AMERICAN JOURNAL OF INDUSTRIAL MEDICINE 55:1078–1086 (2012)

GSTM1 and *GSTP1* Gene Variants and the Effect of Air Pollutants on Lung Function Measures in South African Children

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Background *Several genes are associated with an increased susceptibility to asthma, which may be exacerbated by ambient air pollution. These genes include GSTM1 (glutathione-S-transferase M1 gene) and GSTP1 (glutathione-S-transferase P1 gene), which may modulate the response to epithelial oxidative changes caused by air pollutant exposure. This study evaluated fluctuations in the forced expiratory volume in one second (FEV_1) in relation to lagged daily averages of ambient air pollutants (SO_2, NO_2, NO, and PM_{10}) while considering genotype as an effect modifier.*

Methods *A longitudinal cohort of 129 schoolchildren of African descent from Durban, South Africa was assessed. GSTM1 (null vs. present genotype) and GSTP1 (Ile105Val; AA → AG/GG) genotypes were determined using standard techniques. SO_2, NO_2, NO, and PM_{10} were measured continuously over a year using validated methods. The outcome was intraday variability in FEV_1. Data were collected daily over a 3-week period in each of four seasons (2004–2005).*

Results *Among the children tested, 27% had the GSTM1 null genotype and 81% carried the GSTP1 G allele. Approximately 26 out 104 children (25%) showed evidence of bronchial hyperreactivity, 13% reported having symptoms in keeping with persistent asthma, and a further 25% reported symptoms of mild intermittent asthma. PM_{10} and SO_2 levels were moderately high relative to international guidelines. Neither GSTM1 nor GSTP1 genotypes alone were significantly associated with FEV_1 intraday variability. In models not including genotype, FEV_1 variability was statistically significantly associated only with NO_2 for 5-day lags (% change in intraday variability in FEV1 per interquartile range = 1.59, CI 0.58, 2.61). The GSTP1 genotype modified the effect of 3 days prior 24-hr average PM_{10} and increased FEV_1 variability. A similar pattern was observed for lagged 3 day SO_2 exposure (P interaction < 0.05). Adverse*

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Contract grant sponsor: National Research Foundation (NRF), South Africa, supplemented by Durban University of Technology (DUT) KwaZulu-Natal, Durban, South Africa; Contract grant number: 2068195 Thuthuka;

Contract grant sponsor: Ethekwini Municipality, KwaZulu Natal, Durban, South Africa; Contract grant number: 1a-103—Health Study;

Contract grant sponsor: Intramural Research Program, National Institute of Environmental Health Sciences, NIH, USA; Contract grant numbers: Z01 49019; Z01 ES025045;

Contract grant sponsor: US NIH Fogarty International Center; Contract grant number: 2 D43 TW000812.

Disclosure Statement: All authors declare no conflict of interest.

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Accepted 15 December 2011

DOI 10.1002/ajim.22012. Published online 6 January 2012 in Wiley Online Library (wileyonlinelibrary.com).

effects of these pollutants were limited to individuals carrying the G allele for this polymorphism.
Conclusion *Among this indigenous South African children cohort, the GSTP1 genotype modified the effects of ambient exposures to PM_{10} and SO_2 and lung function. A plausible mechanism for these observed effects is decreased capacity to mount an effective response to oxidative stress associated with the GSTP1 AG + GG genotype.*
Am. J. Ind. Med. 55:1078–1086, 2012.

KEY WORDS: air pollutants; asthma; child respiratory health; gene–environment interaction

INTRODUCTION

Exposure to air pollutants and genetic factors associated with the risk of development or aggravation of asthma are likely to interact in a complex manner that may vary from one population to another. Knowledge of how genes increase susceptibility to environmental exposures may be crucial to understanding asthma causation, management and even prevention. While cohort studies on children's respiratory health have been concluded or are currently underway in several developed countries [Ober et al., 1998; Wjst et al., 1998; Custovic et al., 2002; Phelan et al., 2002], no similar study has been conducted on the African continent. Additionally, there is very limited information on the role of outdoor air pollution in the development or aggravation of allergic diseases in Africa. To the best of our knowledge, the only such report over the last 5 years examined the association of household proximity to roads with wheeze symptoms in an Ethiopian population [Venn et al., 2005]. There are very limited data from the sub-Saharan region that document the association between air pollutant and pulmonary function as modified by genotype.

The adverse effects of air pollutants on airway function have been extensively reviewed [Seaton et al., 1995; Peden, 2003; Bernstein et al., 2004]. Exposure to ambient air pollutants may lead to the development or expression of asthma phenotypes [Gilmour et al., 2006; McConnell et al., 2006] and increasing evidence suggests that the effects of pollutants vary among individuals because of the variation in their genetic susceptibility. However, there are few examples in the literature of specific gene–environment interactions in relation to asthma-related measures. The clearest examples of genetic interactions for air pollutants exist for ozone, environmental tobacco smoke and endotoxin [London, 2007; London and Romieu, 2009], whereas data are limited for particulate matter, and nitrogen oxides (NO and NO_2) and sulfur dioxide (SO_2). The latter pollutants may act as oxidants or induce oxidant responses in the airways. Greater airway or plasma antioxidant status has been associated with protection from the effect of pollutant exposure, and most genetic studies addressing environmental exposures and asthma to date have focused on the genes thought to play a role in inflammation or antioxidant protection [Peden, 2005].

