Interaction Effects of Long-Term Air Pollution Exposure and Variants in the \textit{GSTP1}, \textit{GSTT1} and \textit{GSTCD} Genes on Risk of Acute Myocardial Infarction and Hypertension: A Case-Control Study

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Abstract

Introduction: Experimental and epidemiological studies have reported associations between air pollution exposure, in particular related to vehicle exhaust, and cardiovascular disease. A potential pathophysiological pathway is pollution-induced pulmonary oxidative stress, with secondary systemic inflammation. Genetic polymorphisms in genes implicated in oxidative stress, such as \textit{GSTP1}, \textit{GSTT1} and \textit{GSTCD}, may contribute to determining individual susceptibility to air pollution as a promoter of coronary vulnerability.

Aims: We aimed to investigate effects of long-term traffic-related air pollution exposure, as well as variants in \textit{GSTP1}, \textit{GSTT1} and \textit{GSTCD}, on risk of acute myocardial infarction (AMI) and hypertension. In addition, we studied whether air pollution effects were modified by the investigated genetic variants.

Methods: Genotype data at 7 single nucleotide polymorphisms (SNPs) in the \textit{GSTP1} gene, and one in each of the \textit{GSTT1} and \textit{GSTCD} genes, as well as air pollution exposure estimates, were available for 119 AMI cases and 1310 randomly selected population controls. Population control individuals with systolic blood pressure $\geq$140 mmHg, diastolic blood pressure $\geq$ 90 mmHg or on daily antihypertensive medication were defined as hypertensive ($n = 468$). Individual air pollution exposure levels were modeled as annual means of NO$_2$ (marker of vehicle exhaust pollutants) using central monitoring data and dispersion models, linking to participants’ home addresses.

Results: Air pollution was significantly associated with risk of AMI: OR 1.78 (95%CI 1.04–3.03) per 10 $\mu$g/m$^3$ of long-term NO$_2$ exposure. Three \textit{GSTP1} SNPs were significantly associated with hypertension. The effect of air pollution on risk of AMI varied by genotype strata, although the suggested interaction was not significant. We saw no obvious interaction between genetic variants in the \textit{GST} genes and air pollution exposure for hypertension.

Conclusion: Air pollution exposure entails an increased risk of AMI, and this risk differed over genotype strata for variants in the \textit{GSTP1}, \textit{GSTT1} and \textit{GSTCD} genes, albeit not statistically-significantly.

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Introduction

Exposure to air pollution has been associated with several cardiovascular (CV) outcomes such as atherosclerosis, progression of CV disease (CVD) and CV mortality. [1,2] However, the exact biological mechanisms by which pulmonary air pollution exposure leads to cardiovascular disease outcomes are not fully understood. A proposed pathway is that pulmonary exposure induces pulmonary oxidative stress, which leads to release of pro-thrombotic and inflammatory cytokines into the blood, as well as an increased level of reactive oxygen species (ROS) in the heart. [1,3] This enhances expression of inflammatory cytokine-genes, which in turn induces systemic inflammation and systemic oxidative stress. Inflammation furthers progress of atherosclerosis...
and can potentially trigger acute plaque rupture. The release of pro-thrombotic agents into the blood can also lead to clot formation and put the individual at increased risk of ischemic heart disease. Other pathways have also been suggested, e.g. translocation of particles across the pulmonary epithelium and lung-blood barrier into the cardiovascular system, which has been shown in both animals and humans. [4]

A recent review by the American Heart Association concludes that the evidence is consistent with a causal relationship between exposure to particulate matter (PM) air pollution <2.5 μm in diameter (PM2.5) and cardiovascular disease. [5] However, Europe had no air quality target value for PM2.5 until 2010 and the limit value does not enter into force until 2013, and to date longitudinal studies measuring population exposure to PM and associations with CVD are lacking. Nitrogen dioxide (NO2) is a gaseous air pollutant which is highly correlated with traffic intensity, and often used as indicator for vehicle exhaust in general. [1] The annual average limit for NO2 in the European Union has been set to 40 μg/m3 and has been in force since 2010. Several European studies have found an association between NO2 exposure and cardiovascular mortality, well below 40 μg/m3 [6].

