-wide search for linkage of the inflammation (polymorphonuclear leukocytes) phenotype was performed with informative simple sequence length polymorphisms (SSLPs) distributed at ~10-centi-Morgan (cM) intervals throughout the genome (see [27] for more detail regarding choice of markers). The number and spacing of markers yielded complete coverage of the mouse genome with 95% confidence. Linkage was carried out with individual intercross animals derived from B6 and C3 progenitors ($B6C3F_2$). The phenotyped F_2 progeny were genotyped for each of the SSLPs, and linkage of susceptibility to O_3 was evaluated using Map Manager QT and MAPMAKER-QTL software packages. Interval mapping by simple linear regression in the entire F_2 cohort determined the presence of a susceptibility locus on chromosome 17 in the interval ~16–22 cM (table 1; [27]). An additional QTL was detected on chromosome 11 between D11Mit20 and D11Mit12. Within the chromosome 17 QTL, there are a number of candidate genes, including the pro-inflammatory cytokine, tumour necrosis factor (TNF)-α. Because TNF-α may be postulated to have a role in the inflammatory response to oxidant-related lung injury, preliminary evaluations of this candidate gene were made for determination of differential O_3-induced inflammation in B6 and C3 mice. Pretreatment of susceptible B6 mice with a

Table 1. – Linkage and association studies that have identified quantitative trait loci (QTLs) and/or candidate genes for susceptibility to detrimental effects of air pollutants on the lung

Study type	Species	Pollutant	Phenotype	Chromosomal location(s) of QTLs	Candidate gene	First author [ref no.]
Linkage	Mouse	Ozone	Inflammation (PMNs)	17	TNF-α	KLEEBERGER [27]
				11	Small inducible cytokines	
	Mouse	Ozone	Lung hyperpermeability	4	TLR4	KLEEBERGER [28]
				11	Small inducible cytokines	
	Mouse	Ozone	Acute lung injury, death	11	Small inducible cytokines	PROWS [26]
				13	GPX	
				17	TNF-α, XDH	
	Mouse	Sulphate-associated particles	Immune dysfunction	17	TNF-α	OHTSUKA [29]
				11	Small inducible cytokines	
Association	Human	Ozone	Pulmonary dysfunction, lung hyperpermeability	N/A	NQO1; GSTM1	BERGAMASCHI [30]

GSTM1: glutathione-S-transferase μ-1; GPX: glutathione peroxidase; NQO1: nicotinamide adenine dinucleotide phosphate (NAD(P)): quinone oxidoreductase; TLR4: toll-like receptor 4; TNF-α: tumour necrosis factor α; XDH: xanthine dehydrogenase; O_3: ozone; PMN: polymorphonuclear leukocytes; N/A: not applicable.

monoclonal antibody to TNF-α significantly attenuated the inflammatory response to O_3 relative to control B6 mice, thus providing support of TNF-α as a candidate susceptibility gene in this model [27].

To further understand the mechanisms of O_3-induced lung injury, a genome-wide linkage analysis for susceptibility QTLs was performed to explain inter-strain differences in hyperpermeability induced by 72-h exposure to 0.3 parts per million (ppm) O_3. Because there is an apparent dissociation between inflammatory cell infiltration and lung hyperpermeability induced by O_3 [31], it was hypothesised that different loci control the hyperpermeability response. To determine the susceptibility QTLs, a genome screen was performed using recombinant inbred (RI) strains of mice derived from B6 and C3 progenitors (see [21] for explanation of the use of RIs for QTL mapping). A significant QTL was identified on chromosome 4, and suggestive QTLs were identified on chromosomes 3 and 11 (table 1; [28]). The chromosome 4 QTL contains a candidate gene, toll-like receptor 4 (TLR4) that has recently been implicated in innate immunity and endotoxin susceptibility [32–34]. As a "proof of concept" that TLR4 has an important functional role in susceptibility, the hyperpermeability responses to O_3 in C3H/HeOuJ (OuJ) and C3 mice were compared. These strains differ only at a polymorphism in the coding region of the TLR4 gene and the polymorphism confers resistance to endotoxin-induced injury in the C3 mouse compared to wild type OuJ. Significantly greater protein concentrations were found in OuJ mice compared with C3 mice after exposure to O_3 [28]. Furthermore, reverse transcriptase polymerase chain reaction analysis demonstrated that TLR4 message levels in the lungs of C3 mice were markedly downregulated, while levels increased in the OuJ strain after O_3 exposure [28]. Together, results indicate that a QTL on chromosome 4 explains a significant portion of the genetic variance in O_3-induced hyperpermeability, and support a role for TLR4 as a strong candidate susceptibility gene. This is the first demonstration that innate immune mechanisms modulated by TLR4 are involved in the pulmonary response to oxidant exposure.

