Chapter 12
Genetics

Talat Islam and Frank Gilliland

12.1 Introduction

In previous chapters we discussed the chemical and physical properties of the different airborne pollutants (AP) and their potential health effects. Air pollutants with potential detrimental health effects can be gasses (i.e., Ozone, oxides of nitrogen (NOx)) or solid particles (i.e., PM_{10}, $PM_{2.5}$) that can elicit myriad of biological responses and affect cardio-respiratory system, immune system, CNS, and reproductive system. Despite the epidemiological and toxicological evidence of AP-related health effects, the biological mechanisms and pathways involved are not well established. The particular biological pathways and process involved in AP-mediated health effects can vary by the involved organs/systems and may include oxidative stress, local or systemic inflammation, neural-mediated responses, and oxygen insufficiency. Many of these pathways have been identified through toxicological studies and discussed in the earlier chapters. However, toxicological studies often involve exposures that are limited in reproducing exposures encountered in the real world. One approach to investigating the biological pathways has been to identify the genes that modify sensitivity to AP.

If a *specific* pathway is important for *specific* air pollutant mediated health effects, then functional polymorphisms in gene(s) involved in the pathway can affect the magnitude of the health effects of that air pollutant. Therefore, gene-environment interaction (GxE) studies can confirm or identify the important mediating biological pathways, as well as, serve to identify susceptible groups. Multiple genes are likely to be involved in a single biological pathway and functional polymorphisms of the different genes in the pathway might provide synergistic or antagonistic effect on the air pollutant mediated health effect.

T. Islam, M.B.B.S., Ph.D. (✉) • F. Gilliland, M.D., M.P.H., Ph.D.
Division of Environmental Health, Department of Preventive Medicine, Keck School of Medicine, University of Southern California (USC), Los Angeles, CA, USA
e-mail: islam@usc.edu

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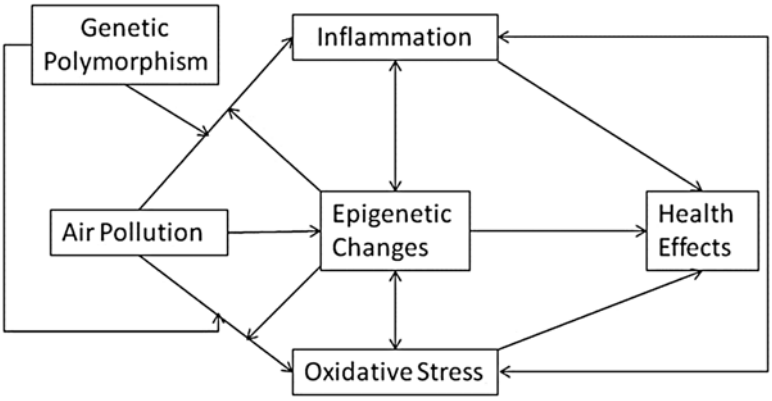

Fig. 12.1 Schematic representation of genetics and air pollution on human health. The health effects of AP exposure can be mediated through inflammation, oxidative stress and epigenetic changes; all of which are interrelated. Furthermore the effects of AP can be modified by genetic and epigenetic variants

Beyond identifying the biological pathways, a better knowledge of GxE can serve multiple purposes in public health and medicine as has been enumerated by Hunter in his review (Hunter 2005). Some of the rationale for studying gene-AP interactions are as follow:

- To have a better estimate of the population attributable risk considering the joint effect of genes and air pollution.
- To identify the susceptible population to AP-mediated disease (i.e., increased susceptibility to allergy due to exposure to diesel exhaust among population with GSTM1 deletion (~50 % Caucasian) (Gilliland et al. 2004))
- Identify the specific factor from a complex pollutant mix that can have a causal effect (i.e., polycyclic aromatic hydrocarbon in traffic exposure for asthma risk) (Salam et al. 2007)
- Utilize the information to develop better preventive and therapeutic approaches

In this chapter, we will review the complex interplay between air pollution, genetic variants and epigenetic changes on human health outcomes. Our discussion will focus on genetic polymorphisms that can modify the effect of AP on local and systemic inflammation and oxidative stress (Fig. 12.1). We will also discuss contributions of AP-induced epigenetic changes that could contribute to AP exposure related health effects.

12.2 Identification of Gene-Environment Interactions

In epidemiological studies, the identification of the genetic polymorphisms that modify the effect of AP (GxAP) involves mainly three major approaches: (i) hypothesis driven candidate gene approach: selecting genes with main effect on the health

outcome or selecting genes that are involved in a purported biological pathway, (ii) agnostic genome-wide interaction studies (GWIS), and (iii) hypothesis driven pathway based approaches. Each of these approaches has merits and limitations (Thomas 2010).

Because the candidate gene approach is hypothesis driven, we often have better study designs that are less subject to bias and residual confounding. These studies require a relatively small sample size compared to GWIS and have sufficient statistical power to detect moderate level of gene-environment interaction. It also provides a better chance for cross validation across studies as researchers are more likely to use the same genetic variants to address similar hypothesis. One of the major limitations of candidate gene approach is, by definition, it will miss any novel genetic pathway. Using candidate GxE approach from the onset can limit the identification of new genes as researchers focus on validating the initial finding and genes that might not have reached threshold p-value in some studies may not surface at all. In the candidate gene approach, we are likely to miss genetic factors that do not have any main effect especially when genetic variants have a 'flip-flop' effect where the effect of the genetic variants on disease is opposite based on presence or absence of environmental factor. This approach is also susceptible to researcher bias as those variants are not agnostically chosen. Many of the limitations of candidate gene approach are adequately addressed using the agnostic GWIS approach. The current availability of 2.5 million SNP chips at a reasonable price has supported the era of genome-wide association studies (GWAS) as well as for GWIS. The major concerns for the GWIS approach are related to exposure assessment, multiple testing and sample size issues. Considering the issues of multiple testing, the common cut-off p-value for GWAS/GWIS level of significance is 10^{-8}. A study would require almost 40,000 participants to have 80 % power to see a moderate level of effect (i.e., both the marginal effects and interaction odds ratio being 1.5) for a genetic variant with minor allele frequency (MAF) of 0.05 and exposure prevalence of 10 %. This is often a challenge for any single study and requires collaboration among multiple studies. Although, the collaboration approach in current state of information technology is quite feasible and beneficial to all involved, methodological challenges is the major hindrance for such approach. Differences in study design as well as availability of the appropriate exposure metrics and covariates limits collaborative GxE work. The participating studies are required to have similar genetic and environmental information that, to date, is often not available. Genetic data often varies between groups based on the choice of array (i.e., half-a-million/million or other, Illumina or Affymetrix platform). Although this may raise technical issues, most of them can be addressed. A greater concern is often the complete lack of, or variability in the collection of exposure data. For example some studies use model based estimate of oxides of Nitrogen (NOx) as a proxy measure for traffic exposure; whereas, another study can use residential distance to freeway. Even the model used by different groups to estimate NOx may not be similar. Often studies have to rely on some basic measure that is available in all of the groups, i.e. residential distance from freeway or major roads, instead of more refined measures. Therefore, measurement misclassification is a major concern in GxE collaborative studies that can result in biased effect estimates, reduced power and or null findings.

12.2.1 An Example of Genetic Polymorphism Modifying the Effect of AP

The gene-AP interactions discussed in this chapter are mostly identified through candidate gene approach in human studies. The following example from Salam et al. (2007) illustrates how candidate gene approach can be utilized in the identification of the modifying effect of multiple genes in the PAH metabolism pathway on the association between traffic related air pollutant (**TRAP**) and asthma prevalence (Salam et al. 2007).

Multiple epidemiologic studies have reported that high level of TRAP exposure is associated with asthma (Chap. 4). The investigators hypothesized that PAHs that are formed due to incomplete combustion of fuel and have been observed to be associated with asthma, might be the major component of TRAP that contributes to TRAP-Asthma association. The researchers hypothesized that genetic polymorphisms of microsomal epoxide hydrolase (EPHX1) and glutathione S-transferase (GST) can modify the effect of traffic exposure on asthma prevalence.

Once PAHs from TRAP enter the body through inhalation, it undergoes metabolic transformation that can lead to detoxification or activation (Fig. 12.2). Reactive PAHs are initially converted to active PAH-epoxide that serves as substrate for

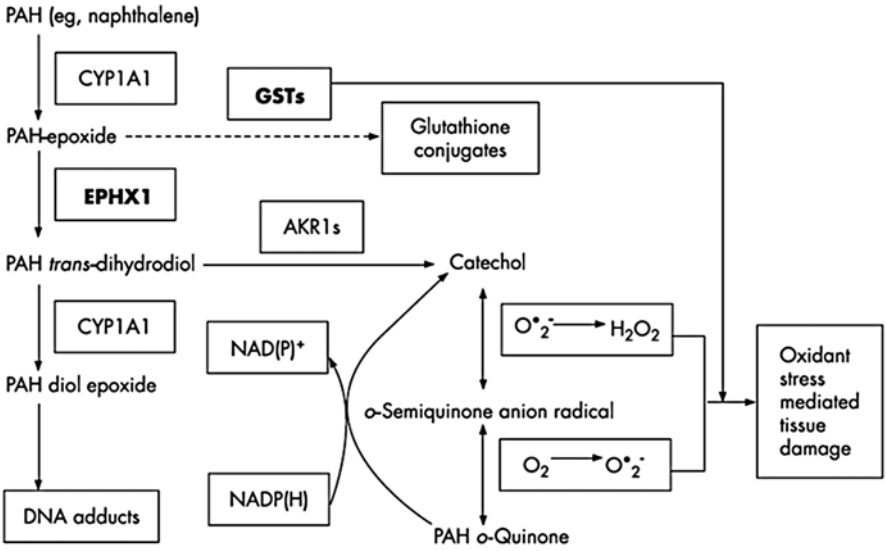

Fig. 12.2 Schematic pathway of PAH metabolism. After inhalation, polycyclic aromatic hydrocarbon (PAH) undergoes metabolic changes with formation of toxic metabolites that can lead to oxidative changes and formation of DNA adducts. PAHs can also be sequestered by the formation of GST–conjugates. Therefore, the activity level of the different involved enzymes, i.e., epoxides or GSTs, can affect the biological effective dose of PAH exposure (Reproduced from Salam et al. (2007), with permission from BMJ Publishing Group Ltd)

GSTs and epoxide hydrolase. GSTs detoxify PAHs by forming epoxide-conjugates and rendering them inactive. Epoxide hydrolases on the other hand form PAH-*trans*-dihyrodiol from PAH-epoxide. The PAH-trans-hydrodiols can be further metabolized to form semiquinones that generate reactive oxygen species (ROS) leading to oxidative stress (Bolton et al. 2000). Based on this pathway it can be purported that people with low level of GST activity and higher level of EPHX1 activity will be more susceptible to TRAP exposure compared to those with high activity of GST activity and low EPHX1 activity. The researchers identified a genetic polymorphism of *GSTP1* (*ile105val)*, where the variant (*val*) is associated with decreased conjugation of PAH-epoxide and functional polymorphisms of EPHX1 are associated with increased activity of epoxide hydrolase.