Genetic polymorphisms may contribute to determining individual susceptibility to air pollution exposure as a promoter of coronary vulnerability and trigger of acute coronary events. [7] The human body can counteract the damaging effects of oxidative stress with detoxifying enzymes, and genetic variants in and surrounding the genes coding for such enzymes may determine the severity of oxidative stress. One such detoxification enzyme is the dimeric Glutathione S-Transferase (GST), whose specific role is to protect against the products of oxidative stress. [8] The GST gene family in humans is complex, consisting of 16 genes in six subgroups including GSTT1, GSTM1 and GSTCD. [9] GST gene expression is induced by compounds linked to chemical stress and carcinogenesis. [10,11]

Variants in the GSTP1 gene have been shown to modify the effect of air pollution exposure on lung function [12], and a few studies have also studied GSTP1 variants regarding interaction with air pollution exposure in CVD outcomes such as blood pressure, but to date no significant interactions have been reported. [13,14] A genome wide association study (GWAS) identified an association between decreased pulmonary function and SNPs in GSTCD (glutathione S-transferase, C-terminal domain containing), a result which was supported by an extended meta-analysis of several GWASs. [10,15] So far, no associations between GSTCD and cardiovascular disease have been reported. The polymorphism in the GSTT1 gene is caused by a gene deletion, resulting in almost no enzymatic activity in individuals with the null genotype; hypothetically putting the individual at increased risk of oxidative stress and downstream effects e.g. atherosclerosis and coronary heart disease. [11,16]

Hence, a probable pathway between air pollution and cardiovascular effects is oxidative stress, but the relationship between variants in antioxidant genes and cardiovascular disease as well as potential interaction between such genetic variants and air pollution exposure in cardiovascular disease is incompletely explored. We hypothesized that there is an interaction between air pollution exposure and genetic variants in the GST genes on risk of acute myocardial infarction (AMI) and thus we aimed to investigate, in a population-based study, a) the association of long-term traffic-related air pollution exposure with risk of acute myocardial infarction (AMI) and hypertension, b) the associations of genetic variants in GSTP1, GSTCD and GSTT1 with risk of AMI and hypertension, and c) if these genetic variants modify the associations between air pollution exposure and the two outcomes.

Methods

Ethics statement

The study was approved by the local ethical committee and all participants provided written informed consent.

Study population and data collection

The INTERGENE/ADONIX (INTERplay between GENetic susceptibility and environmental factors for the risk of chronic diseases in West Sweden/ADult-Onset asthma and exhaled Nitrice OXide) study has been described in detail elsewhere. [17,18] In brief, from April 2001 until December 2004, INTERGENE/ADONIX recruited coronary heart disease (CHD) cases and a population control cohort from the greater Gothenburg area in Sweden. The population control cohort consisted of 3614 randomly selected individuals aged 25–75 years at time of selection. As CHD cases, the study included consecutive patients admitted to wards at 3 locations (Ostra, Molndal and Sahlgrenska) of the Sahlgrenska university hospital, Gothenburg, Sweden or outpatients with significant coronary lesions identified from coronary angiograms. Altogether, the INTERGENE/ADONIX study included 618 patients (73.4% men and 26.6% women) with CHD, 295 with a first episode of acute myocardial infarction (AMI) or unstable angina pectoris, and the rest with chronic CHD, defined as either prior AMI or positive angiogram. The upper age limit was 75 years. 192 patients were individuals presenting with first-time acute myocardial infarction (AMI) and included as cases in the present study.

Study participants received questionnaires and were invited to a medical examination. During the medical examination, height and body weight was measured to the nearest 1 cm and 0.1 kg with the subjects in light clothing and without shoes. Body mass index (BMI) was calculated from weight (kg) and height (m) using the formula BMI = weight/height². Blood pressure measurement was carried out in a sitting position after 5 minutes rest, using a validated blood pressure device (Omron 711 Automatic IS; Omron Healthcare Inc., Vernon Hills, IL). The blood pressure was measured twice and then the mean of the two measurements was used. Blood samples were collected after 4 hours of fasting for immediate serum lipid (total cholesterol, HDL cholesterol and triglycerides) analysis and storage for DNA extraction.

Based on the questionnaire, the individuals’ level of education was classed as primary if highest achieved education was elementary school, lower secondary school, vocational school or girls’ school. Secondary meant that the individual indicated having attended upper secondary school or high school and tertiary signified having attended university or college.

