Gene–Air Pollution Interactions in Asthma

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Genetic and environmental factors interact to cause asthma. However, genetic studies have generally ignored environmental factors and environmental studies have generally ignored genetics. Thus, there are few examples from the literature of specific gene–environment interactions in relation to asthma. The clearest examples of genetic interactions for inhaled pollutants exist for endotoxin, environmental tobacco smoke, and ozone. Endotoxin –genetic interactions in asthma are the focus of two other manuscripts from this conference, so this review focuses on environmental tobacco smoke and ozone. In the sparse literature, there is evidence for the role of specific genes involved in oxidative stress, notably *GSTM1* and *TNF*, in the respiratory responses to ozone and environmental tobacco smoke. There are few data on genes involved in innate immune pathways, which are crucial in response to endotoxin and may play a role in response to ozone and environmental tobacco smoke. Genes involved in oxidative stress may interact with both air pollutants and diet in relation to asthma phenotypes. Future directions to advance the field include whole genome association studies, better assessment of exposure and phenotypes, and consideration of joint interactions with diet and other co-factors that influence individual susceptibility.

Keywords: asthma; genetic; air pollution; tobacco smoke pollution; single nucleotide polymorphism

The prominence of family history among asthma risk factors has long suggested an important role for genetics. Having one parent with asthma confers about a twofold increased risk of asthma and having two parents with asthma increases the risk to about fourfold. The search for specific genes involved in predisposition to asthma has been challenging, due in part to the complex nature of the disease. Several asthma genes have been positionally cloned, and additional candidate genes have emerged from association studies (1). The degree of replication has been variable for genes from both types of studies. Although sample size appears to be the major factor in failure to replicate genetic associations for common diseases (2), for asthma, the variability of disease phenotypes across studies likely contributes.

The marked differences in rates of asthma between countries, even among genetically similar groups, with substantially higher rates in more developed countries (3) clearly suggest the role of environmental factors. Given what we know about genetic predisposition, asthma likely results from a complex interplay between genetic and environmental factors. Differences in exposure profiles between populations may also contribute to failure to replicate some genetic associations.

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Among environmental factors, there is incontrovertible evidence that ambient and indoor air pollutants exacerbate existing asthma. There is increasing evidence that air pollutants also contribute to asthma development. The data are clearest for environmental or secondhand tobacco smoke. Consistent associations have been found for a crude measure of exposure, living with smoking parents, and the development of asthma and asthma-related phenotypes in children (4). Recent evidence suggests that childhood exposure to secondhand smoke can contribute to the development of asthma and respiratory symptoms in adults (5, 6). The likely importance of genetic susceptibility in determining the risk of developing asthma from early childhood exposure was suggested by analyses using parental history of asthma and allergies as an index of genetic risk. In the Children's Health Study, a cohort of California school children, having a mother who smoked during pregnancy was most strongly related to early-onset persistent asthma among children with a parent with asthma or allergies (7). Relatively few studies address the specific genes that underpin this interaction.

Abundant data link ambient air pollution to asthma exacerbation (8). Both fine particles and ozone appear to be important (9). Recent data have demonstrated asthma exacerbation at levels of ozone below U.S. air quality standards (10). Establishing a role of air pollution in the development of asthma requires long-term prospective data, but few exist. However, an increasing body of prospective data supports a role for ambient air pollution in the induction of asthma (11). Few data exist to address interactions between air pollutants and genetics in relation to asthma in humans.

Dietary factors appear to influence effects of air pollution on asthma-related phenotypes. In particular, antioxidant intake was shown to reduce the effects of daily variation in ozone exposure on changes in pulmonary function in children with asthma in Mexico City (12). It is possible that other components of the diet may be important in modulating effects of inhaled pollutants on asthma-related phenotypes. For example, high intake of fruits and vegetables may be protective, whereas a diet high in animal fats and of high glycemic load may be deleterious (13, 14). Genetic factors may modify the interplay between diet, air pollution, and asthma phenotypes (15).

INTERACTION BETWEEN GENETICS AND OZONE IN RELATION TO ASTHMA-RELATED PHENOTYPES

In 1991, McDonnell suggested that acute respiratory response to ozone in humans may be under genetic control, based on the observation, from controlled exposure studies, that between-subject variability is much greater than within-subject variability over time (36). Several years later, two groups identified a genetic basis for several respiratory responses to acute ozone exposure in inbred mouse models (16, 17). These and later studies in mice implicate tumor necrosis factor-α (*TNF*) and Toll-like receptor 4 (*TLR4*) in susceptibility to ozone response. An *in silico* genome scan approach in mice identified a number of additional linkages for a variety of phenotypes of acute ozone response; the specific genes remain to be identified (18). The animal models of gene–ozone interaction have not addressed the chronic exposure situation most relevant to human asthma induction.