Research has mainly focused on a handful of common polymorphisms with well-described functional effects in genes thought to be involved in oxidative stress responses [Romieu et al., 2005; London and Romieu, 2009]. The single most commonly examined is a highly prevalent deletion polymorphism of the glutathione-*S*-transferase M1 gene (*GSTM1*). Deletion of both copies of the *GSTM1* gene, referred to as homozygous deletion or the null genotype, abolishes *GSTM1* activity [Strange et al., 2001]. The high frequency of the *GSTM1* null genotype, ranging from 25% to 60% depending on the ethnic group, enables examination of this polymorphism in studies that were not originally powered to study interactions. The second most commonly studied gene in relation to either ETS or ambient air pollution is glutathione-*S*-transferase P1 (*GSTP1*). A functional polymorphism (Ile105Val; AA → AG/GG) occurs at relatively high frequency [Strange et al., 2001]. The majority of published studies looking at interactions between air pollutant exposures and either or both *GSTM1* and *GSTP1* polymorphisms show positive findings, but not always in the same direction for *GSTP1* [London and Romieu, 2009]. Whether the *GSTM1* and/or *GSTP1* polymorphisms will be confirmed as modifiers of effects of exposure to ETS or other inhaled oxidants as new data accumulate remains to be seen.

We investigated whether polymorphisms in *GSTM1* and *GSTP1* genes, which are expected to modulate the effects of oxidative stress in airway inflammation and tissue damage [Hayes and Strange, 1995; Fryer et al., 2000], contribute to the variation in response to inhaled pollutants. Repeated measures of daily changes in FEV_1 (forced expiratory volume in one second) in response to air pollutants including SO_2, NO, NO_2, and PM_{10} (particulate matter less than 10 μm in aerodynamic diameter) were assessed in a longitudinal study of a cohort of South African schoolchildren residing in the Durban metropolitan area. We hypothesized *GSTM1* or *GSTP1* polymorphisms associated with reduced antioxidant capacity would lead to increases in the intraday variability of FEV_1, a marker

of aggravation of asthma-related conditions that has been associated with air pollutant exposures.

METHODS

Study Population

A sample of 129 indigenous African children (between 9 and 11 years old) from 7 primary schools was recruited for this study from south and north Durban in South Africa. At each of the seven schools, two 4th grade classrooms were randomly prioritized as classroom 1 and 2 and children were randomly selected from these classrooms. Details of the study sampling strategy have been presented elsewhere [Naidoo et al., 2006]. The south Durban region, recognized as one of the most highly industrialized and most heavily polluted areas in Southern Africa [Nriagu et al., 1999], includes extensive residential areas. The schools in north Durban were selected because of a similar socio-economic profile as communities in the south but with little industrial exposure compared to the south. This study was approved by the University of KwaZulu-Natal Ethics Committee, and parental consent for genetic analyses was obtained for all children. Each child completed an informed assent form which was comprehensively explained in the language of their choice.

Symptom and Demographic Data

A questionnaire was administered to all caregivers, which included components addressing demographic information, and respiratory and other relevant symptoms using standardized validated questions from sources such as the British Medical Research Council and the American Thoracic Society. Questions addressing the presence and severity of asthma included information concerning wheezing, coughing, chest tightness, shortness of breath, activity limitations, and medication use; health services utilization; quality of life measures; perinatal history; place of birth and residential history; exercise, viral respiratory infections, exposure to cigarette smoke and pre-existing medical conditions.

Baseline spirometry was performed by experienced technologists using the American Thoracic Society criteria using the Jaeger Flowscreen [ATS, 1995]. Spirometers were calibrated at least twice a day with a 3 L syringe. Spirometry was conducted by technologists who had undergone training in standard technique. Spirometry was performed in a sitting position without nose clips. Those children without a baseline obstructive pattern underwent methacholine nonspecific challenge testing by trained technologists according to an abbreviated protocol used in epidemiological surveys [Yan et al., 1983]. Participants with an obstructive pattern at baseline (FEV_1/FVC < 0.75) were administered an inhaled bronchodilator and had testing repeated.

Bihourly Measures of Pulmonary Function

A central aspect of the health data collection was bihourly measures of lung function (spirometry) collected at school 5 days per week over 3-week periods in each of four seasons (2004–2005) using digital hand-held devices: the AirWatch® (iMetrikus, Carlsbad, CA) brand airway monitor. All schools were studied simultaneously in the same calendar periods. On each of the five schooldays during the week, participants were asked to perform a session of three consecutive forced expiratory maneuvers every one and a half to 2 hr (four times per 5.5-hr school day: at approximately 08:00 hr, 09:45 hr, 11:30 hr, and 13:20 hr). Results of repeated expiratory maneuvers over a period of 12 months were digitally stored in each Air Watch. A unique patient identifier and the time and date of each expiratory maneuver were manually downloaded into a database. Each participant received his/her own device, which was kept at the school, and was clearly labeled with the participant's full name to avoid inadvertent exchange of devices. An intensive training session was conducted at the school with the participants in the proper performance of lung function maneuvers, followed by revision sessions prior to each monitoring cycle. The bihourly pulmonary function tests were completed during classroom hours under the supervision of study personnel.

The highest FEV_1 from a valid expiratory maneuver for each of the four daily sessions was used in data analyses. An expiratory maneuver was considered valid if the FEV_1 result (1) was between 30% and 120% of each child's personal best as defined by that child's highest recorded FEV1 during baseline spirometry (performed by experienced technicians using the American Thoracic Society criteria) and methacholine challenge testing, and, (2) came from expiratory maneuver that was recorded by the Airwatch device as ''error-free.'' The Airwatch device shows two possible error messages, one for length of blow and the other for technique. Almost 40% of the data was error-free and 79.2% of the error-free data were valid blows.