The joint effect of residential distance from a major road (exposure to TRAP decreases with increased distance), *Ile105Val* polymorphism, and two *EPHX1* polymorphisms that defined three metabolic phenotypes (low, medium, and high activity) was investigated in 2,700 children (Table 12.1). They reported statistically significant three way interaction showing that those children who were *GSTP1* variant homozygous (val105 val) with the high metabolic phenotype of EPHX1 and lived within 75 m of a major road, had almost nine times increased risk of asthma

Table 12.1 Functional polymorphisms of GSTP1 and EPHX1 modify the effect of traffic related exposure on lifetime asthma prevalence in children (Salam et al. 2007)

Distance of residence from major road (metres)	GSTP1 ile 105Val	EPHX1 metabolic phenotypes	No asthma (N[a])	Lifetime asthma (N[a])	OR[b] (95 % CI)
≥75	Ile/Ile	Low/intermediate	589	94	1.0
≥75	Ile/Val	Low/intermediate	720	132	1.19 (0.88–1.61)
≥75	Val/Val	Low/intermediate	215	29	0.94 (0.59–1.50)
≥75	Ile/Ile	High	141	23	1.03 (0.61–1.71)
≥75	Ile/Val	High	158	31	1.35 (0.85–2.15)
≥75	Val/Val	High	38	14	2.63 (1.34–5.18)
<75	Ile/Ile	Low/intermediate	144	23	1.01 (0.60–1.69)
<75	Ile/Val	Low/intermediate	171	28	0.89 (0.54–1.44)
<75	Val/Val	Low/intermediate	48	11	1.46 (0.71–3.03)
<75	Ile/Ile	High	31	9	1.71 (0.75–3.87)
<75	Ile/Val	High	30	12	2.61 (1.22–5.58)
<75	Val/Val	High	5	6	8.91 (2.40–33.12)
					p = 0.04[c]

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[a]Children with missing data on distance of residence from major road and GSTP1 Ilel05Val were excluded

[b]ORs adjusted for age, sex, race/ethnicity, in utero exposure to maternal smoking, number of smokers at home, community of residence, parental education, health insurance and parental history of asthma

[c]p value for EPHX1 activity phenotype by distance of residence from a major road and by GSTP1 Ile105Val genotype interaction was obtained from likelihood ratio test from a non-stratified model with appropriate interaction terms and was based on 7df

compared to children who lived beyond 75 m of a major road and were GSTP1 wild type homozygous (*ile105ile)* with low/intermediate activity EPHX1 metabolic phenotype. In fact a dose dependent effect of the genetic polymorphisms and TRAP exposure was observed for asthma prevalence in children. The finding was replicated in a cross-sectional study of Taiwanese children (Tung et al. 2011). This study exemplifies many of the rationale of GxE studies we have mentioned earlier:

- Demonstrates the importance of PAH metabolism pathway in TRAP mediated risk of asthma
- Identification of susceptible population to TRAP exposure: children who are GSTP1 variant (Val) homozygous and EPHX1 high metabolic phenotype (~11 % of US children)
- Identifies PAH as the important chemical of TRAP, which is a complex mix of chemicals, as the risk factor for asthma following TRAP exposure.

12.3 Modification of AP Effect on Respiratory Outcomes by Genetic Factors

Inflammation, both chronic and acute, are central features of many common AP mediated lung conditions, such as asthma, COPD, or decline in lung function (Rahman et al. 2006; Adcock et al. 2005; Rahman and Adcock 2006; Adcock and Lee 2006). The respiratory tract endures the primary insult from air pollutants as air is inhaled into the lungs, thus it also provides the first line of defense against the air pollutants, as has been discussed in Chap. 5. In response to the environmental insults, as well as endogenous metabolism, reactive oxygen species (ROS) and free radicals are formed in the airway (Rahman et al. 2006). The airway, specially the lungs serves as the first line of defense against the effects of air pollutants. The fluid lining of the lung tissue is rich in enzymatic anti-oxidants (i.e., Glutathione-S-transferases (GSTs), heme oxugenase-1 (HMOX1), catalase (CAT), superoxide dismutases (SODs) and others) and non-enzymatic (i.e., vitamin C & E, glutathione (GSH) and other low molecular weight particles) that can neutralize the oxidative effects of air pollutants. Normally a balance is attained between the oxidative and anti-oxidative forces; however, when the anti-oxidative activity is overwhelmed due to exogenous (i.e., high level of air pollutants) or endogenous (i.e., respiratory infection) factors, oxidative stress ensues. The excess ROS can cause local damage to the lung parenchyma leading to a pro-inflammatory state in the lung tissue. Inflammation itself being an oxidative process starts a vicious cycle of oxidative stress-inflammation-oxidative stress (Fig. 12.1). Therefore, healthy abundance of anti-oxidant and anti-inflammatory repertoire in the lung tissue can provide great defense against the air pollutants mediated oxidative stress in the airway; whereas, a lack of such defense can make the lungs susceptible to the air pollutants.

Most of the genetic and gene-environmental works related to AP and respiratory outcomes have focused on the oxidative and inflammatory pathway in the airways.

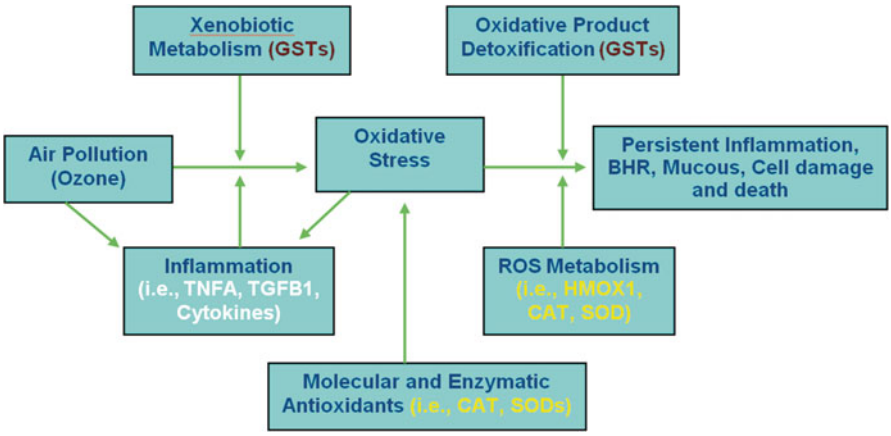

Fig. 12.3 Schematic representation of AP mediated health effects in lung tissue and its possible mediators. Inhalation of air pollutants such as ozone, PM, NOx, or PAHs, can lead to oxidative stress and inflammation of the lung tissue

The framework for AP mediated respiratory health outcomes and it's interaction with the genetic factors is depicted in Fig. 12.3. Air pollutants such as ozone or diesel exhaust upon entering the airway can cause oxidative stress in the lung tissue that if uncontrolled can lead to acute and chronic inflammation of the lung leading to asthma, COPD, decline in lung functions and other ailments. The detrimental effect of the air pollutant depends on the level of exposure and the susceptibility of the lung tissue. The availability of enzymatic and not-enzymatic anti-oxidants in the lung tissue and the inflammatory response mechanism are the major factors in the determination of the individual susceptibility to the effects of air pollutants.

High level of exposure to AP may cause little detrimental effect in presence of abundant anti-oxidants (i.e, HMOX-1, CAT, SODs, & GSTs) repertoire in the lung fluid as well as a well controlled inflammatory mechanism. On the contrary, low level exposure to AP can lead to substantial damage to the lung tissue if there is deficiency in the anti-oxidant activity and hyperactivity of the inflammatory mechanism. Therefore, genetic factors that can affect the level of anti-oxidative activity or inflammation level in the lung have been the genes of choice to investigate gene-environment interaction in the candidate GxE approach.

12.3.1 Anti-oxidant Genes and AP

12.3.1.1 Overview

The most commonly studied genes in the anti-oxidant pathway are the GSTs, HMOX1, NAD(P) Dehydrogenase Quinone 1 (NQO1), CAT and SODs. Figure 12.4 presents a schematic representation of the role of these enzymes in the anti-oxidative

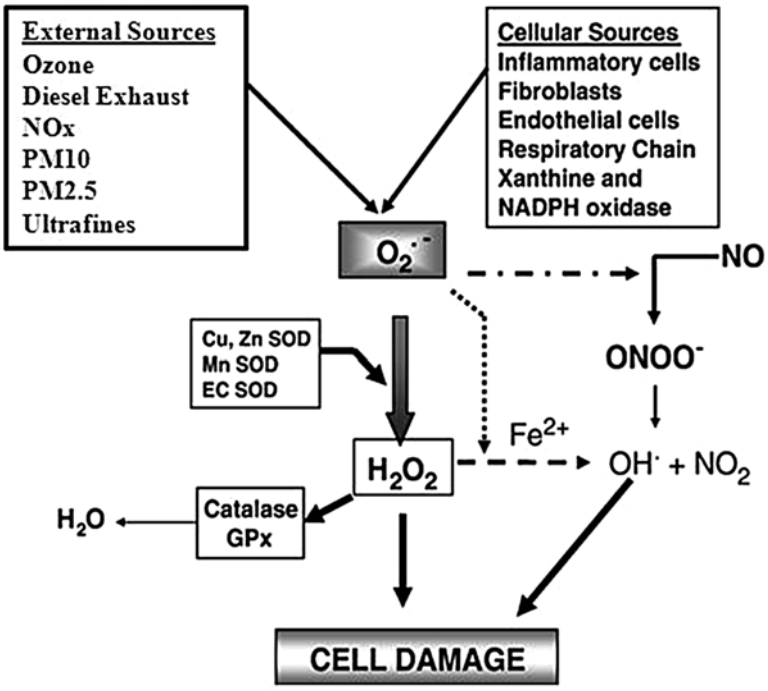

Fig. 12.4 Endogenous and exogenous sources of ROS and cellular anti-oxidative machinery. Intracellular reactive oxygen species $O_2^{\cdot -}$ = superoxide anion, NO = nitric oxide, H_2O_2 = hydrogen peroxide, ·OH = hydroxyl radical, NO_2 = nitrogen dioxide, ONOO– = peroxynitrite, Fe2+= Ferrous oxide, GPx= Glutathione peroxidase (Reprinted from Rahman et al. (2006), Copyright (2006), with permission from Elsevier)

defenses in response to the formation of different intracellular reactive oxygen species. The physiologically important ROSs are superoxide anion ($O_2^{\cdot -}$), hydroxyl radical (OH^-), nitric oxide (NO) and hydrogen peroxide (H_2O_2) with OH^- being most reactive and damaging to the cells (Rahman et al. 2006). The SODs, GSTs, and possibly HMOX-1 act as ROS scavengers in the cell and protects the cell by reducing the availability of ROS to cause cellular damage. SODs form H_2O_2 from two superoxide anions and two hydrogen ion. Catalase neutralizes H_2O_2 by converting it to water. Therefore the anti-oxidative activity of SOD is dependent on catalase/GPx availability and activity. The role of HMOX1 as an anti-oxidant and cytoprotective factor is more complex and enigmatic (Rahman et al. 2006). HMOX1 is purported to exerts its anti oxidant effect by (i) formation of bilirubin from heme protein which is readily converted to biliverdin, potent anti-oxidant (Stocker and Ames 1987; Stocker et al. 1987), (ii) regulating Fe^{2+} transfer (Ferris et al. 1999), and (iii) formation of CO that stimulates a number of anti-oxidant activity (Otterbein et al. 2000).

The GSTs are a group of Phase II iosenzymes that removes secondary ROSs, including products of lipid peroxidation and xenobiotics (i.e., PAH from diesel

exhaust), by catalyzing their conjugation with reduced GSH. The conjugates are sequestered and removed from the cell. The GST superfamily is highly diverse. Based on their substrate specificity, structure, and kinetic behavior eight distinct classes are defined: Alpha (GSTA), Kappa (GSTK), Mu (GSTM), Omega (GSTO), Pi (GSTP), Sigma (GSTS), Theta (GSTT) and Zeta (GSTZ). Each of these classes has subgroups. GSTM1, GSTP1 and GSTT1 have been most studied in the context of AP and environmental effect (Minelli et al. 2011).

12.3.1.2 Genes and the Commonly Studied Polymorphism

GSTs: The GST genes are located in different chromosomes and these are highly polymorphic. The commonly studied *GSTM1*, *GSTP1*, and *GSTT1* is located in the 1p13.3, 11q13, and 22q11.2 region. The most commonly studied functionally relevant polymorphisms are deletion allele of *GSTM1* and *GSTT1* and Ile105Val (rs1695) polymorphism of *GSTP1*. The *GSTM1* and *GSTT1* deletion is often studied as homozygous deletion ('null' genotype) that is associated with total absence of enzymatic activity. The functional effect of *GSTP1*-val variant in more complex as the increased or decreased activity of the polymorphism is substrate specific (Hu et al. 1997; Sundberg et al. 1998) and its effect on asthma can be age specific (Islam et al. 2009). Early onset asthma (by 6 years of age) appears to be associated with the *val*-allele; whereas late onset asthma (after 9) appears to be associated with *Ile*-allele. The inducibility of inducible *HMOX-1*, located in 22q12, is inversely associated with the length of a $(GT)_n$ tandem repeat in the 5′ flanking (promoter) region of the gene (Chen et al. 2002; Hirai et al. 2003; Yamada et al. 2000). The functional 609C>T polymorphism of *NQO1*(16q22.1) results in a serine variant that reduces the activity of the enzyme. Subjects with homozygous variant (Ser/Ser) has no detectable enzyme activity and the enzymatic activity among the heterozygous (Ser/Pro) is significantly lower to those with wild type homozygous (Pro/Pro) (Siegel et al. 1999). Paradoxically, epidemiologic studies observed that Ser allele renders protection against ozone. The observed effect of this polymorphism initially appears paradoxical as the Ser allele provides protection against asthma among children with *GSTM1*-null genotype (David et al. 2003). This is biologically plausible as beside its anti-oxidant activity, NQO1 also catalyzes the bioactivation of quinones to more reactive hydroquinones which are potent oxidants (Minelli et al. 2011). Because NQO1 can activate nitroaromatic compounds and heterocyclic amines present in diesel exhaust (Hajos and Winston 1991a, b; Nakagawa et al. 1983), Ser allele that reduces the enzymatic activity of NQO1 might be protective under certain conditions. Similar to oxidative stress, nitrosative stress mediated through NO can also play role in airway inflammation (Baraldi et al. 2006; Ricciardolo et al. 2006a, b). Genes encoding nitric oxide synthase (NOS) and arginases (ARG) are of particular interest. (Islam et al. 2010; Salam et al. 2009)

12.3.1.3 Gene, AP on Respiratory Health Outcomes

There has been extensive interest in the scientific community to identify genetic variants modifying the effect of air pollutants on health. As the field is quite young, there have been a limited number of studies providing evidence for gene-AP interaction. In 2011, Minelli et al. systemically reviewed 17 articles from 15 different studies that were published prior to April 30 2009 (Minelli et al. 2011). Of the 17 articles reviewed, five were published between 2001–2005 and the other 12 between 2006–2010. Romieu et al. also reviewed 13 articles on children gene-AP interaction that were published prior to April 2009 (Romieu et al. 2010), many of these studies were also in the review of Minelli et al. Following the same MEDLINE search strategy as Minelli et al., a total of 32 studies could be identified with five publications between 2001–2005, 18 between 2006–2010, and 9 between 2011–2013. This shows a rapid growth in this nascent field of gene-AP.