Individuals with a systolic blood pressure (SBP) ≥140 mmHg, or diastolic blood pressure (DBP) ≥90 mmHg or who were taking daily antihypertensive medication were defined as ‘hypertensive’. Individuals of non-European birth (5%) were excluded from the current genetic analyses. Of those reporting European origin and included, 90% reported being of Swedish origin.

Air pollution exposure

Modeled annual average levels of NO2 outside each participant’s baseline home address were used for exposure assessment. Each participant’s home address was translated into geographical coordinates and combined with modeled levels of NO2 in a geographical information system (GIS). The dispersion model, which is hosted by the local authorities, contains both emission data and meteorological information and has been previously validated against actual measurements, showing good agreement. [19] The main output from the model is NO2 values with...
high spatial resolution (20×20 meters), which were then converted to estimated NO₂ using local empirical relationships. Due to the availability of concentration grids, the calculated exposure levels represented the years 2006 and 2007 and not the exact years of inclusion (2001–2004). For individuals with air pollution data for both years, we used the 2007 value because the geographical area covered was increased from 2006. For individuals with exposure data from only one year, this value was used. Correlation between values for individuals with values from both years was 0.98 for NO₂. This high degree of stability over years indicates that 2007 is a good indicator also for the long-term spatial distribution of exposure levels during 2001–2004.

SNP selection and genotyping

7 SNPs from the GSTP1 gene and one SNP from each of the GSTTI and GSTCD genes were selected from the literature. SNPs were genotyped using a Sequenom MassARRAY platform (Sequenom San Diego, CA, USA) or a competitive allele-specific PCR system (KASPar; KBioscience, Hoddesdon Herts, GB), all with a call rate ≥90%. SNPs with a Hardy-Weinberg Equilibrium (HWE) p-value ≤0.05 were excluded, as were individuals missing results for more than two out of seven GSTTI SNPs. The GSTTI SNP rs2266637 (as a marker of the null genotype) was genotyped twice and the resulting concordant pairs of null/non-null genotype individuals from the two assays were used for analysis.

Statistical analysis

Methods are analogous for AMI and hypertension, except that for hypertension the analysis was restricted to the population control sample. All analyses used logistic regression models adjusted for age, age squared and gender. Potential selection bias for cases and controls due to geographical clustering of cases’ home addresses in areas closer to the two source hospitals was accounted for by adjusting for residential area based on the postal code, since within these smaller areas the distribution of cases and controls was representative. Consequently, all analyses concerning air pollution were also adjusted for residential area and educational level (main effects and interaction analyses), while for all analyses involving genotypes, BMI was included in the model (main effects and interaction analyses).

First, effects of NO₂ (as a marker for vehicle exhaust pollutants) on risk of AMI or hypertension were analyzed separately. Thereafter, effects of genetic variants on risk of AMI and hypertension were studied. For GSTP1, each of the 7 SNPs was analyzed coded to the dominant genetic model (0 for two copies of the major allele, 1 for heterogeneous genotype or two copies of the minor allele). [20] For this gene, only the SNP or SNPs with the strongest effects on AMI or hypertension were studied for interaction with air pollution exposure on risk of each respective outcome. For GSTCD, a single variant (rs10516526) was studied, coded to the dominant genetic model. The GSTTI null genotype was studied using the two genotypes captured by the SNP rs2266637. Finally, interaction between air pollution and genetic variants was investigated by estimating effects of air pollution in analyses stratified by genotype in a common regression model. For this model, we also assessed whether smoking might modify the interaction between air pollution and genetic variants on AMI.

Results

In total, 1429 individuals had both valid genotype and air pollution exposure data and could be included in the analyses; 119 of the 192 first-time AMI cases, and 1310 of the 3614 control individuals. The reduction in numbers was mainly due to the geographical area covered by the dispersion model (for details, see Figures S1a–b). As expected, AMI cases were significantly different from controls on several characteristics; in particular, cases were more often male and older than controls (Table 1). In the population control cohort, 468 (36%) individuals were classified as hypertensive. Over 90% of AMI cases were taking daily medication for high cholesterol, but only 17% of hypertensive controls and 2% of normotensive controls; most likely this explains the lower mean cholesterol levels among cases compared to controls. Mean annual value of NO₂ exposure across all subjects was 15.6 with standard deviation 6.1.