Environmental Monitoring of Ambient Pollutants

During each of the four intensive 3-week phases, gaseous air pollutant concentrations were monitored continuously: NO_2 and NO were sampled at seven monitoring sites using continuous gas-phase chemiluminescence detection; and SO_2 was monitored continuously at 16 sites, including all seven schools, using ultraviolet fluorescence spectro-

metry. PM_{10} was monitored gravimetrically at 12 sites: at each school using 24-hr integrated measurements, and at five additional sites using TEOMs. Each pollutant was sampled using standard reference methods and protocols. Details of these measurements and the quality assurance program are reported elsewhere (Naidoo et al., 2006).

Genotyping

All genotyping assays were conducted by a researcher who was blind to child ID and disease status. Genomic DNA was extracted using a PUREGENE DNA isolation kit. The presence or absence of the *GSTM1* gene was determined by using a multiplex PCR method, including the β-globin gene as a positive control [Bell et al., 1993]. Fifteen percent of samples were reassayed as confirmation of results. The *GSTP1* (rs1695) genotype was determined by Taqman® SNP Genotyping Assays (Applied Biosystems, Foster City, CA). The *GSTP1* (rs1695) PCR amplifications were performed using the 5′-nuclease assay on Gene-Amp PCR Systems 9700 (Applied Biosystems). Fluorescent PCR products were detected by the ABI Prism 7900HT sequence detection system and analyzed by SDS software (Applied Biosystems). Both positive genotype and no template controls were included with at least 95% plate efficiency.

Statistical Analysis

The longitudinal design of this study allowed the investigation of how daily and bihourly fluctuations in outdoor contaminant levels affected potential fluctuations in pulmonary function measures. Linear regression models were fitted using generalized estimating equations (GEEs, assuming normal distribution with identity link) using PROC GENMOD for SAS to accommodate the correlation structure arising from repeated measurements on the same individual. An exchangeable correlation working structure was used.

Within-day variability for FEV_1 was defined as $100 \times$ (the maximum best FEV_1 − minimum best FEV_1)/ maximum best FEV_1 where the ''best FEV_1'' is the highest valid value for the specific time of day (08:00 hr, 09:45 hr, 11:30 hr, and 13:20 hr), thus providing a single summary lung function measurement per child, per day. For power reasons, we used a dominant coding model to assess the genotype effects in the statistical models.

Covariates used in the GEE models included race, school, caregiver smoking, caregiver education, household income and season. Effect modification was examined by including genotype and pollutant product terms in the models. The gene–environment interaction was assessed for associations of exposure to SO_2, NO, NO_2, and PM_{10} with FEV_1 intraday variability, using *GSTM1* and *GSTP1* genotypes as the effect modifiers. Daily exposures estimates were based on the child's school: PM_{10} and SO_2 used school-based measurements; and NO and NO_2 used the spatial average across either north (three schools) or south (four schools) Durban, as not all schools had NO_x monitors. Multiple imputation procedures (repeated five times for each exposure parameter) were used to obtain a complete data set. Lag effects were modeled to account for both acute and prior exposure effects, and included lags of 1–5 days as well as the a 5-day average. The percent change in within-day variability in FEV_1 was estimated for an increase of one interquartile range in each pollutant (NO_2: 8.19 ppb, NO: 29.7 ppb, PM_{10}: 29.4 $\mu g\ m^{-3}$ and SO_2: 9.8 ppb). The interquartile range was calculated as the 75th–25th percentile value concentrations, using all of the concentration measurements obtained in the study. The use of the IQR for a specific pollutant, when multiplied by the corresponding estimated coefficient and transformed appropriately (since logarithms are typically used), allows a direct comparison of the effect size among the pollutants used in the study, and it assures the magnitude of the change in exposure being examined for effects is relevant to the study population. This approach also accounts for the differences among concentrations units. An adverse effect in lung function would be denoted by an increase in the estimate for intraday variability in FEV_1. Analyses used SAS (Version 9.1) and STATA (version 9, College Station, TX).

RESULTS

Among this sample of 129 indigenous African schoolchildren, the average age was 10.6 years, and almost two-thirds were female (Table I). The education level among the caregivers was relatively high with 35% having completed high school, with a substantial percentage (>50%) of households belonging in a low socio-economic category earning R30,000 (approximately US$3000) or less per annum, which is regarded as a lower income bracket though slightly above the poverty line in South Africa [National Treasury, 2007]. The frequency of the *GSTM1* null genotype was 27%, and the frequency of the polymorphic *GSTP1* AG + GG genotype was 81%. According to the methacholine challenge tests, 25% of children had evidence of bronchial hyperreactivity. Approximately 13% of all children reported having symptoms in keeping with persistent asthma, and a further 25% reported symptoms of mild intermittent asthma (Table I).

Table II summarizes 24-hr average pollutant levels in Durban. PM_{10} levels during the study period were high relative to current WHO guidelines [WHO, 2006], the 24-hr guideline of 50 $\mu g/m^3$ was frequently exceeded. It should be noted that PM_{10} guidelines have been recently lowered. SO_2 levels varied widely across the study area,

TABLE I. Demographic and Phenotypic and Genotypic Characteristics of Study Population (n = 129)

Category	n (%)
Age, year[a] (n = 129)	10.6 (1.1)
Sex	
Female	84 (65.1)
Caregiver education	
Grade 11 or less	60 (46.9)
High School matriculant	45 (35.2)
Some tertiary education	23 (18.0)
Annual household income	
R10,000 or less	24 (24.7)
R10,000 to R30,000	25 (25.8)
R30,001 to R75,000	24 (24.7)
R75,001 or more	24 (24.7)
Caregiver smokes (% yes), n = 195	34 (17.4)
Genotype	
GSTM1 pos	89 (72.9)
GSTM1 null	33 (27.1)
GSTP1[b]	
Ile-Ile (AA)	21 (18.8)
Ile-Val (AG) + Val-Val (GG)	91 (81.2)
Health outcomes	
Airway hyperreactivity (baseline)[c]	
Marked hyperreactivity	10 (9.6)
Probable hyperreactivity	6 (5.8)
Possible hyperreactivity	10 (9.6)
None	78 (75.0)
Asthma severity from caregiver questionnaire	
Moderate to severe	5 (4.5)
Mild persistent	9 (8.0)
Mild intermittent	28 (25.0)
No asthma	70 (62.5)

There was data unavailable for demographic and phenotypic characteristics due to lack of participant cooperation. There were 10 undetermined samples for GSTP1, which could not be attributed to poor DNA quality as these samples were also used for the GSTM1 determination.