GSTs (*GSTM1, GSTP1 or GSTT1*) were investigated in 18 of those 32 studies. An array of APs were considered including indoor particulate matter to outdoor ozone, particulate matter and TRAP often based on modeled oxides of Nitrogen (Gauderman et al. 2009). Bergamaschi and colleagues (Bergamaschi et al. 2001) first reported on the possible modifying effect *GSTM1* of ozone on lung function. They performed a cross-over experimental study on 24 healthy non-smoker participants. The participants were randomly exposed to above and below 80 ppb ozone. Exposure to ozone level above 80 ppb was associated with decrease in lung function measures and increase in oxidative stress marked by high level of Clara cell (CC18) protein and decreased 8-Hydroxy-2′-deoxyguanosine a biomarker of ROS-DNA interaction. All those associations were much stronger in *GSTM1* and *NQO1 wild type.* Similar joint modifying effect of *GSTM1 null* and *NQO1 wild type* in modifying the effect of biomarker of oxidative stress (Corradi et al. 2002) and lung function (Chen et al. 2007) was substantiated by other studies (Corradi et al. 2002; Chen et al. 2007). Based on their study of young college students, Chen et al. (2007) reported a sex specific modifying effect of the anti-oxidant genes. The detrimental effect of chronic ozone exposure on lung function was modified by *GSTP1 val105* allele in males and the *GSTM1 null-NQO1* genotype in the male. It should be noted that 19 of the 24 participants in the study reported by Bergamaschi et al. (2001) were female. David et al. reported from their 218 case-triads (case + parents) a protective role of the variant *NQO1(Ser)* genotype against asthma among *GSTM1-null* carriers. Although, there was no formal test of interaction for any air pollutants, the authors argued that the observed effect is possibly due to the high background ozone level and particulate mass in Mexico city (David et al. 2003). Although, David et al. did not provide relevant pollution data with appropriate statistical testing to validate their claim; the overall hypothesis is supported by experimental findings of Gilliland et al. (2004). Gilliland and colleagues conducted a single blind placebo controlled cross over study exposing ragweed sensitive study participants in presence of clean air and diesel exhaust. The researchers observed increased level of nasal allergic responses following exposure to diesel exhaust with further amplification of the effects of diesel exhaust among carriers of *GSTM1-null* or *GSTP1-ile*

genotypes. Most pronounced effect was observed among subjects who were both *GSTM1-null* and *GSTP1-ile* genotype carriers. The finding certainly supports the claim by David et al. (2003) that the observed effect in children of highly polluted Mexico City might be due to the effect of ozone and traffic exposure. The group did a follow up study that sheds more light on the purported gene-environment interaction in childhood asthma. Among children with asthma, the researchers observed that recent exposure to high level of ozone was associated with increased breathing difficulties in children with *GSTP1-val* genotype and most pronounced among those with *GSTM1-null* and *GSTP1-val* genotype (Romieu et al. 2006). The group also conducted a clinical trial investigating whether anti-oxidant supplementation (Vit-C and Vit-E) can protect against ozone induced lung function decline. The detrimental effect of ozone was most pronounced among children with *GSTM1-null* genotype who most benefitted from the anti-oxidant supplementation (Romieu et al. 2004). This was confirmed in a recent follow up study (Moreno-Macias et al. 2013).

It is interesting to note that only a few studies reported that *GSTM1*-null alone modifying the effect of AP on respiratory outcomes (Romieu et al. 2004; Moreno-Macias et al. 2013; Alexis et al. 2009). Kim and colleagues (2011) conducted a double blinded experimental study to investigate the effect of ozone exposure on lung function and the possible modifying effect *GSTM1*. They selected 59 healthy adults 19–35 years of age without any history of smoking and performed a double blinded experiment to investigate the effect of ozone exposure on lung function (FEV1 and FVC). Spirometry and blood samples were taken after study participants were exposed to clean air (0.00 ppm ozone) or 0.06 ppm ozone randomly for a period of 6.6 h following moderate level of exercise. Compared to clean air, exposure to 0.06 ppm ozone for 6.6 h lead to decrease in both FEV1 and FVC, pulmonary inflammation marked by increase in polymorphonuclear neutrophils. No statistically significant modifying effect of *GSTM1* was observed for either lung function measures or pulmonary inflammation. Although, in the *GSTM1 null* showed a detrimental effect following ozone exposure compared to clean air, the detrimental effect was no different from that observed for *GSTM1 present* group. Also other studies involving children with asthma (Newcomb et al. 2012; Vagaggini et al. 2010) did not show any modifying effect of *GSTM1-null* on ozone induced airway responses. The modifying effect of *GSTM1* appears to be dependent on health status, exposure conditions, health outcome, and the overall anti-oxidant repository.

The experimental work by Gilliland and colleagues was further followed through utilizing the resources of Children Health Study, a large cohort (~6,000 starting in 1993 (Peters et al. 1999a, b) and another ~5,000 in 2003 (McConnell et al. 2006)) of school aged children (6–18 years of age). Given the large sample size, extensive genetic and exposure data, and longitudinal nature of the study we could investigate gene-AP interaction on both prevalent and incident asthma as well as lung function in this study. In 2002, McConnell et al., reported an important but controversial finding that participating in high level of sports activity (>2 team sports) in high ozone communities (24-h ozone > 30.7 ppb and 8-h ozone (10 am-6 pm) >55.8 ppb) was associated with increased incidence of asthma in children (50/1,000 person year) compared to low ozone communities (asthma incidence ~ 19–33/1,000 person

year) (McConnell et al. 2002). This was followed up to investigate whether the joint effect of participating in team sports and exposure to high level of ambient ozone is further modified by genetic susceptibility (Islam et al. 2009). In this study, both *GSTM1-null* and *GSTP1-Ile* genotype was associated with increased risk of incident asthma; however, the modifying effect was observed only for *GSTP1-Ile* genotype. The increased risk of incident asthma for high outdoor sports mostly restricted to those who were *GSTP1*-Ile *homozygous* (p-value of interaction = 0.02). The carriers of *GSTP1 Ile/Ile* who lived in high ozone communities and were involved in high sports activity were almost sixfold more likely to develop asthma compared to any other groups (Fig. 12.5). As we discussed earlier, the direction of effect for *GSTP-1* Ile105Val might be age and exposure dependent. In a cross sectional study from Taiwan, *GSTP-1* Ile allele appeared to provide protection against asthma and wheeze following exposure to $PM_{2.5}$ and ozone (Hwang et al. 2013). In this study, a strong correlation between $PM_{2.5}$ and ozone ($r = 0.73$) was observed and the interaction appeared to be stronger for $PM_{2.5}$ compared to ozone. Therefore, the observed effect might be due to $PM_{2.5}$, rather than ozone. In a birth cohort study, allergic sensitization in the first four years of life following exposure to modeled NO_x (surrogate measure of TRAP) was most apparent among carriers of *GSTP1-val* allele (Melen et al. 2008). Other studies involving TRAP exposure also noted *GSTP1-val* allele as the risk allele (Salam et al. 2007; Tung et al. 2011).

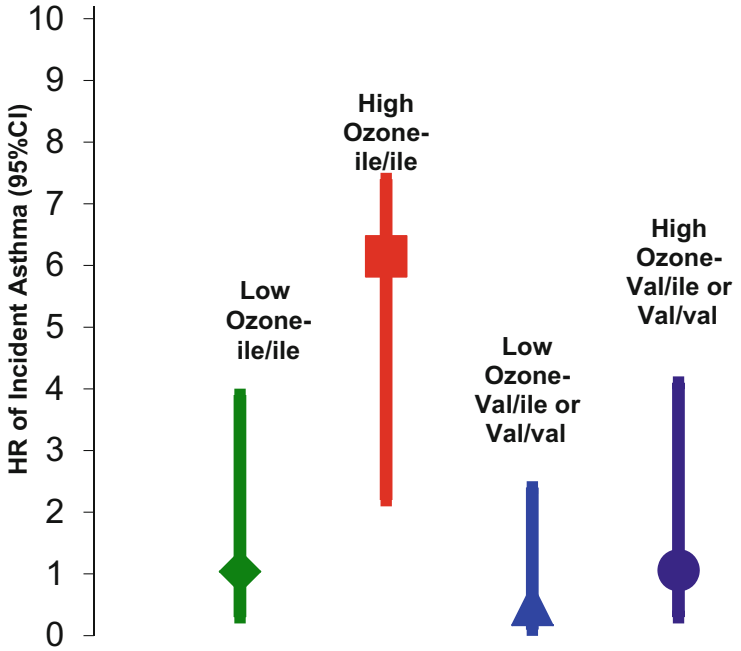

Fig. 12.5 Effect of ozone and GSTP1-Ile allele on asthma incidence among children participating in >2 team sports

Another anti-oxidant polymorphism that has shown some consistent effect have been the (GT)n tandem repeat of *HMOX1*. In the Children Health Study, an ethnicity specific effect of the tandem repeat was observed (Islam et al. 2008). Based on functionality, the *HMOX1* with alleles 23 or less $(GT)_n$ repeats were defined as 'Short' (S) in the study. Non-Hispanic Whites (40 %) were more likely to have one *S-allele*, compared to Hispanic Whites (28 %). Although, no association was observed between the *S-allele* and asthma, *S-allele* appeared to be protective against new onset asthma in non-Hispanic children (Hazard ratio (HR): 0.64, 95 % confidence interval (CI):0.41–0.99) but not among Hispanic Whites (HR: 1.25, 95%CI: 0.64–2.47). Among the non-Hispanic Whites, the *S-allele* appears to modify the effect of ozone on asthma risk (interaction p-value = 0.003). The *S-allele* appears to be more protective in the low-ozone communities (HR = 0.44) compared to high-ozone communities (HR: 0.88). Although, PM_{10} exposure appeared to be associated with increased risk of asthma in children without any *S-allele* (HR: 1.79, 95%CI: 0.69–4.61) compared to those with *S-allele* (HR: 0.62, 95%CI: 0.20–1.87); no statistically significant interaction was observed (p-value of interaction: 0.18). A large cohort study (N = 4,365) of Swiss Caucasian adults (18–65 years of age) (the SAPALDIA study) reported that the beneficial effect of PM_{10} reduction on lung function was modified by *HMOX1* polymorphisms including the $(GT)_n$ repeat (Curjuric et al. 2010). Each 10 μgm–m^3 decrease of PM_{10} level was associated with a 26.5 (11.7–41.2) mL-sec^{-1}-$year^{-1}$ improvement of $FEF_{25–75}$ values compared to 11.7 (95%CI: 4.5–19.2) mL-sec^{-1}-$year^{-1}$ among those without any long allele. Although the definition of short and long allele differed between the CHS and SAPALDIA study, both noted the presence of shorter allele in each population to be protective against the detrimental effect of air pollutants (ozone and PM_{10}).

Although, GSTT1 (Hong et al. 2007; Castro-Giner et al. 2009), CAT (Islam et al. 2008; Wenten et al. 2009), and *SOD* (superoxide dismutase) (Islam et al. 2008; Yang et al. 2005) have been studied in the context of gene-environmental interaction for respiratory health outcomes, currently limited evidence exists for such modifying effect. In the CHS cohort, a statistically significant three-way interaction was observed between a haplotype (based on 8 tag-SNPs) of *ARG1*, atopy, and ozone exposure for asthma risk (Salam et al. 2009). Presence of each copy of the *ARG1* haplotype was associated with 0.55 fold reduction in asthma risk among children; however the protective effect was much larger among children with atopy and living in high ozone communities (Odds Ratio (OR):0.12, p-value of interaction = 0.008).

