EPIDEMIOLOGICAL AND GENETIC RISK FACTORS ASSOCIATED WITH ASTHMA AMONG CHILDREN IN THE SOUTH DURBAN REGION, KWAZULU-NATAL

Poovendhree Reddy

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Occupational and Environmental Health, School of Family and Public Health, Nelson R Mandela School of Medicine, University of KwaZulu-Natal

SUPERVISOR: Prof Rajen Naidoo (Head of Department, Department of Occupational and Environmental Health, UKZN)

CO-SUPERVISOR: Prof Richard Naidoo (Head of Department, Pfizer Molecular Biology Institute, UKZN)

DEDICATION

For Colin, Hayley, Teagan and Mom

Thanks for your patience, love and understanding through all the trying times

PUBLICATIONS OR PRESENTATIONS

- Poster. Gluthathione-S-Transferase polymorphisms (GSTM1 and GSTP1) and respiratory health in a South African population. Poovendhree Reddy[1], Rajen Naidoo[2], Richard Naidoo[2], Thomas G. Robins[3] Graciela Mentz[3] Stephanie J London[4], and Stuart Batterman[3]

  (1) Durban Institute of Technology, Durban, South Africa; (2) University of Kwa-Zulu Natal, Durban, South Africa; (3) University of Michigan, Ann Arbor, USA, (4) National Institute of Environmental Health, NC, USA.

  International Society for Environmental Epidemiology (ISEE) 19th conference. Mexico City, September 5-9, 2007.

- Paper presented. Effect modification of respiratory responses to ambient air pollutants by GSTM1, GSTP1 and NQO1 polymorphisms.

  Poovendhree Reddy[1], Rajen Naidoo[1], Richard Naidoo[1], Thomas G. Robins[2] Graciela Mentz[2] Stephanie J London[3], and Stuart Batterman[2]

  [1]Nelson R Mandela Medical School, University of Kwa-Zulu Natal, South Africa; [2]University of Michigan, USA. [3]National Institute of Environmental Health Sciences, USA. American Thoracic Society (ATS) Congress (2007), San Francisco, USA.

- Paper presented. The gene-environment interaction.

  Poovendhree Reddy[1], Rajen Naidoo[1], Richard Naidoo[1], Thomas G. Robins[2] Graciela Mentz[2] Stephanie J London[3]

  [1]Nelson R Mandela Medical School, University of Kwa-Zulu Natal, South Africa; [2]University of Michigan, USA. [3]National Institute of Environmental Health

  AstraZeneca College of Health Sciences Research Symposium, University of KwaZulu-Natal, Nelson R Mandela Medical School, September 12-13, 2006.

- Poster Presentation: Allelic frequencies of GSTM1, GSTP1 and NQO1 polymorphisms in a South African population.

  Poovendhree Reddy, Rajen Naidoo, Richard Naidoo, Stephanie London and Tom Robins.

  [1]Nelson R Mandela Medical School, University of Kwa-Zulu Natal, South Africa; [2]University of Michigan, USA. [3]National Institute of Environmental Health

  Public Health 2006, Public Health Association of South Africa (PHASA) Congress. 15-17 May 2006, Gauteng, South Africa

- Poster Presentation. Glutathione-s-transferase gene polymorphisms (GSTM1 and GSTP1) as increased risk factors for asthma.

  Reddy, P. (1); Naidoo, R. (2); Naidoo, R. (2); London, S. (3); Robins, T. (4)

  Durban Institute of Technology, Durban, South Africa; (2) University of Kwa-Zulu Natal, Durban, South Africa; (3) National Institute of Environmental Health, Bethesda, USA; University of Michigan, Ann Arbor, USA. (2005).

ACKNOWLEDGEMENTS

The author would like to extend her appreciation and gratitude to:

Prof Rajen Naidoo, (my supervisor), the Centre of Occupational and Environmental Health (UKZN) for your intellectual and inspirational contribution, and for encouraging me to shape my learning in its entirety;

Prof Richard Naidoo, (my co-supervisor), the Pfizer Molecular Biology Institute, for you encouragement and support;

Dr Stephanie London, the National Institute for Environmental Health Sciences (NIEHS), for your expertise and invaluable input and kindness. **Huiling Li** from the NIEHS for her assistance with genotyping

Prof Thomas Robins, University of Michigan, for your assistance and intellectual support;

Prof Mary Lou Thompson (University of Washington), **Dr Graciela Mentz** (University of Michigan) and **Ms Tonya Esterhuizen** (UKZN), for their invaluable input and guidance through my torment with biostatistics;

Joy Kistnasamy and Anusha Karamchand, thanks for believing in me, the time out, the upliftment, motivation and for your amazing friendship. You are my own personal support group; **Joy**, thanks for your assistance with my literature search.