[a]Mean and SD at study entry.

[b]*GSTP1*: A allele codes for isoleucine, G allele codes for valine.

[c]Marked: $PC_{20} \leq 2$ mg/ml, probable: 2 mg/ml $< PC_{20} \leq 4$ mg/ml, possible: 4 mg/ml $< PC_{20} \leq 16$ mg/ml, none: $PC_{20} > 16$ mg/ml.

TABLE II. Summary of Ambient Pollutant Levels (24 hr Averages) in Durban During 2004–2005

Pollutant	Mean (SD)	Range
PM_{10} ($\mu g/m^3$)	86.8 (1.1)	28.1–266.6
SO_2 (ppb)	5.8 (0.2)	0–40.8
NO_2 (ppb)	22.1 (0.2)	7.5–38.1
NO (ppb)	53.4 (0.8)	1.3–91.7

and the highest concentrations occurred in south Durban, reflecting the location of emitting industries. WHO guidelines for SO_2 also have been lowered recently, and most monitoring sites (except those in north Durban) frequently exceeded the 24-hr guideline value of 8 ppb. NO_2 levels were highest in the city center and reflected traffic emissions. WHO guideline values for NO_2 exist on an annual average (21 ppb) and 1-hr (106 ppb) basis, but not for 24-hr averages. During the study period, the annual average level was similar to guideline value, and 1-hr concentrations at the city center site occasionally exceeded the 1-hr guideline value.

In models adjusted for covariates, neither *GSTM1* nor *GSTP1* genotypes alone were significantly associated (using alpha = 0.05) with FEV_1 intraday variability (Table III).

Effect estimates (ignoring *GSTM1* and *GSTP1* polymorphisms status) for within-day variability in FEV_1 associated with PM_{10}, SO_2, NO, and NO_2 (tested independently) were inconsistent across different lagged times (Table IV). For NO_2 and NO, the within-day variability generally increased with longer lags. FEV_1 variability with NO at lag 5 (% change in intraday variability in FEV1 per interquartile range =1.59, 95% CI 0.58, 2.61) was statistically significant. No other pollutant–lag combinations were statistically significant.

Tables V and VI present models addressing potential effect modification by genotype on the association between air pollutant exposures and FEV_1 intraday variability for, respectively, *GSTM1* and *GSTP1* polymorphisms. Among the *GSTM1* positive children, a statistically significant association of exposure with FEV_1 intraday variability was seen only with PM_{10} for the 5-day average; this association was in the ''unexpected'' protective direction (i.e., higher exposure associated with decreased FEV_1

TABLE III. Adjusted Associations of GST[a] Genotypes With Intraday Variability in FEV_1[b]

Genotype	No. of observations	Intraday variability in FEV_1	
		Coeff.	95% CI
GSTM1 pos	1,179	—	
GSTM1 null		−0.32	(−1.93, 1.29)
GSTP1 AA	1,037	—	
GSTP1 AG + GG		0.54	(−1.11, 2.19)

[a]The effects of the GST genotypes are adjusted for race, school, caregiver smoking, caregiver education, household income, and season.

[b]Intraday variability for FEV_1 is defined as: 100 (maximum best FEV_1-minimum best FEV_1)/maximum best FEV1; where the "best FEV_1" is the highest valid, error-free value for the specific time of day (08:00 hr, 09:45 hr, 11:30 hr, and 13:20 hr). An increase in the estimate for intraday FEV_1 is indicative of a negative impact on lung function as compared to the reference group.

TABLE IV. Percent Change[a] in Intraday Variability[b] of FEV_1 Associated With One Inter-Quartile Range Change of Ambient Exposure From Single Pollutant[c] Linear Regression Models Using Generalized Estimating Equations (GEE)

Outcome	Lag	PM_{10}, estimate (CI)	SO_2, estimate (CI)	NO_2, estimate (CI)	NO, estimate (CI)
Intraday variability of FEV_1	Lag 1	0.19 (−0.48, 0.86)	1.59 (−0.03, 3.20)	0.68 (−0.95, 2.30)	−0.30 (−1.63, 1.11)
	Lag 2	0.80 (−0.33, 1.93)	0.26 (−1.25, 1.77)	0.12 (−1.30, 1.55)	−0.42 (−1.83, 0.98)
	Lag 3	0.41 (−0.58, 1.41)	−0.77 (−2.35,0.81)	0.30 (−1.25, 1.84)	0.21 (−1.54, 1.96)
	Lag 4	0.55 (−0.21, 1.31)	−0.09 (−1.88, 1.69)	0.73 (−1.42, 1.88)	0.55 (−0.72, 1.81)
	Lag 5	0.38 (−0.29, 1.05)	−0.08 (−1.61, 1.45)	0.90 (−0.53, 2.32)	*1.59 (0.58, 2.61)
	5 day average	1.74 (−0.14, 3.62)	0.93 (−2.99, 4.86)	1.15 (−1.28, 3.58)	1.33 (−1.75, 4.42)

Covariates in each model: race, school, caregiver smoking, caregiver education, household income, season.