12.3.2 Inflammatory Genes and AP

Exposure to air pollutants leads to airway inflammation either indirectly due to oxidative stress or directly due to induction of inflammation that plays an important role in air pollutant induced detrimental respiratory health effects (Kelly 2003; Tubby et al. 2011). Air pollutants like ozone, particulate matter or oxides of nitrogen cause activation of transcription factors such as nuclear factor – κB (NF-κB),

resulting in increased expression of different cytokines, chemokines, and adhesion molecules that can lead to a proinflammatory state. Both innate and adaptive immune responses within the lung contribute to the inflammatory process (Tubby et al. 2011). Therefore, genes of the inflammatory pathway have often been considered as potential modifier of AP effect on respiratory health. Animal studies suggest that tumor necrosis factor α (TNF-α) and toll like receptor (TLR4) are possible susceptible region to ozone effect (Cho and Kleeberger 2007; Hollingsworth et al. 2004; Kleeberger et al. 1997).

A functional polymorphism of TNF-α, *TNF-α G308A* (rs1800629), where the variant allele can lead to higher level of TNF-α: a proinflammatory cytokine, has been reported to modify the effect of air pollutants (Melen et al. 2008; Yang et al. 2005; Li et al. 2006; Lee et al. 2009). Yang et al. reported that there is a decline in FEV_1 following acute exposure to varying level of ozone (400 ppb for 2 h to 200 ppb for 24 h) during moderate exercise, and the decline differed by *TNFαG308A* status (Yang et al. 2005). The percentage decline following the ozone exposure was about 3 % among those with at least one *A*-allele; whereas the decline was almost 9 % among *G/G* individuals. On the contrary, in the CHS the *G*-allele was identified as a protective factor for asthma and wheezing in children (Li et al. 2006). The protective effect of the *G*-allele was most pronounced among children living in the low ozone communities. The GG homozygous children were 50 % less likely to ever suffer from wheezing compared to GA or AA carriers in the low ozone communities; however, there was no difference in risk of wheezing based on *TNFG308A* status in the high ozone communities. Similar protective effect of the *GG* allele for bronchitic symptoms in children with asthma living in low zone communities but not in high ozone communities have been reported from Taiwan (Lee et al. 2009). Melena et al. reported that children with *GSTP1-val* allele had almost 2.5 fold increased risk of sensitization when exposed to elevated level of NOx; whereas, no heighted risk of sensitization was reported for *GSTP1-Ile* homozygous (Melen et al. 2008). This GSTP1-NOx interaction was mostly limited to those with *TNF-308 A* allele (OR = 22), with no substantial effect among the *GG* homozygous. Except for the study reported by Yang et al., which was unique being an experimental study in adults, a consistent protective effect of *TNF-308 GG* homozygous against air pollutant mediated health effect is observed.

Toll like receptors are important gatekeepers of host immunity in response to gram-positive (TLR2) and gram-negative (TLR4) bacteria. Therefore they can play important role in AP mediated airway inflammation as pathogens can get access to lower airway with inhaled particulate matter. Kerkhof and colleagues (2010) investigated whether *TLR2* and *TLR4* modifies the susceptibility to AP mediated asthma in the well established birth cohort of Prevention and Incidence of Asthma and Mite Allergy (PIAMA). They identified multiple polymorphisms of *TLR2* and *TLR4* to increase the susceptibility of prevalent asthma following exposure to $PM_{2.5}$ and soot.

12.3.3 Summary

The respiratory tract being the first system to deal with the onslaught of air pollutants, gene-AP studies have mostly focused on respiratory diseases. In the process multiple genes such as *GSTM1, GSTP1, HMOX1, NQO1, TNF-α* and *TLRs(TLR2 & TLR4)* have been identified to modify individual susceptibility to air pollutants in respect to airway diseases. The observations from the epidemiological studies often fall short to provide conclusive evidence, owing to methodological and biological issues discussed later in this chapter. The genetic factors discussed in the context of AP and respiratory health also plays a role in other health outcomes because the systemic effect of air pollutants often starts from lung parenchyma (Brook et al. 2009, 2010).

12.4 Gene, AP and Cardiovascular Health

Over time epidemiological studies have shown the impact of AP on cardiovascular health as has been discussed in Chaps. 8 and 9. Acknowledging the importance of AP, specially particulate matter in cardiovascular morbidity and mortality, the American Heart Society first issued scientific statement underlining the importance of a better understanding of the role of particulate matter in cardiovascular disease (CVD) (Brook et al. 2010). Despite the accumulating evidence of AP mediated cardiovascular health effects; the biological mechanism is not well understood. The possible biological pathway for AP mediated health effects include (i) systemic inflammation following oxidative stress and inflammation in the lungs (Systemic spillover effect), (ii) imbalance of the automatic nervous system (ANS) following activation of respiratory ANS reflex arc and (iii) direct effect of the ultra-fine particles (UFP) that can pass from through the alveolar membrane and reach blood from alveolar air (Brook et al. 2009, 2010).

12.4.1 Gene, AP, and CVD: Human Studies

Unlike respiratory health outcomes, the gene-AP studies in CVD are extremely limited. In a review by Zanobetti et al. (2011) reviewed 16 publications in the context of gene-AP interaction in CVD, were from three cohort studies. Thirteen were from the Normative Aging Study (NAS), two from AIRGENE, and one from MESA study. Only the MESA study had racial/ethnic diversity with NAS and AIRGENE being totally Caucasian population. Sex representation were also highly skewed in those studies, with 100 % of NAS participants being male, 80 % of AIREGENE and about 48 % of MESA. Average age range in these studies was 40–85. Most of the studies investigated the effect of short term pollutant exposure rather than chronic AP exposure (Van Hee et al. 2010). The health endpoints also varied widely in these

studies, ranging from biomarkers such as plasma homocysteine (Ren et al. 2010a) or heart rate variability (HRV) (Ren et al. 2010b; Park et al. 2006) to disease endpoints such as blood pressure (Wilker et al. 2009) and left ventricular mass (Van Hee et al. 2010). Homocysteine concentrations at high levels are an independent risk factor for cardiovascular disease. Elevated levels in the blood may be associated with atherosclerosis, stroke, blood clot and heart attacks. Exposure to air pollutants are associated with decreased heart rate variability and related markers of oxidative stress may play an important role in cardiac toxicity of particles.

The oxidative stress genes had been the early focus of gene-AP studies in CVD. In 2005, Schwartz and colleagues reported an increased susceptibility to $PM_{2.5}$ mediated decreased variability in HRV among *GSTM1-null* individuals with no apparent effect of $PM_{2.5}$ on HRV among *GSTM1-positive* individuals (Schwartz et al. 2005). Each 10μgm-m^{-1} increase in $PM_{2.5}$ was associated with 34 % (95%CI: −9 to −52 %) decrease in HRV among *GSTM1-null* carriers; whereas, no effect was observed among *GSTM1-positive* individuals. The detrimental effect of $PM_{2.5}$ was even more prominent among *GSTM1-null* individuals who did not use statin or had high level of neutrophil or were obese. This initial provocative finding was followed up by multiple investigations in the NAS exploring the role of oxidative-stress genes as potential modifiers for AP mediated CV outcomes (Ren et al. 2010a; Park et al. 2006, 2009; Baja et al. 2010; Mordukhovich et al. 2009; Chahine et al. 2007). Chahine et al. (2007) investigated the role of the *HMOX1* promoter region (GT)n repeat in addition to *GSTM1*, modifying the effect of $PM_{2.5}$ on HRV. They observed no statistically significant interaction for *GSTM1* (interaction p-value: 0.13, although HRV decrease in response to $PM_{2.5}$ was significant only in the *null* group) and the interaction p-value for *HMOX1* was 0.06. The largest detrimental effect of $PM_{2.5}$ was observed among the *GSTM1-null* individuals with long(GT)n repeats ($\geq$25) (p-value of interaction $<$ 0.04). This modifying effect of *HMOX1* was supported by two other studies from the same group in the context of tibia lead level and QT interval (Park et al. 2009) and $PM_{2.5}$ and plasma level of homocystiene (Ren et al. 2010a); however, not in the context of exposure to black carbon (BC)/$PM_{2.5}$ and blood pressure (Mordukhovich et al. 2009). In the elderly male population of NAS, 1-standard deviation increase in 7-day moving average of BC from the time of clinical examination was associated with 1.46 mmHg increase in systolic blood pressure (SBP) and 0.87 mmHg increase in diastolic blood pressure (DBP); however, this detrimental effect of BC was not modified by any of the tested polymorphisms of the anti-oxidant genes: namely, *GSTM1-null*, *GSTT1-null*, *GSTP(Ile105Val)*, *HMOX1 tandem repeat, NQO1(C609T)* and three SNPs of *CAT* (Mordukhovich et al. 2009). In another study from the NAS, the effect of ambient $PM_{2.5}$ on changes in the postural blood pressure was modified by genetic variants of genes (PHD finger protein 11 (PHF11), matrix metalloprotease 1 (MMP1) and inositol 1,4,5-triphosphate receptor, type 2 (ITPR2) in the renin-angiotensin pathway (Wilker et al. 2009).

The AIRGENE study recruited 1,031 survivors of myocardial infarctions (MI) to investigate the role of particulate exposure on inflammation in this vulnerable population. The researchers were interested in fibrinogen and IL-6 as fibrinogen is

an essential component in cardiovascular related inflammation and coagulation and established risk factor of CVD (Danesh et al. 2005) and IL-6 is key regulator of fibrinogen (Fuller and Zhang 2001). Among these survivors of MI, different measures of TRAP related pollutants were associated with elevated plasma level of IL-6 and fibrinogen, underlining the acute effect of particulate matter in MI related inflammation (Ruckerl et al. 2007; Peters et al. 2009; Ljungman et al. 2009). This effect of the TRAP on plasma IL-6 and fibrinogen level was modified by multiple genetic variants of fibrinogen beta-chain gene (FBG). In a sub-cohort of 2,880 of the MESA study, researchers investigated the possible modifying effect of genetic variants of 12 different genes on the detrimental effect of TRAP on left ventricular mass, a strong marker of adverse cardiovascular outcomes (Bluemke et al. 2008). Of the 12 different genes investigated, genetic variants of type 1 angiotensin II receptor (*AGTR1*) gene and 15-lipoxygenase gene (*ALOX15*), modified the effect of residential proximity, a marker of TRAP, on LVM (Van Hee et al. 2010). Those genes are involved in vascular inflammation, oxidative stress, and vasoconstriction.

12.4.2 Summary

One of the major concerns regarding the current state of evidence for gene-AP interactions for CVD is lack of replication of findings across studies. The lack of diversity and the different endpoints and exposures of interest in the studies are certainly a major limitation in the replication of findings across studies. While NAS and AIRGENE were optimized to identify acute (hours) to sub-chronic (days) effect of air pollutants, MESA is best positioned to investigate chronic exposure (i.e., residential distance from major roads). The endpoints investigated also differed considerably between the studies, ranging from HRV, blood pressure, QT intervals and markers of inflammation. Although, these outcomes are related to each other on a continuum, the genes and pathways involved can differ considerably thus making it difficult to have replication across these studies. There is also considerable variation between study population make replication of findings quite difficult between those studies.

Despite those limitations, both the NAS and the AIRGENE study provided evidence that genetic variants of genes in the rennin-angiotensin pathway may modify the effect of air pollutant on CV outcomes. All these studies investigated the short term effect of air pollutant on CV health outcomes. The observations of a consistent effect of *GSTM1(null)* and *HMOX1 (GT)n* repeat in modifying the effect of different air pollutants on multiple CV health outcomes within NAS provides confidence to the observed effect, specially as the findings were in line with the reported respiratory effects (Sect. 12.3.1). Further studies investigating gene-AP for CV outcomes utilizing both candidate gene approach and GWIS are required to reach a definitive conclusion.

12.5 Gene, AP and Non Cardio-respiratory Health Outcomes

Understanding the role of AP in the causation of health effects beyond cardio-respiratory health outcomes is relatively new and there are only a limited number of studies investigating gene-AP interaction on such health outcomes. One major interest has been the role of genes and AP in pregnancy outcomes.