Mr Zakeer Gafoor (UKZN) for your research assistance and your tolerance of my annoying schedules;

Mrs Jenny Pillay and Mrs Ruby Govender for the financial administration;

Prof Gansen Pillay, who has made research for women working at the DUT possible with his innovative ideas, and **his team** at the Centre for Research and Development, for always accommodating my requests and providing assistance;

Postgraduate students at the Pfizer Molecular Biology Institute from 2004-2006; I really needed the coffee breaks to keep sane.

Learners, parents and study team of the South Durban Health Study, thanks for your committed participation;

Kameshree Govender, for editorial assistance and **my extended family and friends** who tolerated my self centered behavior during this study. Special thanks to my **Mother,** for holding down the fort whenever I am away, and for your constant support in all aspects in my life.

The **Staff of the Department of Environmental Health, DUT,** for their support and for never denying me time off when I needed it.

DUT and NRF (Thuthuka) for funding; especially for the training visits to the NIEHS and Johns Hopkins School of Public Health; you made it possible for me to complete this in the minimum period;

Colin, for your love, patience and understanding, I know that you would like a "normal" wife, but you grin and bear it anyway and don't ask too many questions. I appreciate that. **Hayley,** for the sunshine you bring into my life and **Teagan,** for the beautiful smile that makes everything better.

Finally, for all the smart women I know who have made multitasking an art form, it is not easy to juggle all the different roles in your lives and still remain sane, but you do it with such aplomb anyway.

TABLE OF CONTENTS

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 6

CHAPTER 7

LIST OF FIGURES

LIST OF TABLES

LIST OF ANNEXURES

LIST OF ABBREVIATIONS AND DEFINITIONS

Allele:	alternative forms of a gene or locus marker due to changes at the level of the DNA
Allergy:	is an immunological state where ubiquitous harmless substances are recognized as allergens by the immune system leading to complex defense mechanisms of chronic inflammation
ASHMOG:	Seventh day Aventists in California
ATS:	American Thoracic Society
BHR	Bronchial Hyperresponsiveness
BMRC:	British Medical Research Council
CHS :	Children's health study
CI:	Confidence interval
CO:	Carbon monoxide
COPD:	Chronic Obstructive Pulmonary Disease
DEAT	South African Department of Environmental Affairs and Tourism
DEPs:	Diesel Exhaust Particles
EAACI	European Academy of Allergy and Clinical Immunology
ECHRS	European Community Respiratory Health Survey
EDTA:	Ethylene diamine tetraacetic acid
EPA:	Environmental Protection Agency

ETS: passive smoking or exposure to secondhand smoke is defined as the exposure of a nonsmoking person to tobacco combustion products from smoking by others

Exposure: Exposure is defined as the "contact between a target organism and a pollutant at the outer boundary of the organism", which is specifically the inhalation route in this study. Exposure may be quantified as the amount of pollutant available at the boundary of the receptor organism per specified time period.

FEV_1: Forced expiratory volume in one second

FVC: Forced Vital Capacity

GEE: Generalised estimating equations

Gene : an individual unit of hereditary. It is a specific instruction that directs the synthesis of a protein or ribonuclease acid product. Each gene is located at a specific site (locus) on a chromosome.

Genetic Model: The overall specification of how the diseases alleles act to influence the disease

Genome: the sum of all genetic information of an organism.

Genotype: Refers to the precise allelic makeup of an organism or cell. In reference to a specific gene, the genotype is the pair of alleles that occur at the chromosomal site of the gene, which may be the same (homozygous) or different (heterozygous).

GM-CSF: Granulocyte macrophage colony stimulating factor

GSTs: Glutathione-S-tranferase enzymes

GSTM1: Glutathione-S-transferases are involved in phase 2 xenobiotic and reactive oxygen species metabolism and have coordinate regulation based on antioxidant response element in their promoter region. GSTM1 is a member of the M family of glutathione-S-transferases. The gene is located at 1p13.3 and has a common allele that results in no protein production.