[a]The percent change value shown is for an increase of one inter-quartile range in each respective pollutant: NO_2: 8.19 ppb, NO: 29.7 ppb, PM_{10}: 29.4 μg m^{-3}, SO_2: 9.8 ppb.

[b]Intraday variability for FEV_1 is defined as: 100 (maximum best FEV_1-minimum best FEV_1)/maximum best FEV1; where the "best FEV_1" is the highest valid, error-free value for the specific time of day (08:00 hr, 09:45 hr, 11:30 hr, and 13:20 hr). An increase in the estimate for intraday FEV_1 is indicative of a negative impact on lung function.

[c]Pollution levels used in regression models combine measured and imputed values.

*P-value for the change in estimate <0.05.

intraday variability) (Table V). No statistically significant associations were seen among the *GSTM1* null children. In three instances, the interaction terms *GSTM1* genotype were statistically significant, however, in only one (SO_2 5-day average) of these three scenarios was the interaction in the expected direction, that is, higher exposure associated with greater FEV_1 intraday variability among those *GSTM1* null as compared to those *GSTM1* pos.

In five exposure-response scenarios, individuals with the *GSTP1* AG + GG polymorphism showed statistically significant associations of exposure with FEV_1 intraday variability (Table VI). In three of these (PM_{10} lag 3, and SO_2 lags 1 and 3), the GSTP1 genotype modified the association between exposure and increased FEV_1 intraday variability (P interaction <0.05). With PM_{10} (lags 2 and 3) and SO_2 (lag 3), higher exposure was associated with greater FEV_1 intraday variability among those with *GSTP1* AG + GG as compared to those with *GSTP1* AA. Adverse effects of these pollutants were limited to individuals carrying the G allele for this polymorphism.

TABLE V. Effect of Pollutant Exposure by *GSTM1* Genotype (Percent Change[a] in Intraday Variability[b] of FEV_1 Associated With Ambient Levels[c] of Pollutants From Single Pollutant Linear Regression Models Using Generalized Estimating Equations (GEE))

		PM_{10}		SO_2		NO_2		NO	
GSTM1		Est	CI	Est	CI	Est	CI	Est	CI
Pos	Lag 1	−2.03	−6.95, 288	0.31	−0.31, 0.93	1.40	−0.36, 3.17	1.34	−2.63, 5.31
Null		−1.38	−7.52, 4.75	0.44	−0.78, 1.66	−0.17	−0.29, 2.62	−2.19	−5.93, 1.55
Pos	Lag 2	−2.57	−8.60, 3.47	0.09	−0.44, 0.61	1.17	−0.69, 3.03	0.85	−3.47, 5.17
Null		0.36	−7.17, 7.89	1.06	−0.36, 2.48	−0.04	−2.97, 2.89	−1.56	−5.38, 2.26
Pos	Lag 3	−0.52	−5.78, 4.74	−0.56	−1.44, 0.32	1.68	−0.58, 3.95	1.52*	−2.11, 5.16 (***0.05**)
Null		−5.66	−14.45, 3.14	1.46	−0.75, 3.66	−0.73	−3.77, 2.32	−3.06	−6.61, 0.49
Pos	Lag 4	0.17	−2.05, 2.38	6.28	−2.04, 14.60	0.7	−1.22, 2.61	1.34	−0.67, 3.36
Null		−0.12	−3.26, 3.02	−4.10	−13.84, 5.64	1.82	−1.82, 5.46	0.30	−3.32, 2.92
Pos	Lag 5	1.83	−0.22, 3.88	−0.23	−7.15, 6.67	1.39	−0.98, 3.75	−0.69	−3.26, 1.89
Null		−1.04	−4.49, 2.40	−2.47	−14.07, 9.12	1.88	−2.53, 6.34	0.34	−2.41, 3.10
Pos	5-day average	−0.98†	−1.56, −0.41 (†**<0.00**)	1.08*	0.44, 2.60 (***<0.00**)	−5.75*	−11.63, 0.12 (***0.01**)	1.27	−1.03, 3.58
Null		−0.15	−1.50, 1.20	−1.18	−2.89, 0.53	1.63	−5.14, 8.40	0.51	−3.68, 4.70

Covariates in each model: race, school, caregiver smoking, caregiver education, household income, season, interaction between genotype and pollutants. Bold denotes significant P-value.

[a]The percent change value shown is for an increase of one interquartile range in each respective pollutant: NO_2: 8.19 ppb; NO: 29.7 ppb, PM_{10}: 29.4 μg m^{-3}; SO_2: 9.8 ppb.

[b]Intraday variability for FEV_1 is defined as: 100 (maximum best FEV_1-minimum best FEV_1)/maximum best FEV1; where the "best FEV_1" is the highest valid, error-free value for the specific time of day (08:00 hr, 09:45 hr, 11:30 hr, and 13:20 hr). An increase in the estimate for intraday FEV_1 is indicative of a negative impact on lung function.

[c]Pollution levels used in regression models combine measured and imputed values.

*P-value for genotype–pollutant interaction term ≤ 0.05.

†P-value for the change in estimate ≤ 0.05.