There is substantial body of evidence noting the importance of AP specially ozone, TRAP, or CO in adverse pregnancy outcomes, such as low birth weight (LBW), preterm birth or intrauterine growth retardation (IUGR) (Backes et al. 2013; Stieb et al. 2012; Misra et al. 2012; Shah and Balkhair 2011; Bonzini et al. 2010). Because oxidative stress as well as reduced oxygen supply to the fetus are the presumed mechanism for AP mediated adverse pregnancy health outcomes, the aforementioned sets of genes (Sect. 12.3.1.2) have been investigated as the usual suspects for gene-AP interaction. In 2008, Suh et al. (2008) reported on the possible modifying effect of three xenobiotic genes, *GSTM1 (null), GSTT1(null),* and cytochrome P450IA1 (CYP1A1) on PM_{10} mediated preterm delivery (before 37th week). The researchers recruited 117 women with preterm delivery and living in Seoul during 2003–2007 and matched them randomly with 118 women who had full term delivery and were living in Seoul. All women were recruited from the same hospital. An increased risk for preterm delivery was observed for women with *GSTM1(null)* genotype compared to positives (OR > 2.0, p-value < 0.008) and women who were exposed to high level of ambient PM_{10} (≥75th percentile) during the 3rd trimester compared to those with lower level of exposure (OR > 2.3, p-value 0.02). The effect of PM_{10} on preterm birth appeared to be modified by maternal *GSTM1* status. The risk of preterm delivery was almost 6 fold greater (OR: 6.22, p-value = 0.001) among women who were *GSTM1(null)* and were exposed to high level of PM_{10} compared to those who were *GSTM1* positives and were exposed to low level of PM_{10} during the last trimester; however, no overall p-value for test of interaction was not available. Although the data has been adjusted for a number of confounders, it was not adjusted for maternal respiratory infection or asthma/wheeze status. It is possible that *GSTM1(null)* women exposed to high level of pollutant experienced more respiratory problems leading to oxygen deficiency to both the mother and the fetus resulting in preterm birth. In 2011, Rossner and colleagues (2011) also reported possible gene-AP interaction on pregnancy outcomes. Data on 1200 women were randomly selected from a large case control study, but, genetic (placental), AP and covariate information was available only for 891 women. Unlike the South Korean study, the researchers investigated the role of placental genes in modifying the effect of $PM_{2.5}$ and PAH on IUGR and LBW in this study. Besides *GSTM1 a*nd *GSTT1*, they also investigated the possible role of 94 (577 SNPs) other genes using an *Illumina* array. They failed to observe any statistically significant interaction between AP and *GSTM1* or other genetic polymorphism in this study. The identification of gene-AP interaction for pregnancy outcomes are specially

challenging as both maternal, placental and fetal physiology and genetics can play important role as well as the time and type of exposure. Therefore, well designed studies are needed.

12.6 AP and Epigenetics

Epigenetics is the study of mitotically or meiotically heritable states of gene expression potential that occur without directly altering the DNA sequence. DNA methylation, histone modifications and miroRNAs are major types of epigenetic variations that are currently being investigated in relation to AP and health outcomes. The epigenetic process is schematically presented in Fig. 12.6, and briefly described in the following paragraphs. (for further reading: Yang and Schwartz (2012)).

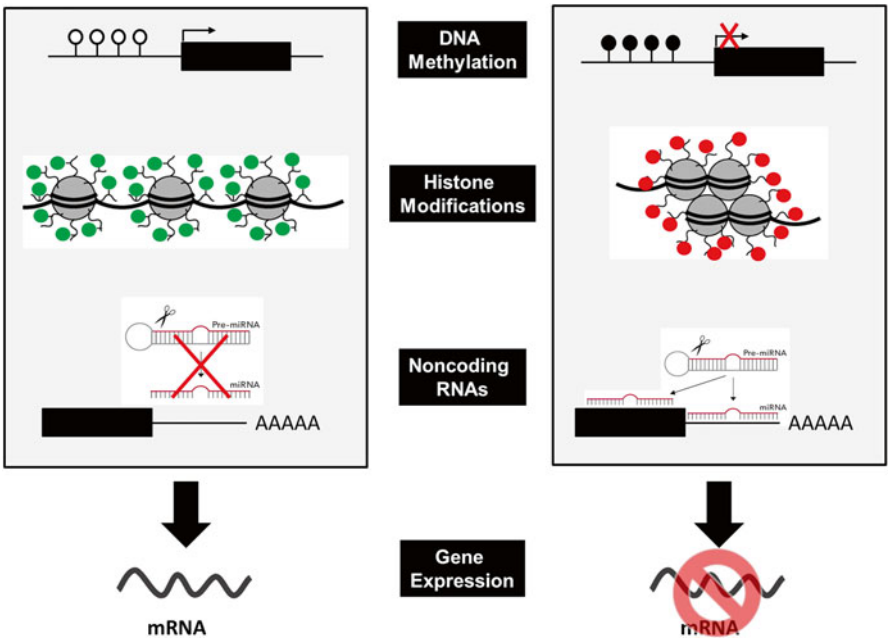

Fig. 12.6 Effect of epigenetic marks (DNA methylation, histone modifications, and miRNAs) on gene expression. *White circles* denote unmethylated CpGs, and *black circles* denote methylated CpGs. *Green circles* refer to permissive histone modifications, and *red circles* indicate repressive histone marks. miRNAs can affect gene expression through either RNA degradation (perfect complementarity and binding) or inhibition of protein translation (imperfect complementarity and partial binding) (Reprinted from Yang and Schwartz (2012), Copyright (2012), with permission from Elsevier)

DNA methylation refers to the covalent addition of a methyl group to cytosine nucleotides (5-methylcytosine or 5mC) adjacent to guanine residues in the DNA sequence – so-called CpG sites (Irizarry et al. 2009). Methylation leads to 'switching off' of the genes and thus inhibiting expression of the involved genes

Histone modifications refers to the epigenetic changes that controls the accessibility of a particular gene during transcription. In cells, the DNA is tightly packaged and ordered in nucleosomes by histone proteins (H2A, H2B, H3 and H4) to form chromatin structure. Posttranslational histone modifications such as acetylation, methylation, phosphorylation, ubiquitination, SUMOylation and ADP-ribosylation on the tails of core histones are important epigenetic modifications for gene transcription. For example, acetylation of histone tails often increases the accessibility of binding sites to transcription factors and thus the activation of gene expression. The status of histone acetylation is reversibly regulated by two distinct enzymes, histone acetyltransferase (HAT) and histone deacetylase (HDAC). Increased histone acetylation by HATs leads to the unwinding of chromatin structure and transcriptional activation, whereas removal of acetyl groups by HDACs causes chromatin condensation and transcriptional silencing.

MicroRNAs (miRNAs) are small (~22 nucleotides), non-coding RNAs that negatively regulate gene expression at posttranscriptional level by RNA degradation or inhibition of protein translation (Fig. 12.6). The miRNAs are generated from much longer primary miRNA by a multi-step process which is regulated by RNase III endonuclease (Drosha and Dicer). One miRNA sequence can regulate the expression of multiple genes in a coordinated fashion.

There is a growing body of evidence that exposure to air pollutants such as particulate matter or diesel exhaust can lead to epigenetic changes that can have potential detrimental health effects (Sonkoly and Pivarcsi 2011; Jardim 2011; Jardim et al. 2012). A pubmed search for articles on AP (or particulate matter, ozone and traffic exposure) and epigenetics identified 50 articles (2013, September) with 27 of them being review articles. Most of the original research articles are focused on cardio-respiratory health outcomes.

12.6.1 AP and Methylation

There is a growing body of evidence that exposure to AP results in epigenetic changes in human (Jardim 2011; Jardim et al. 2012; Bellavia et al. 2013; Hou et al. 2012; Bind et al. 2012; Carugno et al. 2012; Cantone et al. 2011; Baccarelli and Bollati 2009; Bollati et al. 2007). Multiple studies have noted that exposure to AP, specially **particulate matter or diesel exposure** can lead to ***methylation*** changes that can have potential health effects. These findings have generated great interest in identifying epigenetic changes that can modify or mediate AP effect on health outcomes. A study by Bollati et al. demonstrated that exposure to low level of benzene among gas station attendants and traffic police in Italy were associated with both genome-wide and gene-specific changes in methylation (Bollati et al. 2007). In controlled experiment, short term exposure to particulate matter was associated

with both genome-wide (Alu) and gene-specific (TLR4) hypomethylation (Bellavia et al. 2013). Recent findings in the Children Health study (Breton et al. 2012; Salam et al. 2012) also demonstrates that exposure to particulate matter can result in changes in methylation in the inducible nitric oxide synthase promoter region that can lead to health effects (Breton et al. 2011). These findings provide the impetus to investigate the effect of AP on epigenetic changes that can explain the observed health effects of AP.

Perera and colleagues reported that maternal exposure to polyaromatic hydrocarbon (PAH; a constituent found in diet, tobacco smoke and traffic-related pollution) was associated with DNA methylation level in a CpG site in *ACSL3* in umbilical cord blood white cells (Perera et al. 2009) and that DNA methylation level was associated with increased asthma risk in children (odds ratio [OR] = 3.9; 95 % confidence interval (CI): 1.14–14.3).

A recent study involving 141 participants of NAS observed that ambient SO_2 and black carbon level was associated with methylation pattern in the asthma pathway suggesting possible mediative role of methylation in AP related respiratory effect (Sofer et al. 2013).

Another study investigated the possible differential pattern of global methylation in placenta due to $PM_{2.5}$ exposure during pregnancy (Janssen et al. 2013). The investigators reported that $PM_{2.5}$ was associated with global hypomethylation of the placental tissue. Each 5μgm/m^3 increase in $PM_{2.5}$ was associated with 2.13 % (95%CI: −3.71, −0.54) decrease in placental global methylation. Strongest association was observed for 1st trimester especially during the period of implantation. This finding suggests that the observed effects of AP on pregnancy outcomes can be mediated through epigenetic changes of the placental tissue. The major limitations in the interpretation of the study finding is due to (i) unavailability of gene-specific methylation, & (ii) lack of cell specificity. Further studies are required to identify the genes that undergo epigenetic changes and specify the cells where those changes are most marked.

12.6.2 AP, Histone Modification and miRNA

Currently there is very limited data linking AP to histone modification or microRNA and health outcomes. Most of the evidence is indirect and based on *in vitro* studies. Two different i*n vitro* studies have noted increased gene expression of *IL8* and *COX2* genes leading to increased inflammatory activity in human airway epithelial cells exposed to DEP or PM due to increased H4-acetylation (Gilmour et al. 2003; Cao et al. 2007). The few studies investigated the effect of AP on microRNA expression profile have also indicated the involvement of oxidative stress and inflammatory pathway. Jardim et al. investigated the effect of diesel exposure microRNA expression in human bronchial epithelial cells (HBEC) (Jardim et al. 2009). Following exposure to diesel exhaust particle, 1.5 fold change in expression was observed for 63 % (197 of 313) of the detectable microRNAs in the HBECs. Based on bioinformatics analysis, the microRNAs with highest level of changes

were identified to be mostly involved in the regulation of inflammatory and tumorigenic pathways. Inhalation of DEP has also been shown to result in changes in microRNA expression in peripheral blood (Yamamoto et al. 2013). Yamamoto and colleagues investigated the effect of DEP exposure on the microRNA expression in the peripheral blood of 13 participants with asthma, after exposing them to filtered air with placebo (FAP), diesel exhaust (300 μg/l $PM_{2.5}$) with placebo (DEP) and diesel exhaust with anti-oxidant supplementation (DEN) in a double blinded cross-over experiment. The researchers initially identified differential expression of miR-21, miR30e, miR215 and miR-144 in the peripheral blood following DEP exposure and validated increased expression of miR-144 through RT-qPCR. The increased expression of miR-144 was associated with decreased activity of NRF2 and other downstream anti-oxidant genes (NQO1 and GCLC) and increase in oxidative stress measured by 8-hydroxy 2′-deoxyguanosine inn plasma. This finding further provides evidence that exposure to diesel exhaust can result in differential microRNA expression may regulate genes of the oxidative pathway. The different sets of microRNAs identified in HBEC (Jardim et al. 2009) and peripheral blood (Yamamoto et al. 2013) is expected given tissue specificity of microRNA. Although miR-21 was only marginally (p-value = 0.06, one-sided test) associated with DEP exposure in this study, a body of evidence suggests it could be associated with inflammation, oxidative stress, atherosclerosis, carcinogenesis, asthma and allergic pathway and toll-receptor pathway (Yamamoto et al. 2013). mir21 and two other micro RNA (miR222 and miR-146a) was also found to be differentially exposed following three days exposure to particulate matter at workplace among foundry workers (Bollati et al. 2010). Another line of evidence for the possible role of microRNA in AP mediated health effect comes from the NAS population, where genes involved in microRNA processing (DICER, Gem-associated Protein 4(*GEMIN4*), & Diegeorge syndrome critical region 8 (*DGCR8*)) was found to modify the effect of exposure to black carbon on blood pressure (Wilker et al. 2010) and one of the SNPs of *GEMIN4*, rs1062923, also modified the effect of particulate matter on the level of soluble intracellular and vascular adhesion molecules (sICAM & sVCAM, respectively), that are markers of inflammation and endothelial function (Wilker et al. 2011). However, the observed effect modification of the rs1062923 was paradoxical. Exposure to BC was associated with increase in diastolic blood pressure among homozygous carriers whereas; $PM_{2.5}$ was associated with lower level of sICAM and sVCAM, compared to homozygous variant and heterozygous. Further studies are required to provide credence to the findings and address any apparent inconsistencies.