Main effects of air pollution exposure and genetic variants on risk of AMI and hypertension

Air pollution was significantly associated with increased risk of AMI; odds ratio (OR) 1.78 (95% confidence interval (CI) 1.04–3.03) per 10 mg/m³ of NO₂ (Table 2).

None of the seven GSTP1 SNPs showed a significant association with AMI; the most strongly associated with AMI was rs596603, with OR 0.77 (95% CI 0.51–1.16) (Table 3). In contrast, 5 of the 7 GSTP1 SNPs had a p-value ≤0.05 for hypertension; the 3 strongest of these (rs1871042, rs749174 and rs762803) remained significant after Bonferroni-correction (p-value ≤0.007; Table 3). Neither the GSTCD SNP rs10516526 nor the GSTTI null genotype showed significant main effect results for AMI or hypertension. All SNPs in Table 3 were investigated further in the interaction analyses.

Interaction analysis

We saw no significant overall interaction between any of the genetic variants in the GST genes and air pollution exposure for AMI or hypertension. However, for all SNPs tested in the case of AMI (GSTP1:rs596603, GSTCD:rs10516526 and GSTTI: rs2266637), one genotype stratum has an effect estimate for air pollution exposure on risk of AMI that is significant and almost twice that of the other stratum, suggesting that the genotype may affect the adverse effect of NO₂ exposure. (Table 4)

Discussion

This study’s main findings are an association between indicators of long-term vehicle exhaust exposure and acute myocardial infarction, and an effect for several variants in the GSTP1 gene on risk of hypertension. Further, this study of interaction between long-term vehicle exhaust exposure and genetic susceptibility in CVD further suggests that cardiovascular response to air pollution exposure may be modified by genetic variants in the GST genes, although the power for interaction analysis was limited.

Which constituents in the heterogeneous traffic-related air pollution mix are actually responsible for the adverse effect is unknown, and for comparability with other European studies, we have used NO₂ as a marker for vehicle air pollution exposure in the association analyses. The finding of an association between long-term traffic-related air pollution exposure and AMI is consistent with some earlier studies. Several European cohort studies have found significant associations between NO₂ and cardiovascular mortality, with estimates ranging from 1.05 to 1.39 per 10 mg/m³.[6] These estimates are lower than those found in the present study, but considering the important difference in endpoint this may be expected. Rosenlund et al [22] found a suggested association between long-term average air pollution and fatal MI (OR 1.51 95% CI 0.96–2.16 for 5th to 95th percentile difference in 30-year average exposure to NO₂ in Stockholm, but none for non-fatal MI, which gives a null effect overall (OR 0.99
95% CI 0.76–1.30 for 5th to 95th percentile difference in 30-year average exposure to NO₂).

While American studies have focused more on between-city studies, European studies have mostly investigated within-city contrasts. This makes the comparability with American studies

### Table 1. Demographic characteristics of the 1429 individuals in the study population.

| Characteristic | AMI cases | Population controls |
|---------------|-----------|---------------------|
|               | n = 119   | n = 468             | n = 842             |
| Age, years    | Mean (SD) | Mean (SD)           | Mean (SD)           |
| BMI, kg/m²    | 27.6 (3.80) | 27.6 (4.43) | 24.8 (3.73) |
| LDL cholesterol, mmol/l | 2.38 (0.91) | 3.51 (0.99) | 3.13 (0.99) |
| HDL cholesterol, mmol/l | 1.28 (0.36) | 1.61 (0.48) | 1.67 (0.45) |
| Total cholesterol, mmol/l | 4.47 (1.12) | 5.82 (1.08) | 5.34 (1.10) |
| SBP, mmHg     | 133 (21.9) | 149 (18.9) | 116 (11.6) |
| DBP, mmHg     | 81 (11.1) | 89 (10.2) | 76 (7.20) |
| NO₂, µg/m³   | 14.9 (5.75) | 14.9 (5.93) | 16.1 (6.16) |

* p-values refer to test of equality of means between AMI cases and all controls.

While American studies have focused more on between-city studies, European studies have mostly investigated within-city contrasts. This makes the comparability with American studies

### Table 2. Effect of long-term traffic-related air pollution exposure (using annual mean of NO₂ as exposure indicator) on risk of acute myocardial infarction (AMI) and hypertension.

| Effect per 10 µg/m³ of NO₂ | AMI | Hypertension |
|-----------------------------|-----|--------------|
| OR                          | 1.78| 0.82         |
| 95% CI                      | 1.04–3.03 | 0.57–1.19   |
| p-value                     | 0.03| 0.30         |

*Model covariates are sex, age, age squared, level of education and residential area.
AMI: acute myocardial infarction; OR: odds ratio; CI: confidence interval.