TABLE VI. Effect of Pollutant Exposure by *GSTP1* Genotype (Percent Change[a] in Intraday Variability[b] of FEV_1 Associated With Ambient Levels[c] of Pollutants From Single Pollutant Linear Regression Models Using Generalized Estimating Equations (GEE))

		PM_{10}		SO_2		NO_2		NO	
GSTP1		**Est**	**CI**	**Est**	**CI**	**Est**	**CI**	**Est**	**CI**
AA	Lag 1	−0.14	−5.31, 5.0	−0.11	−0.62, 0.39	1.86*	−0.14, 3.87 (***0.04**)	0.58	−4.00, 5.17
AG + GG		0.50	5.35, 6.34	1.17†	0.16, 2.18 (†**0.03**)	−3.40	−5.95, −8.85	−1.56	−5.53, 2.41
AA	Lag 2	−4.47*	−10.70, 1.18 (***0.01**)	0.22	−0.34, 0.77	2.29†,*	0.01, 4.56 (*‹**0.00**; †**0.04**)	−0.15	−4.94, 4.64
AG + GG		9.0	−0.83, 18.84	0.32	−1.13, 1.78	−3.82†	−6.53, −1.11 (†**0.01**)	−0.78	−4.92, 3.37
AA	Lag 3	−2.52*	−10.23, 5.20 (***0.02**)	−0.72*	−2.14, 0.69 (***0.03**)	2.33	−0.36, 5.02	−0.77	−5.32, 3.78
AG + GG		7.98†	1.14, 14.78 (†**0.02**)	3.05†	1.53, 4.56 (†‹**0.00**)	−3.05†	−6.02, −0.08 (†**0.04**)	−0.32	−4.37, 3.74
AA	Lag 4	0.35	−1.55, 2.24	−4.06	−13.87, 5.34	−0.41	−2.91, 2.09	−1.40	−3.55, 0.78
AG + GG		−0.86	−3.73, 2.02	4.37	−3.06, 11.81	2.04	−1.36, 5.45	0.73	−1.51, 2.96
AA	Lag 5	−0.18	2.37, 2.02	−0.09	−7.81, 7.63	−0.46	−3.25, 2.33	−1.43	−4.00, 1.14
AG + GG		1.08	−2.62, 4.77	−1.03	−8.29, 6.25	2.66	−0.93, 2.63	2.04	−1.01, 5.09
AA	5-day average	−0.40	−1.42, 0.62	0.63	−1.01, 2.26	−3.52	−11.45, 4.40	1.91	−0.85, 4.67
AG + GG		0.20	−1.18, 1.63	−1.80	−3.87, 0.26	4.42	−3.27, 12.11	0.63	−2.09, 3.36

Covariates in each model: race, school, caregiver smoking, caregiver education, household income, season, interaction between genotype and pollutants. Bold denotes significant *P*-value.

[a]The percent change value shown is for an increase of one interquartile range in each respective pollutant: NO_2: 8.19 ppb; NO: 29.7 ppb, PM_{10}: 29.4 $\mu g\ m^{-3}$; SO_2: 9.8 ppb.

[b]Intraday variability for FEV_1 is defined as: 100 (maximum best FEV_1-minimum best FEV_1)/maximum best FEV1; where the "best FEV_1" is the highest valid, error-free value for the specific time of day (08:00 hr, 09:45 hr, 11:30 hr, and 13:20 hr). An increase in the estimate for intraday FEV_1 is indicative of a negative impact on lung function.

[c]Pollution levels used in regression models combine measured and imputed values.

*P-value for genotype–pollutant interaction term ≤ 0.05.

†P-value for the change in estimate ≤ 0.05.

DISCUSSION

Few studies have addressed interactions between air pollutants and genetics in relation to respiratory health, and studies from Africa are especially rare. The present study found relatively modest, and not entirely consistent, interaction effects of the *GSTM1* and *GSTP1* polymorphisms. Among the 24 pollutant–lag combinations examined, for *GSTM1*, in only one of the four that showed statistically significant pollutant–genotype interactions, was the effect in the expected direction of those with *GSTM1* null having greater pollution-associated increases in FEV_1 intraday variability (Table V). Moreover, as shown in the table, none of the 48 *GSTM1*-genotype-specific point estimates for the effect of pollutants on FEV_1 intraday variability were statistically significant in the expected direction of increased intraday variability with increased pollution.

As shown in Table VI, for *GSTP1*, three of four pollutant–lag combinations that showed a statistically significant pollutant–genotype interaction found greater pollution-associated increases in FEV_1 intraday variability for the *GSTP1* AG + GG genotype. When examining the pattern among specific pollutants for GSTP1 polymorphisms, there is a suggestion that the AG + GG genotype is associated with increased pollutant associated effects on FEV_1 intraday variability for both PM_{10} and SO_2: in the case of each pollutant, three of the six lags examined showed either a statistically significant interaction term with greater effect for this genotype, or a significant point estimate for pollutant effect on FEV_1 intraday variability among this genotype. However with NO_2, a decreased FEV_1 intraday variability was shown for children with the G allele. One of the more significant effect among pollutants and NO_2 is its negative correlation with ozone (O_3), an effect due to scavenging (removal of O_3 due to NO and NO_2). In Durban, at the central site that collected both NO_2 and O_3 measurements during the study, these pollutants had moderate negative correlation ($r = -0.35$). In Durban and elsewhere, protective effects are sometimes found for NO_2 due to this negative correlation with ozone. While one must be circumspect in overinterpreting these results, they appear particularly notable given that *GSTP1* genotype was not associated with intraday variability in FEV_1 when pollutants were not included in the models (Table III), and that only 1 of 20 pollutant–lag combinations were significantly associated with intraday variability in FEV_1 when genotype was not considered in the models (Table IV).