PROWS *et al.* [26] performed a linkage analysis of susceptibility to death induced by exposure to high concentrations of O_3. Using susceptible A/J and resistant B6 mice, these investigators identified a significant QTL on chromosome 11, and suggestive QTLs on chromosomes 13 and 17 (table 1). Interestingly, the QTLs on chromosomes 11 and 17 are similar to those described by KLEEBERGER *et al.* [27] for susceptibility to inflammation induced by exposure to 0.3 ppm O_3.

Evidence also exists for genetic determinants of susceptibility to O_3 in human subjects. A number of laboratories have reported inter-individual variation in pulmonary function responses to O_3 in otherwise normal, healthy human subjects [35–37]. Inter-individual variation in the inflammatory response to O_3 has also been described [38–42]. A second line of evidence is the demonstration that specific gene polymorphisms associate with response phenotypes in exposed human subjects. BERGAMASCHI *et al.* [30] found that polymorphisms in genes for quinone-metabolising enzymes may have an important role in the pulmonary function and epithelial permeability responses to O_3 in nonsmoking exercising subjects.

Genetic determinants of susceptibility to particle-induced lung inflammation and injury

Considerable attention has been focused on the adverse respiratory effects caused by inhalation of particles. Epidemiological studies have reported significant association of acute and chronic respiratory and nonrespiratory health effects with increases in particulate exposure throughout the industrialised world [4, 43, 44]. Susceptible subpopulations include the aged ($\geqslant$65 yrs) and patients with cardiopulmonary disease, such as chronic heart disease, chronic obstructive pulmonary disease, and asthma [4, 45–47]. To determine whether genetic background is an important determinant of pulmonary responses to particulates, the inter-strain variance of lung responses to acid sulphate-coated particles (ACP) in inbred strains of mice was studied [48]. Although the 4-h challenge to ACP did not elicit a detectable inflammatory response, significant inter-strain differences were found in Fc receptor-mediated phagocytosis of alveolar macrophages (an indicator of innate immune defence). A genome scan similar to that described for O_3 susceptibility (see above) was then performed with susceptible B6 and resistant C3 mice [29]. Interestingly, linkage analyses identified a significant QTL on chromosome 17 and a suggestive QTL on chromosome 11 that nearly overlapped similar QTLs identified for O_3 susceptibility. The common linkages suggest that similar genetic mechanisms may control pulmonary responses to O_3-induced inflammation and macrophage phagocytic dysfunction induced by ACP; however, further genetic analyses are required to confirm this hypothesis.

Contribution of age and genetic susceptibility to airborne pollutants

To date, few studies have examined the influence of age on genetic susceptibility to pulmonary diseases, and none have examined the interaction of these host factors on pollutant susceptibility. GAUDERMAN and MORRISON [49] performed a segregation analysis suggesting that the effect of genotype on lung cancer varies by age, such that age-specific relative risks are greatest in the young and decline thereafter. Though no specific genes were identified, this study strongly suggests that age and genetic background may be important co-determinants of pulmonary disease. Both factors should be considered in future proposals/studies to understand susceptibility to air pollutant-induced lung disease.

Conclusion

Air pollution-induced morbidity and mortality continue to be important public health concerns worldwide, and identification of susceptible subpopulations is of critical importance. Numerous factors may contribute to inter-individual susceptibility to the detrimental effects of air pollution, including age and genetic background. Linkage analyses with inbred mice and association studies with human subjects have led to identification of candidate susceptibility genes for pulmonary responses to ozone and sulphate-associated particles. The role of age as an interacting factor with genetic background has not been thoroughly examined in air pollution-induced lung disease, but likely has an important role in genetic susceptibility, and future studies should be designed to investigate the interaction of these two factors. An understanding of the biology of candidate genes will lead to an understanding of the genetic basis for differential responses to pollutant exposures. Furthermore, characterisation of a polymorphism in a pollutant susceptibility gene(s) may thus provide the means to identify individuals who are genetically susceptible to the development of injury.

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