GSTP1: Gluthathione-S-tranferase P1 is a member of the P family of glutathione-S-transferase. GSTP1 is located at 11q13.3 and has a common single nucleotide polymorphism t codon (A105G) that results in an amino acid change in the protein from isoleucine to valine. This aminoacid substitution has pleotropic effects on the enzyme function.

H_2O_2: Hydrogen Peroxide

Heterozygous: The alleles at a genetic locus are different from one another on the two partners of a chromosome pair.

Homozygous: the alleles at a genetic locus are identified on the two partners of a chromosome pair.

IgE: Immunoglobin E

Interquartile range: The interquartile range (IQR) is the distance between the 75^{th} percentile and the 25^{th} percentile. The IQR is essentially the range of the middle 50% of the data. Because it uses the middle 50%, the IQR is not affected by outliers or extreme values.

ISAAC: International Study of Asthma and Allergies in Childhood

NADPH: Nicotinamide Adenine Dinucleotide phosphate

$(NH_4)_2SO_4$: Ammonium Sulphate

NHANES III: The third national health and nutrition examination survey

NO: Nitrogen oxide

NO_2: Nitrogen Dioxide

NQO1: NAD(P)H: quinone oxidoreductase 1

O_2^-: Superoxide radical

O_3: Ozone

OR: Odds ratio

PC_{20}: Dose of methacholine causing a 20% drop in baseline FEV_1

PCR: Polymerase Chain Reaction

PEACE Pollution effects on asthmatic children in Europe

PF: Peak Expiratory Flow

Phenotype: Refers to the observed attribute, in this case, asthma or a component of asthma such as bronchial hyperresponsiveness. The phenotype is the manifestation of the underlying genotype.

PIAMA: Prevention and incidence of asthma and mite allergy

PM_{10}: Respirable particulate matter less than 10 microns

Polymorphism: A tendency for a gene to exist in more than one form, or the specific alleles thereof.

ppb: parts per billion

ROS:	(Reactive oxygen species) important reactive species for biological systems include superoxide, hydrogen peroxide, and hydroxyl radical. At low levels, these species may function in cell signalling process. At higher levels, reactive oxygen species damage cellular macromolecules and participate in apoptotic processes.
RR:	Relative Risk
SDHS:	South Durban Health Study
SDIB:	South Durban Industrial Basin
SDSDMSC	South Durban Sulphur Dioxide Management Systems Committee
SNP:	Single Nucleotide Polymorphism
SO_2:	Sulphur Dioxide
SA:	South Africa
Susceptibilty:	the degree to which a person or a population is sensitive to either adverse or protective exposures in developing asthma.
VOCs:	Volatile organic compounds
WHO	World Health Organization

ABSTRACT

Several genes are associated with an increased susceptibility to respiratory diseases, including asthma, which may be exacerbated by ambient air pollution. These genes include the Gluthathione-S-Transferase family (GSTM1 and GSTP1) and the NAD(P)H quinone oxidoreductase (NQO1). This, the first genetic epidemiological study conducted in Sub-Saharan Africa had 2 main objectives: 1) to evaluate whether the above genotypes confer susceptibility to asthma and related phenotypes; and 2) to investigate if polymorphisms in these genes known to modulate the response to or protect from epithelial oxidative damage modify pulmonary response to ambient air pollutants.

A total of 369 schoolchildren from seven primary schools in a heavily industrialized region of south Durban and a demographically similar area in north Durban, Kwa-Zulu Natal, South Africa during the period May 2004- October 2005, participated in the study. DNA was extracted from whole blood using the GENTRA Puregene kit. Genotyping for the GSTM1 (null vs present genotype) was done using multiplex PCR while the GSTP1 (Ile105Val; AA→AG/GG) and the NQO1 (Pro/Ser; CC →CT/TT) genotypes were determined using real time PCR and Taqman probes (Applied Biosystems). Persistent asthma and asthma of "any severity" was determined by questionnaires based on the ATS and BRMC questionnaires. Positive atopy was determined by at least one positive skin test reaction to the seven allergens tested. Other health assessments included spirometry, methacholine challenge testing and four cycles of three-week serial peak flow measurements. Acute respiratory measures included within day variability in FEV_1 and

PF and the lowest valid values on a given day. SO_2, NO_2, NO and PM_{10} were measured over a year using ultraviolet fluorescence, gas-phase chemiluminescence and gravimetric methods respectively. STATA (version 9, College Station, TX, USA) was used for data analysis. Multiple logistic models and Pearson's chi-squared tests were used to evaluate the association between asthma, BHR, atopy and genotype. Covariate-adjusted generalised estimating equations (GEE) with lags of 1-5 days were used to evaluate genotype effect modification of exposure-response.