12.7 Challenges and Limitations

Given that most of the diseases are considered to be a complex interplay between individual susceptibility and environmental exposure, the role of gene-environmental interaction in the causation or exacerbation of disease has gained momentum in the

last decade. Genetics certainly plays an important role in defining individual susceptibility to both disease and effect of the environmental exposure. This has ushered in new wave of research in AP and health studies, investigating the joint and interactive effect of AP and genetics on different health outcomes. Given the scientific importance of, and interest in genetics in AP related health effects, this is a burgeoning field that is currently at its infancy facing many challenges that needs to be addressed in future.

As discussed earlier, currently only a **limited number of studies** are available investigating the interplay between AP-Gene in health outcomes and majority of these studies are focused on cardio-respiratory health. Furthermore, most of the findings are based on couple of studies (i.e., CHS, NAS, SAPALDIA and others) thus limiting the external validity and generalizability of the findings. **Lack of replication** of the findings has been one major concern. The published evidence of lack of replication can be considered as the tip of iceberg given the publication bias against ***null*** findings. Reviewing the studies that have reported gene-AP interactions, it is evident that many of the studies had similar gene, environment and outcome information to replicate findings from other studies; however, only few such replications are reported. One of the major challenges in replicating the gene-AP interaction studies is the availability of similar exposure matrix given the intrinsic and extrinsic **heterogeneity in exposure assessment**. Population from different region, age group, and birth cohort can have substantially different level of exposure to the same pollutant; therefore, it can be quite challenging in replicating GxE findings. Furthermore, the exposure assessment of the same pollutant was carried out differently in these studies, i.e. using residential distance to major roads, traffic density, or NOx as a measure of '***traffic exposure***', making replication challenging. Sample size is another major concern in investigating GxAP models (Fig. 12.7). It is evident that to observe modest GxE effect (OR ~ 1.2) quite a substantial sample size (N > 5,000) is required unless the minor allele frequency is high (MAF > 0.4) or there is substantial variability in exposure. If the standard deviation of the outcome variable is large, the required sample size will also increase substantially. The sample size calculation in the table considered that the outcome variable is continuously distributed with the standard deviation being 1/10th of the mean; however, if it is even 1/5th of the mean, then the required sample size will almost double. This illustrates the challenges faced in identifying GxAP health effects majority of these studies. Moreover, if we are to consider possible multiple GxG interactions in GxAP models the required sample size can increase substantially. This sample size estimations are based on hypothesis based GxAP models; however, for agnostic approach of GWIS will increase substantially. Currently there is also scarcity of studies that fully utilizes **different genomic approaches in a single study**, i.e. investigating the GxAP effect including possible epigenetic changes and gene expression data to clearly elucidate the biological pathway involved in GxAP health effects. The availability of tissue specific epigenetic and gene expression data is specially challenging in large epidemiological studies. To fill the paucity in data for demonstration of Gene-AP interactions observed in epidemiological studies warrant additional research including using non-invasive approaches such as buccal and blood samples for biomarker identification.

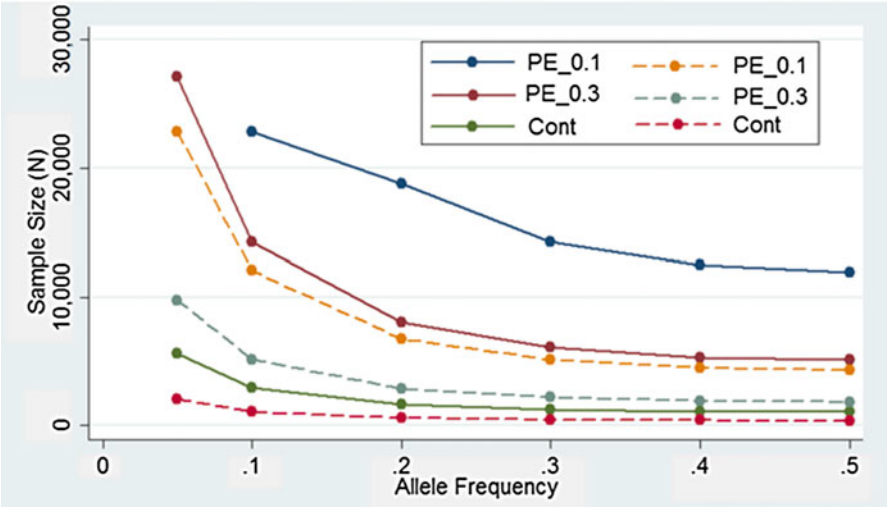

Fig. 12.7 Sample size calculations are based on using individual subject study design for additive effects of genes, with gene and AP marginal effect being 1.5 for continuous outcome with mean = 100 and std = 10. For dichotomous exposure, exposure prevalence was 10 % (PE_0.1) and 30 % (PE_0.3). Type I and II errors were 0.05 (2-df) and 0.20, respectively. *Solid* and *Dash lines* represent GxE interaction OR = 1.2 and 2, respectively for dichotomous exposure and interaction $\beta = 1.2$ and 2, respectively for continuous variable

12.8 Summary

The availability of new technologies and tools paves the way for innovative GxAP studies in future that may identify the underlying biological pathway in disease causation or exacerbation. Gene-AP studies in addition to identifying susceptible populations will also guide developing preventive and intervention strategies. With all the limitations discussed above, we are already witnessing the intricate interplay between AP and genomics in wide range of health outcomes starting from cardio-respiratory health outcomes to neurological conditions and pregnancy outcomes. These initial findings should motivate the development of larger new studies or consortiums among studies that collect consistent exposure and outcome data.

References

Adcock IM, Lee KY (2006) Abnormal histone acetylase and deacetylase expression and function in lung inflammation. Inflamm Res 55(8):311–321. doi:10.1007/s00011-006-0081-1

Adcock IM, Ito K, Barnes PJ (2005) Histone deacetylation: an important mechanism in inflammatory lung diseases. COPD 2(4):445–455

Alexis NE, Zhou H, Lay JC, Harris B, Hernandez ML, Lu TS et al (2009) The glutathione-S-transferase Mu 1 null genotype modulates ozone-induced airway inflammation in human subjects. J Allergy Clin Immunol 124(6):1222–1228.e5. doi:10.1016/j.jaci.2009.07.036, S0091-6749(09)01145-2 [pii]

Baccarelli A, Bollati V (2009) Epigenetics and environmental chemicals. Curr Opin Pediatr 21(2):243–251

Backes CH, Nelin T, Gorr MW, Wold LE (2013) Early life exposure to air pollution: how bad is it? Toxicol Lett 216(1):47–53. doi:10.1016/j.toxlet.2012.11.007, S0378-4274(12)01380-X [pii]

Baja ES, Schwartz JD, Wellenius GA, Coull BA, Zanobetti A, Vokonas PS et al (2010) Traffic-related air pollution and QT interval: modification by diabetes, obesity, and oxidative stress gene polymorphisms in the normative aging study. Environ Health Perspect 118(6):840–846. doi:10.1289/ehp.0901396

Baraldi E, Giordano G, Pasquale MF, Carraro S, Mardegan A, Bonetto G et al (2006) 3-Nitrotyrosine, a marker of nitrosative stress, is increased in breath condensate of allergic asthmatic children. Allergy 61(1):90–96. doi:10.1111/j.1398-9995.2006.00996.x, ALL996 [pii]

Bellavia A, Urch B, Speck M, Brook RD, Scott JA, Albetti B et al (2013) DNA hypomethylation, ambient particulate matter, and increased blood pressure: findings from controlled human exposure experiments. J Am Heart Assoc 2(3):e000212. doi:10.1161/JAHA.113.000212, jah3232 [pii]

Bergamaschi E, De Palma G, Mozzoni P, Vanni S, Vettori MV, Broeckaert F et al (2001) Polymorphism of quinone-metabolizing enzymes and susceptibility to ozone-induced acute effects. Am J Respir Crit Care Med 163(6):1426–1431. doi:10.1164/ajrccm.163.6.2006056

Bind MA, Baccarelli A, Zanobetti A, Tarantini L, Suh H, Vokonas P et al (2012) Air pollution and markers of coagulation, inflammation, and endothelial function: associations and epigene-environment interactions in an elderly cohort. Epidemiology 23(2):332–340. doi:10.1097/EDE.0b013e31824523f0

Bluemke DA, Kronmal RA, Lima JA, Liu K, Olson J, Burke GL et al (2008) The relationship of left ventricular mass and geometry to incident cardiovascular events: the MESA (Multi-Ethnic Study of Atherosclerosis) study. J Am Coll Cardiol 52(25):2148–2155. doi:10.1016/j.jacc.2008.09.014, S0735-1097(08)03231-2 [pii]

Bollati V, Baccarelli A, Hou L, Bonzini M, Fustinoni S, Cavallo D et al (2007) Changes in DNA methylation patterns in subjects exposed to low-dose benzene. Cancer Res 67(3):876–880. doi:10.1158/0008-5472.CAN-06-2995, 67/3/876 [pii]

Bollati V, Marinelli B, Apostoli P, Bonzini M, Nordio F, Hoxha M et al (2010) Exposure to metal-rich particulate matter modifies the expression of candidate microRNAs in peripheral blood leukocytes. Environ Health Perspect 118(6):763–768. doi:10.1289/ehp.0901300

Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ (2000) Role of quinones in toxicology. Chem Res Toxicol 13(3):135–160. doi:tx9902082 [pii]

Bonzini M, Carugno M, Grillo P, Mensi C, Bertazzi PA, Pesatori AC (2010) Impact of ambient air pollution on birth outcomes: systematic review of the current evidences. Med Lav 101(5):341–363

Breton CV, Byun HM, Wang X, Salam MT, Siegmund K, Gilliland FD (2011) DNA methylation in the arginase-nitric oxide synthase pathway is associated with exhaled nitric oxide in children with asthma. Am J Respir Crit Care Med 184(2):191–197. doi:10.1164/rccm.201012-2029OC, 201012-2029OC [pii]

Breton CV, Salam MT, Wang X, Byun HM, Siegmund KD, Gilliland FD (2012) Particulate matter, DNA methylation in nitric oxide synthase, and childhood respiratory disease. Environ Health Perspect 120(9):1320–1326. doi:10.1289/ehp.1104439

Brook RD, Urch B, Dvonch JT, Bard RL, Speck M, Keeler G et al (2009) Insights into the mechanisms and mediators of the effects of air pollution exposure on blood pressure and vascular function in healthy humans. Hypertension 54(3):659–667. doi:10.1161/HYPERTENSIONAHA.109.130237, HYPERTENSIONAHA.109.130237 [pii]

Brook RD, Rajagopalan S, Pope CA 3rd, Brook JR, Bhatnagar A, Diez-Roux AV et al (2010) Particulate matter Air pollution and cardiovascular disease. An update to the Scientific Statement from the American Heart Association. Circulation. doi:10.1161/CIR.0b013e3181dbece1

Cantone L, Nordio F, Hou L, Apostoli P, Bonzini M, Tarantini L et al (2011) Inhalable metal-rich air particles and histone H3K4 dimethylation and H3K9 acetylation in a cross-sectional study of steel workers. Environ Health Perspect 119(7):964–969. doi:10.1289/ehp.1002955

Cao D, Bromberg PA, Samet JM (2007) COX-2 expression induced by diesel particles involves chromatin modification and degradation of HDAC1. Am J Respir Cell Mol Biol 37(2):232–239. doi:10.1165/rcmb.2006-0449OC, 2006-0449OC [pii]

Carugno M, Pesatori AC, Dioni L, Hoxha M, Bollati V, Albetti B et al (2012) Increased mitochondrial DNA copy number in occupations associated with low-dose benzene exposure. Environ Health Perspect 120(2):210–215. doi:10.1289/ehp.1103979

Castro-Giner F, Kunzli N, Jacquemin B, Forsberg B, de Cid R, Sunyer J et al (2009) Traffic-related air pollution, oxidative stress genes, and asthma (ECHRS). Environ Health Perspect 117(12):1919–1924. doi:10.1289/ehp.0900589