Given the modest effects of air pollution detectable in large population studies of asthma incidence, identification of genetically susceptible groups may aid in the determination of health effects in humans. However, there are few studies on genetics of ozone response in humans. The first study was done by Bergamaschi and coworkers (19), who measured pulmonary function and a marker of increased lung epithelial permeability (serum clara cell protein, CC16) in 24 healthy volunteers before and after a 2-hour bicycle ride at ambient summer ozone concentrations (32–103 ppb). They examined the effects of ozone on these parameters in relation to two common functionally significant polymorphisms in genes involved in oxidative stress—homozygous deletion (null genotype) of glutathione S-transferase M1 (*GSTM1*), and a base substitution (C to T) in the coding region of NAD(P)H: quinone oxidoreductase (*NQO1*). The authors found that decreases in lung function parameters, including FEV_1, were more pronounced in subjects with the combined *GSTM1*-null and *NQO1* wild-type (CC) genotype. They also found that CC16 in serum increased in relation to ozone exposure only among subjects with this combined genotype. In a controlled ozone exposure study of 22 volunteers, the same group found that subjects with the combined *GSTM1*-null plus *NQO1* CC genotype had greater increases in putative biomarkers of oxidative stress in the exhaled breath condensate (8-isoprostanes, LTB-4, and thiobarbituric reactive substances) than subjects with other genotypes (20).

Based on these findings from acute exposure studies, we examined the relationship between the combined genotypes of *GSTM1* and *NQO1* in relation to childhood asthma in a Mexico City population with high lifetime exposure to ozone. Our results were analogous to those found for acute ozone responses in the two previous papers (20). The NQO1 TT reduced activity genotype was protective for asthma only among the *GSTM1*-null individuals (21).

GSTM1 genotype may modify the acute respiratory response to ozone in individuals with asthma. We assessed *GSTM1* genotype among 158 children enrolled in a randomized double-blind trial of antioxidant supplementation (vitamin C 250 mg/day and vitamin E 50 mg/day) in Mexico City. Children performed pulmonary function measures twice per week for 12 weeks. Measurements of ambient ozone were obtained from the Mexican government's air monitoring stations. All children resided within 5 km of a monitoring station whose values were assigned to that child. In the placebo group, ozone levels were significantly and inversely associated with $FEF_{25–75}$ in children with the *GSTM1*-null genotype (39%); no significant decrement was observed in *GSTM1*-positive children. Expressed as a percentage of baseline $FEF_{25–75}$, for an increase of 50 ppb in 1 h maximum ozone concentration, this decrement in children with the *GSTM1*-null genotype taking placebo corresponded to −2.9% (95% CI, −5.2 to −0.6, $P = 0.01$). Conversely, the beneficial effect of antioxidant supplementation was seen primarily in the *GSTM1*-null individuals. To our knowledge, this was the first example of a gene–diet–air pollution interaction. Although this study was modest in size, the availability of repeat measures of pulmonary function and daily air pollution measurements increased the power to detect interactions with this common polymorphism. The results suggest that children with genetic reduction of antioxidant defenses are at increased risk of pulmonary impairment from ozone exposure and that these effects might be mitigated by antioxidant supplementation. Conversely, the data suggest that benefits of interventions may differ by genetic factors, such as susceptibility to oxidative stress.

We also evaluated ozone-related changes in respiratory symptoms in relation to genotypes of both *GSTM1* and another glutathione S-transferase, *GSTP1*. We found that children with the *GSTM1*-null or the *GSTP1* Val/Val genotype had greater increases in difficulty breathing in relation to ozone than other children (22). Increases were enhanced among children with both of these genotypes. These results add to evidence that ozone-related pulmonary impairment may be greater in individuals genetically at risk for greater susceptibility to oxidative stress.

Based on mouse data suggesting *TNF* as a susceptibility gene for ozone response (16, 17), Yang and colleagues (23) studied four polymorphisms across *TNF* and the adjacent lymphotoxin α (*LTA*) gene in 51 subjects who had undergone controlled acute ozone exposure. The functionally significant TNF-308 genotype appeared to modify FEV_1 response to ozone challenge. Functionally significant polymorphisms in manganese superoxide dismutase (*SOD2*) and glutathione peroxidase (*GPX1*) genes did not alter ozone responses.