The GSTM1 gene deletion (GSTM1null) was detected in 28.9% of the study population while the distribution of GSTP1 AG/GG and the NQO1 CT/TT polymorphisms were 64.9% and 36.0% respectively. Multiple regression with the adjustment for relevant covariates indicated that individuals carrying one or more copies of the GSTP1 minor allele had a statistically significant risk for persistent asthma. GSTM1 and NQO1 genotypes showed no significant association with any of the respiratory outcomes tested. However, we found a protective effect for those individuals carrying the GSTM1null genotype and at least one Ser allele (NQO1 CT/TT) for persistent asthma and marked BHR (OR = 0.7, CI: 0.3-1.5 and OR= 0.3, CI: 0.0-1.9 respectively). This protective effect is consistent with the role of NQO1 in metabolic activation. Children from the south schools had almost twice the risk of persistent asthma (OR=2.0, CI: 1.2-3.2, p<.005) and 3 times the risk of BHR (OR=3.5, CI: 1.4-8.4, p<.005) than those from the schools in the north. Based on symptoms, 20.4% of children from the random sample had persistent asthma and 10.3% had marked BHR ($PC_{20} \leq 2$ mg/ml).

The GEE model results were consistent with modification of air pollutant-pulmonary function relationships by oxidative stress associated genotypes. Statistically significant gene*environment interactions with NO_2, NO, and PM_{10} using FEV_1 and PEF outcomes in the expected direction were more frequent for GSTP1 AA and NQO1 CC genotypes (interaction p-values <0.05). There were very few gene*environment interactions for SO_2 and any of the 3 SNPs tested. The most striking finding in our study was that pollutant exposure, especially oxides of nitrogen and PM_{10}, even at levels below the recommended limits of South African guidelines, is associated with poorer lung function and that this association is significantly modified by an individual's genotype, particularly the GSTM1null, GSTP1AA and NQO1CC genotypes. Children with the GSTM1null GSTP1AG/GG, GSTP1AG/GG NQO1 CC and GSTM1pos NQO1CC gene-gene combinations showed a significant interaction with NO_2, NO, and PM_{10} with decrement in lung function measures.

The increased risk to air pollution conferred by the GSTP1 and GSTM1 genotypes may have clinical and public health importance because this variant is common in most populations. The findings suggest that the risk of developing respiratory symptoms is increased when genetic susceptibility is included with environmental exposures. Our models suggest significant gene*environment interactions i.e the response to the level of air pollutants, as indicated by variability in pulmonary function measures, is modified by genotype. The heightened allergic airway response may be a consequence of a decreased capacity to mount an effective cytoprotective response to oxidative stress. Studying genes may inform us about the biology of asthma which may lead to new therapies or

CHAPTER 1:

INTRODUCTION

The South Durban Industrial Basin (SDIB) is situated on the east coast of South Africa and has one of the highest concentrations of industrial activity in Africa containing among others, 2 large petroleum refineries, a paper mill, an international airport, a large chemical tank farm and a landfill site. Discriminatory land use planning during the Apartheid era had placed a large residential community very close to industry and community in this region had previously requested an independent investigation into the air quality and health status in the SDIB.

In response to this outcry and the economic growth potential of this area, the National Government initiated the Multi-Point Plan in 2000 which proposed to evaluate and monitor pollution levels in the SDIB and to determine the extent to which pollutants adversely impacted on the health of the resident community. As part of the Multi-point Plan, the South Durban Health Study (SDHS), conducted by the Center for Occupational and Environmental Health (University of Kwa-Zulu Natal) and the Department of Environmental Health Sciences (University of Michigan), consisted of a health risk assessment and an epidemiological study. The current study adds the genetic component to the SDHS to: 1) evaluate whether certain genotypes confer susceptibility to asthma and related phenotypes; and 2) if these genotypes modify respiratory response to certain environmental pollutants.