Chahine T, Baccarelli A, Litonjua A, Wright RO, Suh H, Gold DR et al (2007) Particulate air pollution, oxidative stress genes, and heart rate variability in an elderly cohort. Environ Health Perspect 115(11):1617–1622. doi:10.1289/ehp.10318

Chen YH, Lin SJ, Lin MW, Tsai HL, Kuo SS, Chen JW et al (2002) Microsatellite polymorphism in promoter of heme oxygenase-1 gene is associated with susceptibility to coronary artery disease in type 2 diabetic patients. Hum Genet 111(1):1–8

Chen C, Arjomandi M, Tager IB, Holland N, Balmes JR (2007) Effects of antioxidant enzyme polymorphisms on ozone-induced lung function changes. Eur Respir J 30(4):677–683. doi:10.1183/09031936.00160806, 09031936.00160806 [pii]

Cho HY, Kleeberger SR (2007) Genetic mechanisms of susceptibility to oxidative lung injury in mice. Free Radic Biol Med 42(4):433–445. doi:10.1016/j.freeradbiomed.2006.11.021, S0891-5849(06)00758-1 [pii]

Corradi M, Alinovi R, Goldoni M, Vettori M, Folesani G, Mozzoni P et al (2002) Biomarkers of oxidative stress after controlled human exposure to ozone. Toxicol Lett 134(1–3):219–225. doi:S0378427402001698 [pii]

Curjuric I, Imboden M, Schindler C, Downs SH, Hersberger M, Liu SL et al (2010) HMOX1 and GST variants modify attenuation of FEF25-75 % decline due to PM10 reduction. Eur Respir J 35(3):505–514. doi:10.1183/09031936.00044309, 35/3/505 [pii]

Danesh J, Lewington S, Thompson SG, Lowe GD, Collins R, Kostis JB et al (2005) Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. JAMA 294(14):1799–1809. doi:10.1001/jama.294.14.1799, 294/14/1799 [pii]

David GL, Romieu I, Sienra-Monge JJ, Collins WJ, Ramirez-Aguilar M, del Rio-Navarro BE et al (2003) Nicotinamide adenine dinucleotide (phosphate) reduced: quinone oxidoreductase and glutathione S-transferase M1 polymorphisms and childhood asthma. Am J Respir Crit Care Med 168(10):1199–1204. doi:10.1164/rccm.200305-684OC, 200305-684OC [pii]

Ferris CD, Jaffrey SR, Sawa A, Takahashi M, Brady SD, Barrow RK et al (1999) Haem oxygenase-1 prevents cell death by regulating cellular iron. Nat Cell Biol 1(3):152–157

Fuller GM, Zhang Z (2001) Transcriptional control mechanism of fibrinogen gene expression. Ann N Y Acad Sci 936:469–479

Gauderman W, Vora H, Avol E, McConnell R (2009) Predictors of intra-community variation in air quality. Technical report. Report No.: 180. University of Southern California Keck School of Medicine Division of Biostatistics – Research Section 2009, Los Angeles.

Gilliland FD, Li YF, Saxon A, Diaz-Sanchez D (2004) Effect of glutathione-S-transferase M1 and P1 genotypes on xenobiotic enhancement of allergic responses: randomised, placebo-controlled crossover study. Lancet 363(9403):119–125. doi:10.1016/S0140-6736(03)15262-2, S0140-6736(03)15262-2 [pii]

Gilmour PS, Rahman I, Donaldson K, MacNee W (2003) Histone acetylation regulates epithelial IL-8 release mediated by oxidative stress from environmental particles. Am J Physiol Lung Cell Mol Physiol 284(3):L533–L540. doi:10.1152/ajplung.00277.2002, 00277.2002 [pii]

Hajos AK, Winston GW (1991a) Dinitropyrene nitroreductase activity of purified NAD(P)H-quinone oxidoreductase: role in rat liver cytosol and induction by Aroclor-1254 pretreatment. Carcinogenesis 12(4):697–702

Hajos AK, Winston GW (1991b) Purified NAD(P)H-quinone oxidoreductase enhances the mutagenicity of dinitropyrenes in vitro. J Biochem Toxicol 6(4):277–282

Hirai H, Kubo H, Yamaya M, Nakayama K, Numasaki M, Kobayashi S et al (2003) Microsatellite polymorphism in heme oxygenase-1 gene promoter is associated with susceptibility to oxidant-induced apoptosis in lymphoblastoid cell lines. Blood 102(5):1619–1621

Hollingsworth JW 2nd, Cook DN, Brass DM, Walker JK, Morgan DL, Foster WM et al (2004) The role of Toll-like receptor 4 in environmental airway injury in mice. Am J Respir Crit Care Med 170(2):126–132. doi:10.1164/rccm.200311-1499OC, 200311-1499OC [pii]

Hong YC, Hwang SS, Kim JH, Lee KH, Lee HJ, Yu SD et al (2007) Metals in particulate pollutants affect peak expiratory flow of schoolchildren. Environ Health Perspect 115(3):430–434. doi:10.1289/ehp.9531

Hou L, Zhang X, Wang D, Baccarelli A (2012) Environmental chemical exposures and human epigenetics. Int J Epidemiol 41(1):79–105. doi:10.1093/ije/dyr154, dyr154 [pii]

Hu X, Xia H, Srivastava SK, Herzog C, Awasthi YC, Ji X et al (1997) Activity of four allelic forms of glutathione S-transferase hGSTP1-1 for diol epoxides of polycyclic aromatic hydrocarbons. Biochem Biophys Res Commun 238(2):397–402. doi:10.1006/bbrc.1997.7311, S0006-291X(97)97311-3 [pii]

Hunter DJ (2005) Gene-environment interactions in human diseases. Nat Rev Genet 6(4):287–298. doi:10.1038/nrg1578, nrg1578 [pii]

Hwang BF, Young LH, Tsai CH, Tung KY, Wang PC, Su MW et al (2013) Fine particle, ozone exposure, and asthma/wheezing: effect modification by glutathione S-transferase P1 polymorphisms. PLoS One 8(1):e52715. doi:10.1371/journal.pone.0052715, PONE-D-12-03383 [pii]

Irizarry RA, Ladd-Acosta C, Wen B, Wu Z, Montano C, Onyango P et al (2009) The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. Nat Genet 41(2):178–186. doi:10.1038/ng.298, ng.298 [pii]

Islam T, McConnell R, Gauderman WJ, Avol E, Peters JM, Gilliland FD (2008) Ozone, oxidant defense genes, and risk of asthma during adolescence. Am J Respir Crit Care Med 177(4):388–395. doi:10.1164/rccm.200706-863OC, 200706-863OC [pii]

Islam T, Berhane K, McConnell R, Gauderman WJ, Avol E, Peters JM et al (2009) Glutathione-S-transferase (GST) P1, GSTM1, exercise, ozone and asthma incidence in school children. Thorax 64(3):197–202. doi:10.1136/thx.2008.099366, thx.2008.099366 [pii]

Islam T, Breton C, Salam MT, McConnell R, Wenten M, Gauderman WJ et al (2010) Role of inducible nitric oxide synthase in asthma risk and lung function growth during adolescence. Thorax 65(2):139–145. doi:10.1136/thx.2009.114355, thx.2009.114355 [pii]

Janssen BG, Godderis L, Pieters N, Poels K, Kici Ski M, Cuypers A et al (2013) Placental DNA hypomethylation in association with particulate air pollution in early life. Part Fibre Toxicol 10(1):22. doi:10.1186/1743-8977-10-22, 1743-8977-10-22 [pii]

Jardim MJ (2011) microRNAs: implications for air pollution research. Mutat Res 717(1–2):38–45. doi:10.1016/j.mrfmmm.2011.03.014, S0027-5107(11)00080-7 [pii]

Jardim MJ, Fry RC, Jaspers I, Dailey L, Diaz-Sanchez D (2009) Disruption of microRNA expression in human airway cells by diesel exhaust particles is linked to tumorigenesis-associated pathways. Environ Health Perspect 117(11):1745–1751. doi:10.1289/ehp.0900756

Jardim MJ, Dailey L, Silbajoris R, Diaz-Sanchez D (2012) Distinct microRNA expression in human airway cells of asthmatic donors identifies a novel asthma-associated gene. Am J Respir Cell Mol Biol 47(4):536–542. doi:10.1165/rcmb.2011-0160OC, rcmb.2011-0160OC [pii]

Kelly FJ (2003) Oxidative stress: its role in air pollution and adverse health effects. Occup Environ Med 60(8):612–616. doi:10.1136/oem.60.8.612

Kerkhof M, Postma DS, Brunekreef B, Reijmerink NE, Wijga AH, de Jongste JC et al (2010) Toll-like receptor 2 and 4 genes influence susceptibility to adverse effects of traffic-related air pollution on childhood asthma. Thorax 65(8):690–697. doi:10.1136/thx.2009.119636, 65/8/690 [pii]

Kim CS, Alexis NE, Rappold AG, Kehrl H, Hazucha MJ, Lay JC et al (2011) Lung function and inflammatory responses in healthy young adults exposed to 0.06 ppm ozone for 6.6 hours. Am J Respir Crit Care Med 183(9):1215–1221. doi:10.1164/rccm.201011-1813OC, 201011-1813OC [pii]

Kleeberger SR, Levitt RC, Zhang LY, Longphre M, Harkema J, Jedlicka A et al (1997) Linkage analysis of susceptibility to ozone-induced lung inflammation in inbred mice. Nat Genet 17(4):475–478. doi:10.1038/ng1297-475

Lee YL, McConnell R, Berhane K, Gilliland FD (2009) Ambient ozone modifies the effect of tumor necrosis factor G-308A on bronchitic symptoms among children with asthma. Allergy 64(9):1342–1348. doi:10.1111/j.1398-9995.2009.02014.x, ALL2014 [pii]

Li YF, Gauderman WJ, Avol E, Dubeau L, Gilliland FD (2006) Associations of tumor necrosis factor G-308A with childhood asthma and wheezing. Am J Respir Crit Care Med 173(9):970–976. doi:10.1164/rccm.200508-1256OC, 200508-1256OC [pii]

Ljungman P, Bellander T, Schneider A, Breitner S, Forastiere F, Hampel R et al (2009) Modification of the interleukin-6 response to air pollution by interleukin-6 and fibrinogen polymorphisms. Environ Health Perspect 117(9):1373–1379. doi:10.1289/ehp.0800370

McConnell R, Berhane K, Gilliland F, London SJ, Islam T, Gauderman WJ et al (2002) Asthma in exercising children exposed to ozone: a cohort study. Lancet 359(9304):386–391. doi:10.1016/S0140-6736(02)07597-9, S0140-6736(02)07597-9 [pii]

McConnell R, Berhane K, Yao L, Jerrett M, Lurmann F, Gilliland F et al (2006) Traffic, susceptibility, and childhood asthma. Environ Health Perspect 114(5):766–772

Melen E, Nyberg F, Lindgren CM, Berglind N, Zucchelli M, Nordling E et al (2008) Interactions between glutathione S-transferase P1, tumor necrosis factor, and traffic-related air pollution for development of childhood allergic disease. Environ Health Perspect 116(8):1077–1084. doi:10.1289/ehp.11117

Minelli C, Wei I, Sagoo G, Jarvis D, Shaheen S, Burney P (2011) Interactive effects of antioxidant genes and air pollution on respiratory function and airway disease: a HuGE review. Am J Epidemiol 173(6):603–620. doi:10.1093/aje/kwq403, kwq403 [pii]

Misra P, Srivastava R, Krishnan A, Sreenivaas V, Pandav CS (2012) Indoor air pollution-related acute lower respiratory infections and low birthweight: a systematic review. J Trop Pediatr 58(6):457–466. doi:10.1093/tropej/fms017, fms017 [pii]

Mordukhovich I, Wilker E, Suh H, Wright R, Sparrow D, Vokonas PS et al (2009) Black carbon exposure, oxidative stress genes, and blood pressure in a repeated-measures study. Environ Health Perspect 117(11):1767–1772. doi:10.1289/ehp.0900591

Moreno-Macias H, Dockery DW, Schwartz J, Gold DR, Laird NM, Sienra-Monge JJ et al (2013) Ozone exposure, vitamin C intake, and genetic susceptibility of asthmatic children in Mexico City: a cohort study. Respir Res 14:14. doi:10.1186/1465-9921-14-14, 1465-9921-14-14 [pii]