Given data in mice demonstrating *TLR4* as an ozone response locus (24) and in humans showing that a nonsynonymous coding single-nucleotide polymorphism (SNP) in *TLR4* (Asp299Gly) modifies endotoxin response, this SNP would be a logical candidate to examine in human ozone studies. However, this SNP occurs at a relatively low frequency, limiting power in modest-sized studies. Yang and coworkers genotyped this SNP but numbers were too small for analysis. Larger studies, or controlled exposure studies targeting individuals with the variant allele, will be necessary to evaluate its role in ozone response. Variation in other genes involved in innate immunity merit evaluation.

ENVIRONMENTAL TOBACCO SMOKE AND GENETICS IN RELATION TO ASTHMA-RELATED PHENOTYPES

Although environmental tobacco smoke (ETS) is a common exposure that is relatively well-assessed by questionnaire and is important in the etiology of childhood asthma, there are relatively few studies examining genetic interactions. Linkage studies of asthma have generally ignored environmental exposures. Recently, in two family studies, regions of linkage to asthma phenotypes differed by ETS exposure in childhood (25, 26).

The few studies of specific genes have generally considered the same polymorphisms in oxidative stress genes examined in response to ozone: glutathione S-transferases M1 and P1. TNF has also been examined because it is important in lung inflammatory responses to tobacco smoke (27).

In a study of 19 individuals, nasal response to allergens appeared to differ by *GSTM1* and *GSTP1* genotypes (28). In the Children's Health Study cohort, the effects of maternal smoking in pregnancy on asthma risk differed by *GSTM1* genotype (29). In another study, *GSTM1* and *GSTP1* genotypes modified asthma risk and changes in peak expiratory flow in relation to ETS in children (30).

With respect to *TNF*, the −308 SNP modified the relationship between ETS exposure and school absence in the Children's Health Study (31). We examined polymorphisms in *TNF* and *LTA* in relation to asthma in Mexico City children and examined modification by exposure to a smoking parent. The functional TNF-308 SNP was related to asthma risk predominantly among subjects from nonsmoking families (32). Given that TNF expression is increased after exposure to both ozone and tobacco smoke, we believe that the modest effect of this SNP on TNF expression may be masked in subjects with both of these exposures. Children in our study had high lifetime exposure to ozone by virtue of residence in Mexico City. This result is similar to a finding in the Children's Health Study, in which an association between TNF-308 and asthma risk was seen only among children in low–ozone exposure communities (33).

Innate immune pathways may be important in the response to ETS (34). Genetic variation in innate immunity as a modifier of effects of ETS has been explored in one report. Choudhry and colleagues reported an interaction between ETS exposure and polymorphisms in CD14 in relation to two asthma phenotypes: FEV_1 and IgE (35).

FUTURE DIRECTIONS

Studies to date of genetic interactions between ETS or ozone in relation to asthma phenotypes have examined single genes within pathways. The few studies available have focused mostly on a handful of genes involved in oxidative stress. As the knowledge of the biological response mechanisms for these two exposures evolve, future studies can consider more complete gene pathways. However, the examination of pathways involves looking at gene–gene interactions and thus presents challenges of sample size and power. Cooperation between investigators with studies of comparable asthma phenotypes can facilitate these investigations.

High-density SNP arrays are now commercially available for whole genome association studies of complex diseases such as asthma. To date, there are no published whole genome SNP association studies of asthma phenotypes. Given the increasing awareness of the need to consider environmental exposures in gene discovery studies, future studies will likely consider exposure to ETS. Most existing studies of childhood asthma likely include simple questionnaire items on parental smoking that are reasonably valid measures of exposure (4). Statistical methods for evaluating gene–environment interactions in whole genome association studies are likely to evolve. However, given the large sample size required for genetic main effects in whole genome association studies, the power to examine interactions will be an issue. Combined analyses across studies with similar characterization of asthma phenotypes will aid in the investigation of gene–environment interactions

While large sample sizes will improve power for studies of gene–environment interaction, the power can also be enhanced by better measures of exposure. This includes better characterization of individual exposure and repeated measures over time. Likewise, more refined phenotyping will also improve the power to study gene–environment interactions. As many asthma phenotypes change over time, incorporation of repeated measures of outcomes will also be beneficial.

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