In a pilot study among students and teachers at the Settlers' Primary School in the SDIB, Robins *et al.,* (2002) reported unusually high prevalence rates for asthma, with 53.5% of any type of symptoms based asthma to 16.8% of moderate/severe persistent asthma among children. In addition, approximately 20% of the study sample had marked airway hyper-responsiveness as diagnosed by methacholine challenge testing, this prevalence being higher than that of any other reported in literature. In addition, they found statistically significant associations between prior day and prior 48 hour PM_{10}, SO_2, and NO_2 levels and this gave impetus to the SDHS which used a cohort of children from 7 primary schools in south and north Durban (areas which were demographically similar, but with assumed lower levels of ambient pollution in north Durban compared to south Durban).

Asthma, which is a substantial public health burden, is the most common chronic disease during childhood in modern society (Kabesch, 2006). Increases in asthma incidence and morbidity may be attributed to genetic predisposition, exposure to allergens and air pollution, socioeconomic effects, psychosocial stress, culture, and access to and quality of medical care (Miller, 1999). There is increasing evidence that the effects of air pollution vary among individuals because of the variation in their genetic susceptibility. Experimental studies in mice indicate that pulmonary responses to specific pollutants, including ozone and particles, are under genetic control (Kleeberger, 2003). There are two major reasons to investigate genetic susceptibility to air pollution effects in humans. The first is that the effects of air pollution on respiratory outcomes are modest in the general population because the population includes individuals relatively resistant to air

pollution. Thus, the ability to detect subtle effects of air pollution may depend on the ability to identify susceptible subpopulations. Second, susceptible groups might experience health effects at levels below current exposure standards. An essential question is: are environmental influences associated with asthma more likely to affect people with certain genetic profiles? It is therefore necessary to simultaneously study genetic variants in association with asthma related phenotypes and environmental exposures.

In the context of candidate genes for asthma, inflammation the airways is associated with oxidative stress and the formation of reactive oxygen species (ROS). Although host antioxidant defenses should detoxify ROS, individuals differ in their ability to deal with an oxidant burden, and such differences are in part genetically determined. Inability to detoxify ROS should perpetuate the inflammatory process, activate bronchoconstrictor mechanisms and precipitate asthma symptoms (Fryer *et al.*, 2000) and oxidant stress is recognized as a mechanism that underlies the toxic effects of most air pollutants (Kelly, 2003). Cells in the lung are protected against oxidative stress by an extensive range of intracellular defenses, especially members of the Glutathione-S-Transferase enzymes (GSTM1 and GSTP1) and NAD(P)H quinone oxidoreductase 1.

This study focused on these above genes because they have common functional polymorphic variants and they have been implicated in oxidative defense pathways (Gilliland *et al.*, 2002b). These variant alleles result in either total absence or a substantial change in enzyme activity. Given the overall widespread prevalence of the

polymorphisms under study in the general population, there are a substantial number of people who constitute a genetically susceptible population.

Research focused on gene-environment interactions hold great promise in preventing and managing asthma. However there are few studies that examine the relationship between genetic risk factors and environmental exposures in the exacerbation of asthma. Most of these studies were conducted mainly in the Northern hemisphere with Caucasian, Hispanic and Asian populations. In order to compare asthma prevalence with environmental exposures, other authors have used mortality data, admission records, absenteeism and activity limitation with cross sectional designs. The advantage of this study is the use of a longitudinal cohort design with repeated measures of lung function (FEV_1 and PF) as markers of respiratory health and simultaneous detailed air pollutant monitoring as close to the experimental sites as possible. Other asthma related phenotypes investigated included BHR and atopy (defined as skin test responsiveness to common allergens). This study had several important advantages. 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, the 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. Thirdly, the sample deliberately selected persistent asthmatics, which provided additional power to identify specific impacts on susceptible groups. Our investigations with the genetic component was dual pronged: we hypothesised that individual response to oxidative stress as determined by polymorphisms in GSTs and

NQO1 are associated with asthma, BHR and atopy; we further hypothesised that these polymorphic genotypes may modify pulmonary response to environmental pollutants.

CHAPTER 2:

LITERATURE REVIEW

2.1 The South Durban Industrial Basin

The South Durban Industrial Basin (SDIB) is located on the east coast of South Africa. This shallow basin, an approximately 4 x 24 km coastal strip, sits in a flat alluvial corridor defined to the north east by the inland Berea Ridge and Southern Freeway, to the south west by the Bluff Ridge, and to the south east by the Indian Ocean. Land use in the SDIB is primarily residential and industrial and this area is recognized as one of the most highly industrialized and most heavily polluted areas in Southern Africa (Nriagu *et al.*, 1999; Matooane and Diab, 2002).