While American studies have focused more on between-city studies, European studies have mostly investigated within-city contrasts. This makes the comparability with American studies

95% CI 0.76–1.30 for 5th to 95th percentile difference in 30-year average exposure to NO₂).
Table 3. Effects of the most significant variants in GSTP1, the GSTCD SNP rs10516526 (dominant genetic model) and the GSTT1 SNP rs2266637 (null genotype) on risk of acute myocardial infarction (AMI) and hypertension.

| Genotype frequencies | Cases | Controls | Genetic | Effect estimates and precision* |
|----------------------|-------|----------|---------|---------------------------------|
|                      | no. (%) | no. (%) | Model   | OR   | 95% CI | p-value |
| **GSTP1:**           |        |          |         |      |        |         |
| AMI                  |        |          |         |      |        |         |
| TT                   | 20 (17%) | 245 (19%) | (TT+GT) vs. | 0.77 | 0.51–1.16 | 0.21 |
| GT                   | 53 (45%) | 659 (51%) | GG      |      |        |         |
| GT                   | 45 (38%) | 398 (30%) |         |      |        |         |
| **GSTCD:**           |        |          |         |      |        |         |
| AMI                  |        |          |         |      |        |         |
| GG                   | 0       | 10 (1%)  | (GG+AG) vs. | 0.69 | 0.34–1.38 | 0.29 |
| AG                   | 10 (9%)  | 128 (10%) | AA      |      |        |         |
| AA                   | 108 (91%) | 1152 (89%) |         |      |        |         |
| **GSTT1:**           |        |          |         |      |        |         |
| AMI                  |        |          |         |      |        |         |
| Null                 | 11 (10%) | 168 (14%) | Null vs. | 0.65 | 0.33–1.27 | 0.20 |
| Non-null             | 101 (90%) | 1053 (86%) | Non-null |      |        |         |
| Hypertension         |        |          |         |      |        |         |
| Null                 | 58 (13%) | 110 (14%) | Null vs. | 0.88 | 0.59–1.33 | 0.55 |
| Non-null             | 382 (97%) | 671 (86%) | Non-null |      |        |         |

*Adjusted for age, age squared, BMI and sex.

AMI: acute myocardial infarction, OR: odds ratio, CI: confidence interval, BMI: body mass index.

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poor. It has been suggested that within-city contrasts are more strongly associated with cardiovascular events than between-city differences.[23] A recent Danish study [24] also using modeled NO2 exposure at residential addresses found that NO2 at the baseline address showed weaker associations than when using all addresses from 1 January 1971 until death or 31 December 2009 – presumably due to non-differential misclassification of exposure when using one address as a proxy for longer-term exposure. Since we have used NO2 data from the baseline address only, our estimate may similarly be more likely to underestimate the association than overestimate it.

Due to the properties of the gases and particles constituting vehicle exhaust, inhalation of the exhaust may cause oxidative stress [7,25,26,27], and this is potentially the mechanism for the association between such air pollution exposure and cardiovascular disease, especially when antioxidant and reductive response is weakened. This connection of inhaled air pollution to CVD is sometimes called the indirect pathway. The association between hypertension and these SNPs has been published to date. What has been reported is a positive association of variants in the GSTCD gene with cardiovascular outcomes. What has been reported is a positive association between the G-allele in the same GSTCD SNP and forced expiratory volume in the first second (FEV1); [10,15] Thus it seems that the G-allele may be beneficial both for lung function and protection against cardiovascular disease.