The examined pollutants are related to oxidative stress: NO, NO_2, and SO_2 may produce free oxidative radicals, while the mechanism for PM_{10} is via its metal components which may contribute to damage to the respiratory system through the generation of free radicals

[Hong et al., 2007]. The effects of pollutant exposure are mediated by complex interactive processes of oxidative, radical and enzymatic attack on the respiratory extracellular lining fluid, epithelial cells and macrophages. These processes are coupled to a persistent inflammatory response that produces tissue damage, decreased ventilatory capacity, increased airway reactivity, decreased macrophage clearance and altered immune functions [Gilliland et al., 1999; Kelly, 2003; Kunzli and Tager, 2005]. Genes involved in antioxidant and detoxifying reactions such as the GST are thus important in the response to oxidative stress. Polymorphisms in these genes may result in total absence or a substantial change in enzyme activity, which compromises biological reaction to environmental pollutants [Peden, 2005].

Results for gene–environment studies with the *GSTP1* polymorphism have been inconclusive. In accordance with the findings in our study, Melen et al. [2008] found an interaction effect on allergic sensitization in children with the *GSTP1* AG + GG genotypes when exposed to traffic-NO_x during their first year of life; a study in Mexico City has shown that asthmatic children with the GG genotype seem more likely to experience respiratory symptoms in response to acute exposure to ozone as compared to the AA genotype [Romieu et al., 2006]. In contrast a group from Taiwan [Lee et al., 2004] found that *GSTP1* AA conferred an increased risk of asthma in moderate (OR = 1.5, 95% CI 0.7–3.1) and high (OR = 2.9, CI 1.4–6.0) pollution districts compared to a low pollution district (based on mean values of NO_x and SO_2 over 7 years previous to the study).

This study has several strengths. First, the study population of children exposed to ambient pollutants was confined to defined areas, each area with its own monitoring site, allowing a more precise estimation of exposure. Second, pollutants were analyzed in a systematic manner over the duration of the study, which allowed the correlation between increases in exposure and decrements in lung function measures. The few studies that have examined the relationship between genetic risk factors and environmental exposures in the exacerbation of asthma have used mortality data, admission records, absenteeism and activity limitation and the resultant phenotypic definitions could not always be replicated in other studies. By using pulmonary function tests, this study provides a more rigorous phenotypic characterization. A limitation in this study was our small sample size. While larger sample sizes will improve power for gene–environment interaction studies, power is also enhanced by better measures of exposure, characterization of individual exposure and repeated measures over time [London, 2007]. All these factors were included in our study design. With the number of multiple comparisons in this study, false positives may occur. We therefore interpret our results with caution and in addition, a complete set of genotype and symptom data could not be obtained for all participants.

In addition to the factors discussed above (prevalence of persistent asthma in the study population, consistency in performance of expiratory maneuvers, choice of pulmonary function measure), limited consistency in findings across lags 1–5 with the four pollutants tested could be attributed to several other factors. We studied the effects of four pollutants independently, using a model approach of one gene with one exposure. This approach did not account for the fact that air pollution is a complex mixture of pollutants which may interact and modify respective effects on lung function, although repeated measures of FEV_1 and PEF provided an advantage in the GEE models. Additionally, in complex diseases such as asthma, several or many alterations in different genes likely contribute to the genetic predisposition of an individual to develop atopic diseases and asthma, potentially dampening the signal from a single involved polymorphism [Peden, 2005]. Gene–environment effects may also vary from one setting to another which may be due to varying patterns of exposure and different genotype frequencies in ethnically different populations.

The present study supports some previous work that suggests that GSTP1 polymorphisms might play a role in modifying acute effects of air pollutants on variability in lung function. A suggested mechanism is that children with compromised oxidative defense ability are at increased risk of adverse pulmonary outcomes. In light of the found associations between ambient pollutant concentrations and adverse effects on lung function among children who are genetically predisposed, strategies for reducing ambient environmental pollution in these regions should be considered. Further research on these interactions could lead to more accurate estimates of disease risk, intervention and prevention among susceptible populations, including children.

ACKNOWLEDGMENTS

We wish to thank the children and parents of the South Durban Health Study cohort for their dedicated cooperation and commitment. Members of the study team are gratefully acknowledged. We also thank the following sponsors: Grant sponsor: National Research Foundation (NRF) supplemented by Durban University of Technology (DUT); Grant no: 2068195 Thuthuka; Grant sponsor: Ethekwini Municipality, KwaZulu Natal, Durban, South Africa, Grant no: 1a-103—Health Study; Grant sponsor: Supported in part by the Intramural Research Program, National Institute of Environmental Health Sciences, NIH, USA; Project numbers Z01 49019 and Z01 ES025045; Grant sponsor: Sponsored in part by US NIH Fogarty International Center grant 2 D43 TW000812.

REFERENCES

American Thoracic Society (ATS). 1995. Standardization of spirometry—1994 Update. Am J Respir Crit Care Med 152:1107–1136.

Bell DA, Taylor JA, Paulson DF, Robertson CN, Mohler JL, Lucier JW. 1993. Genetic risk and carcinogen exposure: A common inherited defect of the carcinogen-metabolism gene glutathione-S-transferase M1 (*GSTM1*) that increases susceptibility to bladder cancer. J Natl Cancer Inst 85:1159–1164.

Bernstein JA, Alexis N, Barnes C, Bernstein IL, Nel A, Peden D, Diaz-Sanchez D, Tarlo SM, Williams PB. 2004. Health effects of air pollution. J Allergy Clin Immunol 114:1116–1123.

Custovic A, Murray CS, Lowe L, Woodcock A. 2002. NAC Manchester Asthma and Allergy Study Group. The National Asthma Campaign Manchester Asthma and Allergy Study. Pediatr Allergy Immunol 13:32–37.

Fryer AA, Bianco A, Hepple M, Jones PW, Strange RC, Spiteri MA. 2000. Polymorphism at the glutathione S-transferase *GSTP1* locus: A new marker for bronchial hyperresponsiveness and asthma. Am J Respir Crit Care Med 161:1437–1442.