Nakagawa R, Kitamori S, Horikawa K, Nakashima K, Tokiwa H (1983) Identification of dinitropyrenes in diesel-exhaust particles. Their probable presence as the major mutagens. Mutat Res 124(3–4):201–211. doi:0165-1218(83)90191-X [pii]

Newcomb P, Hunt A, Rast P, Cauble D, Rowe N, Li J (2012) Acute effects of walking environment and GSTM1 variants in children with asthma. Biol Res Nurs 14(1):55–64. doi:10.1177/1099800410389167, 1099800410389167 [pii]

Otterbein LE, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M et al (2000) Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. Nat Med 6(4):422–428

Park SK, O'Neill MS, Wright RO, Hu H, Vokonas PS, Sparrow D et al (2006) HFE genotype, particulate air pollution, and heart rate variability: a gene-environment interaction. Circulation 114(25):2798–2805. doi:10.1161/CIRCULATIONAHA.106.643197, CIRCULATIONAHA.106.643197 [pii]

Park SK, Hu H, Wright RO, Schwartz J, Cheng Y, Sparrow D et al (2009) Iron metabolism genes, low-level lead exposure, and QT interval. Environ Health Perspect 117(1):80–85. doi:10.1289/ehp.11559

Perera F, Tang WY, Herbstman J, Tang D, Levin L, Miller R et al (2009) Relation of DNA methylation of 5′-CpG island of ACSL3 to transplacental exposure to airborne polycyclic aromatic hydrocarbons and childhood asthma. PLoS One 4(2):e4488. doi:10.1371/journal.pone.0004488

Peters JM, Avol E, Gauderman WJ, Linn WS, Navidi W, London SJ et al (1999a) A study of twelve Southern California communities with differing levels and types of air pollution. II. Effects on pulmonary function. Am J Respir Crit Care Med 159(3):768–775. doi:10.1164/ajrccm.159.3.9804144

Peters JM, Avol E, Navidi W, London SJ, Gauderman WJ, Lurmann F et al (1999b) A study of twelve Southern California communities with differing levels and types of air pollution. I. Prevalence of respiratory morbidity. Am J Respir Crit Care Med 159(3):760–767. doi:10.1164/ajrccm.159.3.9804143

Peters A, Greven S, Heid IM, Baldari F, Breitner S, Bellander T et al (2009) Fibrinogen genes modify the fibrinogen response to ambient particulate matter. Am J Respir Crit Care Med 179(6):484–491. doi:10.1164/rccm.200805-751OC, 200805-751OC [pii]

Rahman I, Adcock IM (2006) Oxidative stress and redox regulation of lung inflammation in COPD. Eur Respir J 28(1):219–242. doi:10.1183/09031936.06.00053805, 28/1/219 [pii]

Rahman I, Biswas SK, Kode A (2006) Oxidant and antioxidant balance in the airways and airway diseases. Eur J Pharmacol 533(1–3):222–239. doi:10.1016/j.ejphar.2005.12.087, S0014-2999(05)01384-1 [pii]

Ren C, Park SK, Vokonas PS, Sparrow D, Wilker E, Baccarelli A et al (2010a) Air pollution and homocysteine: more evidence that oxidative stress-related genes modify effects of particulate air pollution. Epidemiology 21(2):198–206. doi:10.1097/EDE.0b013e3181cc8bfc

Ren C, Baccarelli A, Wilker E, Suh H, Sparrow D, Vokonas P et al (2010b) Lipid and endothelium-related genes, ambient particulate matter, and heart rate variability–the VA Normative Aging Study. J Epidemiol Community Health 64(1):49–56. doi:10.1136/jech.2008.083295, jech.2008.083295 [pii]

Ricciardolo FL, Nijkamp FP, Folkerts G (2006a) Nitric oxide synthase (NOS) as therapeutic target for asthma and chronic obstructive pulmonary disease. Curr Drug Targets 7(6):721–735

Ricciardolo FL, Di Stefano A, Sabatini F, Folkerts G (2006b) Reactive nitrogen species in the respiratory tract. Eur J Pharmacol 533(1–3):240–252. doi:10.1016/j.ejphar.2005.12.057, S0014-2999(05)01387-7 [pii]

Romieu I, Sienra-Monge JJ, Ramirez-Aguilar M, Moreno-Macias H, Reyes-Ruiz NI (2004) Estela del Rio-Navarro B et al. Genetic polymorphism of GSTM1 and antioxidant supplementation influence lung function in relation to ozone exposure in asthmatic children in Mexico City. Thorax 59(1):8–10

Romieu I, Ramirez-Aguilar M, Sienra-Monge JJ, Moreno-Macias H, del Rio-Navarro BE, David G et al (2006) GSTM1 and GSTP1 and respiratory health in asthmatic children exposed to ozone. Eur Respir J 28(5):953–959. doi:10.1183/09031936.06.00114905, 09031936.06.00114905 [pii]

Romieu I, Moreno-Macias H, London SJ (2010) Gene by environment interaction and ambient air pollution. Proc Am Thorac Soc 7(2):116–122. doi:10.1513/pats.200909-097RM, 7/2/116 [pii]

Rossner P Jr, Tabashidze N, Dostal M, Novakova Z, Chvatalova I, Spatova M et al (2011) Genetic, biochemical, and environmental factors associated with pregnancy outcomes in newborns from the Czech Republic. Environ Health Perspect 119(2):265–271. doi:10.1289/ehp.1002470

Ruckerl R, Greven S, Ljungman P, Aalto P, Antoniades C, Bellander T et al (2007) Air pollution and inflammation (interleukin-6, C-reactive protein, fibrinogen) in myocardial infarction survivors. Environ Health Perspect 115(7):1072–1080. doi:10.1289/ehp.10021

Salam MT, Lin PC, Avol EL, Gauderman WJ, Gilliland FD (2007) Microsomal epoxide hydrolase, glutathione S-transferase P1, traffic and childhood asthma. Thorax 62(12):1050–1057. doi:10.1136/thx.2007.080127, thx.2007.080127 [pii]

Salam MT, Islam T, Gauderman WJ, Gilliland FD (2009) Roles of arginase variants, atopy, and ozone in childhood asthma. J Allergy Clin Immunol 123(3):596–602.e1–8. doi:10.1016/j.jaci.2008.12.020, S0091-6749(08)02435-4 [pii]

Salam MT, Byun HM, Lurmann F, Breton CV, Wang X, Eckel SP et al (2012) Genetic and epigenetic variations in inducible nitric oxide synthase promoter, particulate pollution, and exhaled nitric oxide levels in children. J Allergy Clin Immunol 129(1):232–239e1–7. doi:10.1016/j.jaci.2011.09.037, S0091-6749(11)01560-0 [pii]

Schwartz J, Park SK, O'Neill MS, Vokonas PS, Sparrow D, Weiss S et al (2005) Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. Am J Respir Crit Care Med 172(12):1529–1533. doi:10.1164/rccm.200412-1698OC, 200412-1698OC [pii]

Shah PS, Balkhair T (2011) Air pollution and birth outcomes: a systematic review. Environ Int 37(2):498–516. doi:10.1016/j.envint.2010.10.009, S0160-4120(10)00225-4 [pii]

Siegel D, McGuinness SM, Winski SL, Ross D (1999) Genotype-phenotype relationships in studies of a polymorphism in NAD(P)H:quinone oxidoreductase 1. Pharmacogenetics 9(1):113–121

Sofer T, Baccarelli A, Cantone L, Coull B, Maity A, Lin X et al (2013) Exposure to airborne particulate matter is associated with methylation pattern in the asthma pathway. Epigenomics 5(2):147–154. doi:10.2217/epi.13.16

Sonkoly E, Pivarcsi A (2011) MicroRNAs in inflammation and response to injuries induced by environmental pollution. Mut Res 717(1–2):46–53. doi:http://dx.doi.org/10.1016/j.mrfmmm.2011.02.002

Stieb DM, Chen L, Eshoul M, Judek S (2012) Ambient air pollution, birth weight and preterm birth: a systematic review and meta-analysis. Environ Res 117:100–111. doi:10.1016/j.envres.2012.05.007, S0013-9351(12)00176-4 [pii]

Stocker R, Ames BN (1987) Potential role of conjugated bilirubin and copper in the metabolism of lipid peroxides in bile. Proc Natl Acad Sci U S A 84(22):8130–8134

Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN (1987) Bilirubin is an antioxidant of possible physiological importance. Science 235(4792):1043–1046

Suh YJ, Ha EH, Park H, Kim YJ, Kim H, Hong YC (2008) GSTM1 polymorphism along with PM10 exposure contributes to the risk of preterm delivery. Mutat Res 656(1–2):62–67. doi:10.1016/j.mrgentox.2008.07.006, S1383-5718(08)00204-0 [pii]

Sundberg K, Johansson AS, Stenberg G, Widersten M, Seidel A, Mannervik B et al (1998) Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1-1 towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons. Carcinogenesis 19(3):433–436

Thomas D (2010) Methods for investigating gene-environment interactions in candidate pathway and genome-wide association studies. Annu Rev Public Health 31(1):21–36. doi:10.1146/annurev.publhealth.012809.103619

Tubby C, Harrison T, Todd I, Fairclough L (2011) Immunological basis of reversible and fixed airways disease. Clin Sci 121(7):285–296. doi:10.1042/cs20110062

Tung KY, Tsai CH, Lee YL (2011) Microsomal epoxide hydroxylase genotypes/diplotypes, traffic air pollution, and childhood asthma. Chest 139(4):839–848. doi:10.1378/chest. 10-2479, chest.10-2479 [pii]

Vagaggini B, Bartoli ML, Cianchetti S, Costa F, Bacci E, Dente FL et al (2010) Increase in markers of airway inflammation after ozone exposure can be observed also in stable treated asthmatics with minimal functional response to ozone. Respir Res 11:5. doi:10.1186/1465-9921-11-5, 1465-9921-11-5 [pii]

Van Hee VC, Adar SD, Szpiro AA, Barr RG, Diez Roux A, Bluemke DA et al (2010) Common genetic variation, residential proximity to traffic exposure, and left ventricular mass: the multi-ethnic study of atherosclerosis. Environ Health Perspect 118(7):962–969. doi:10.1289/ehp.0901535

Wenten M, Gauderman WJ, Berhane K, Lin PC, Peters J, Gilliland FD (2009) Functional variants in the catalase and myeloperoxidase genes, ambient air pollution, and respiratory-related school absences: an example of epistasis in gene-environment interactions. Am J Epidemiol 170(12):1494–1501. doi:10.1093/aje/kwp310, kwp310 [pii]

Wilker E, Mittleman MA, Litonjua AA, Poon A, Baccarelli A, Suh H et al (2009) Postural changes in blood pressure associated with interactions between candidate genes for chronic respiratory diseases and exposure to particulate matter. Environ Health Perspect 117(6):935–940. doi:10.1289/ehp.0800279

Wilker EH, Baccarelli A, Suh H, Vokonas P, Wright RO, Schwartz J (2010) Black carbon exposures, blood pressure, and interactions with single nucleotide polymorphisms in MicroRNA processing genes. Environ Health Perspect 118(7):943–948. doi:10.1289/ehp.0901440

Wilker EH, Alexeeff SE, Suh H, Vokonas PS, Baccarelli A, Schwartz J (2011) Ambient pollutants, polymorphisms associated with microRNA processing and adhesion molecules: the Normative Aging Study. Environ Health 10:45. doi:10.1186/1476-069X-10-45, 1476-069X-10-45 [pii]

Yamada N, Yamaya M, Okinaga S, Nakayama K, Sekizawa K, Shibahara S et al (2000) Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. Am J Hum Genet 66(1):187–195

Yamamoto M, Singh A, Sava F, Pui M, Tebbutt SJ, Carlsten C (2013) MicroRNA expression in response to controlled exposure to diesel exhaust: attenuation by the antioxidant N-acetylcysteine in a randomized crossover study. Environ Health Perspect 121(6):670–675. doi:10.1289/ehp.1205963

Yang IV, Schwartz DA (2012) Epigenetic mechanisms and the development of asthma. J Allergy Clin Immunol 130(6):1243–1255. doi:10.1016/j.jaci.2012.07.052

Yang IA, Holz O, Jorres RA, Magnussen H, Barton SJ, Rodriguez S et al (2005) Association of tumor necrosis factor-alpha polymorphisms and ozone-induced change in lung function. Am J Respir Crit Care Med 171(2):171–176. doi:10.1164/rccm.200402-194OC, 200402-194OC [pii]

Zanobetti A, Baccarelli A, Schwartz J (2011) Gene-air pollution interaction and cardiovascular disease: a review. Prog Cardiovasc Dis 53(5):344–352. doi:10.1016/j.pcad.2011.01.001, S0033-0620(11)00015-6 [pii]