The null polymorphism of the GSTT1 gene is present in approximately 20% of Caucasians and has been associated with lower levels of high-density lipoprotein (HDL) cholesterol, elevated triglyceride-HDL ratio and hypertriglyceridemia, all risk factors for CVD. [11] A meta-analysis of 19 studies (total of 8020 coronary heart disease (CHD) cases and 11501 controls) gave an overall relative risk of CHD of 1.26 (95% confidence interval (CI): 0.90–1.75) for the GSTT1 null polymorphism. [28] This effect was modified by smoking: for smoking null genotype individuals, there was a high and significantly increased risk of CHD (OR 3.29, 95% CI: 1.46–7.26). In another study of patients suspected of coronary artery disease who had undergone coronary angiography, however, no association between the GSTT1 null genotype and coronary atherosclerosis (defined as >20% obstructive lesion) was seen. [29] In the present study, less obvious air pollution effect variation by genotype was suggested for GSTT1 rs596603 and the GSTT1 null variant, where air pollution exposure was associated with a higher and nominally significant risk of AMI in subjects with the null genotype and for CVD. [11] A meta-analysis of 19 studies (total of 8020 coronary heart disease (CHD) cases and 11501 controls) gave an overall relative risk of CHD of 1.26 (95% confidence interval (CI): 0.90–1.75) for the GSTT1 null polymorphism. [28] This effect was modified by smoking: for smoking null genotype individuals, there was a high and significantly increased risk of CHD (OR 3.29, 95% CI: 1.46–7.26). In another study of patients suspected of coronary artery disease who had undergone coronary angiography, however, no association between the GSTT1 null genotype and coronary atherosclerosis (defined as >20% obstructive lesion) was seen. [29] In the present study, less obvious air pollution effect variation by genotype was suggested for GSTT1 rs596603 and the GSTT1 null variant, where air pollution exposure was associated with a higher and nominally significant risk of AMI in subjects with the null genotype combined (OR 1.01, 95% CI 0.28–3.73). To our knowledge, nothing has previously been reported regarding an association of variants in the GSTCD gene with cardiovascular outcomes. What has been reported is a positive association between the G-allele in the same GSTCD SNP and forced expiratory volume in the first second (FEV1); [10,15] Thus it seems that the G-allele may be beneficial both for lung function and protection against cardiovascular disease.

Table 4. Interaction between long term air pollution (using annual mean of NO2 as exposure indicator) and SNPs in GST genes on risk of acute myocardial infarction and hypertension.

| Gene | SNP | Outcome | Genotype | OR | 95% CI | p-value | Interaction p-value |
|------|-----|---------|----------|----|--------|---------|---------------------|
| GSTP1: | rs96603 | AMI | TT+GT | 2.12 | 1.09–4.10 | 0.03 | 0.27 |
| GSTP1: | rs1871042 | Hypertension | TT+TC | 0.79 | 0.50–1.24 | 0.31 | 0.62 |
| GSTP1: | rs749174 | Hypertension | AA+AG | 0.78 | 0.50–1.22 | 0.28 | 0.57 |
| GSTP1: | rs762803 | Hypertension | AA+CA | 0.96 | 0.63–1.45 | 0.84 | 0.36 |
| GSTT1: | rs2266637 | AMI | Null | 1.40 | 0.33–5.96 | 0.65 | 0.60 |
| GSTCD: | rs10516526 | AMI | GT | 2.12 | 1.09–4.10 | 0.03 | 0.27 |

*Adjusted for age, age squared, sex, BMI, level of education and residential area.

OR: odds ratio; CI: confidence interval, AMI: acute myocardial infarction.

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Conclusions

In addition to giving insight into the specific pathways of disease pathology, results from gene-environment interaction studies can provide additional possibilities of identifying subjects at particularly high risk (i.e. those with high-risk genotypes and high-risk environment), and potentially to give them targeted prevention, health advice and care. This study shows significant association of vehicle air pollution exposure with risk of AMI; effects of variants in the GSTP1 gene on risk of hypertension, and suggests interactions between variants in the GSTP1, GSTT1 and GSTCD genes and vehicle air pollution exposure on risk of AMI.

Supporting Information

Figure S1 a) Geographical area covered by the dispersion model used to calculate annual average NO2 exposure in 2006, b) geographical area covered by the dispersion model used to calculate annual average NO2 exposure in 2007.

Figure S2 Linkage disequilibrium (LD) plot for GSTP1 gene SNPs, with values in each cell representing R2 between pairs of SNPs and coloring representing D’.

Author Contributions

Conceived and designed the experiments: ACO, FN. Performed the experiments: AL. Analyzed the data: AL, FN. Contributed reagents/materials/analysis tools: LB ACO AR FN LM AL. Wrote the paper: AL FN LB AR ACO LM. Critical revision of manuscript: SD.
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