Gilliland FD, McConnell R, Peters J, Gong H. 1999. A theoretical basis for investigating ambient air pollution and childrens respiratory health. Environ Health Perspect 107:403–407.

Gilmour MI, Jaakkola MS, London SJ, Nel AE, Rogers CA. 2006. How exposure to environmental tobacco smoke, outdoor air pollutants, and increased pollen burdens influences the incidence of asthma. Environ Health Perspect 114:627–633.

Hayes JD, Strange RC. 1995. Potential contribution of the glutathione-S-transferase supergene family to resistance to oxidative stress. Free Radic Res 22(3):195–207.

Hong YC, Hwang JHK, Kim JK, Lee K, Lee H, Lee K, Yu S, Kim D. 2007. Metals in particulate pollutants affect peak expiratory flow of schoolchildren. Environ Health Perspect 115(3):430–434.

Kelly FJ. 2003. Oxidative stress: Its role in air pollution and adverse health effects. Occup Environ Med 60:612–616.

Kunzli N, Tager IB. 2005. Air pollution: From lung to heart. Swiss Med Week 135:697–702.

Lee YL, Lin YC, Lee YC, Wang JY, Hsiue TR, Guo YL. 2004. Glutathione S-transferase P1 gene polymorphism and air pollution as interactive risk factors for childhood asthma. Clin Exp Allergy 34: 1707–1713.

London SJ. 2007. Gene-air pollution interactions in asthma. Proc Am Thorac Soc 4:217–220.

London SJ, Romieu I. 2009. Gene by environment interactions in asthma. Ann Rev Public Health 30:55–80.

McConnell R, Berhane K, Yao L, Jerrett M, Lurmann F, Gilliland F, Künzli N, Gauderman J, Avol E, Thomas D, Peters J. 2006. Traffic, susceptibility, and childhood asthma. Environ Health Perspect 14: 766–772.

Melen E, Nyberg F, Lindgren CM, Berglind N, Zucchelli M, Nordling E, Hallberg J, Svartengren M, Morgenstern R, Kere J, Bellander T, Wickman M, Pershagen G. 2008. Interaction between glutathione-S-transferase P1, tumor necrosis factor and traffic related air pollution for development of childhood allergic disease. Environ Health Perspect 116:1077–1084.

Naidoo R, Gqaleni N, Robins TG, Batterman S. 2006. The South Durban Health Study. Multipoint Plan Project 4: Health Study and Health Risk Assessment. University of KwaZulu-Natal, Durban. Accessible at: http://doeh.ukzn.ac.za/SDHealthStudy1299.aspx.

National Treasury. 2007. A national poverty line for South Africa, Statistics South Africa, February 2007. http://www.treasury.gov.za/publications.

Nriagu J, Robins T, Gray L, Liggans G, Davila R, Supuwood K, Harvey, C, Jinabhai CC, Naidoo R. 1999. Prevalence of asthma and respiratory symptoms in south central Durban. Eur J Epidemiol 15: 747–755.

Ober C, Cox NJ, Abney M, Di Rienzo A, Lander ES, Changyaleket B, Gidley H, Kurtz B, Lee J, Nance M, Pettersson A, Prescott J, Richardson A, Schlenker E, Summerhill E, Willadsen S, Parry R. 1998. Genome-wide search for asthma susceptibility loci in founder population. The collaborative study on the genetics of asthma. Hum Mol Genet 7:1393–1398.

Peden DB. 2003. Air Pollution: Indoor and outdoor. In: Adkinson NF Jr, Yunginger JW, Busse WW, Bochner BS, Holgate SK, Simons FE, editors. Middleton's allergy: Principles and practice. Philadelphia: Mosby. p 515–528.

Peden DB. 2005. The epidemiology and genetics of asthma risk associated with air pollution. J Allergy Clin Immunol 115:213–219.

Phelan PD, Robertson CF, Olinsky A. 2002. The Melbourne Asthma Study: 1964–1999. J Allergy Clin Immunol 109:189–194.

Romieu I, Sienra-Monge J, Ramirez M, Moreno-Macias H, Reyes-Ruiz N, del Río-Navarro BE, Hernández-Avila M, London SJ. 2005. Genetic polymorphisms of *GSTM1* and antioxidant supplementation influence lung function in relation to ozone exposure among asthmatic children in Mexico City. Thorax 59:8–10.

Romieu I, Ramirez-Aguilar M, Sienra-Monge JJ, Morena-Macias H, del Rio-Navarro BE, David G, Marzec J, Hernández-Avila M, London SJ. 2006. *GSTM1* and *GSTP1* and respiratory health in asthmatic children exposed to ozone. Eur Respir J 28:953–959.

Seaton A, MacNee W, Donaldson K, Godden K. 1995. Particulate air pollution and acute health effects. Lancet 345:176–178.

Strange RC, Spiteri MA, Ramachandran S, Fryer AA. 2001. Glutathione-S-transferase family of enzymes. Mutat Res 482:21–26.

Venn A, Yemaneberhan H, Lewis S, Parry E, Britton J. 2005. Proximity of the home to roads and the risk of wheeze in an Ethiopian population. Occup Environ Med 62:376–380.

WHO. 2006. Air quality guidelines for particulate matter, ozone, nitrogen dioxide and sulfur dioxide. Global update 2005. World Health Organization. Geneva, Switzerland.

Wjst M, Popescu M, Trepka MJ, Heinrich J, Wichmann HE. 1998. Pulmonary function in children with initial low birth weight. Pediatr Allergy Immunol 9:80–90.

Yan K, Salome C, Woolcock AJ. 1983. Rapid method for measurement of bronchial responsiveness. Thorax 38:760–765.