The Basin has one of the highest concentrations of industrial activity in Africa, containing, for example, two large petroleum refineries, a paper mill, an international airport, a large chemical tank farm, landfill sites, incinerators, processing and manufacturing industries, major trucking, harbor and rail facilities, and other industry (Fig 2.1). The two major petroleum refineries, Engen and Sapref, are within the community, together with a pulp and paper manufacturer, Mondi. Up until 2000, each of these refineries has emitted, on average, in the range of 35 000 to 40 000 kg of SO_2 per day (Ecoserv, 1998). Residential and recreational areas are intermingled with industry, with approximately 200,000 people living in 25 designated "suburbs", most of which remain racially segregated. The mixed residential/industrial community is a result of discriminatory land use planning during the Apartheid era. The peculiarities of

geography and land development strategies for the Durban South region have been documented in numerous reports (Matooane, 2002), strongly implying a lack of appropriate town planning on the part of the local government.

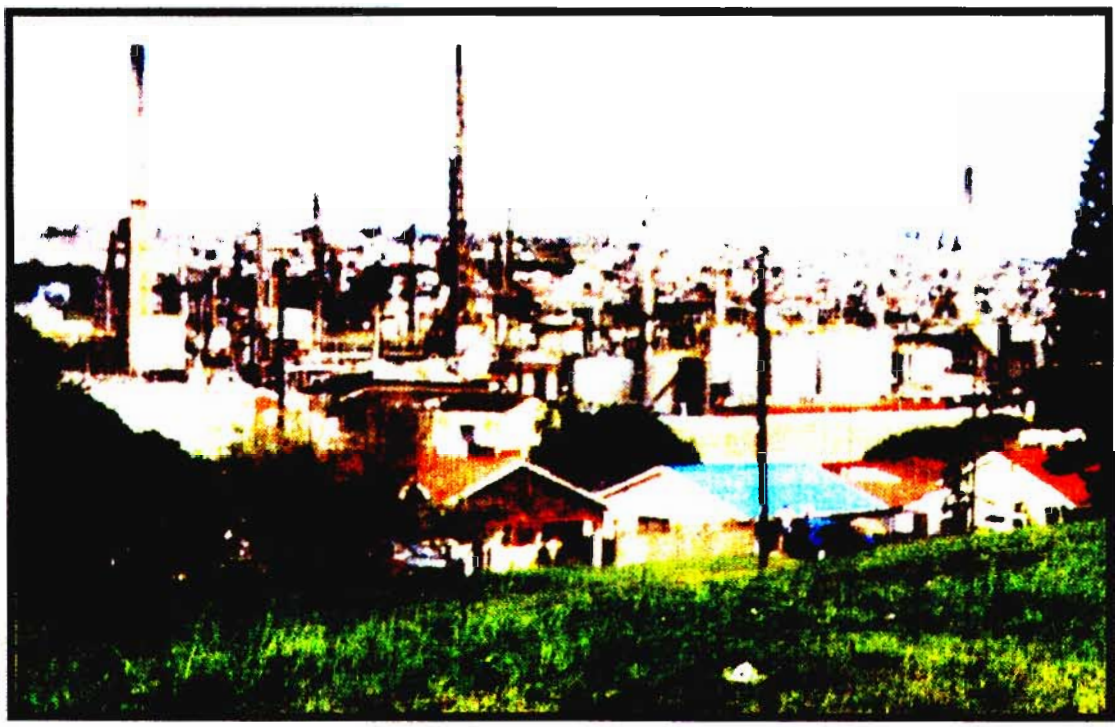


Figure 2.1: Engen Refinery (middle) with community residences of Wentworth (back) and Merebank (front)

Historically, the SDIB is at particularly high risk for exposure to significant levels of ambient air pollution because of its specific topography. Owing to a combination of its geographical relationship to the refineries, land contours, prevailing meteorological conditions, the use of a relatively short emissions stacks at these facilities (50 – 100 meters), the lack of or relative ineffectiveness of emission control devices on refinery stacks, the many sources of so-called fugitive air emissions at refineries, emissions from industrial and passenger vehicles, as well as the proximity of other industries and the

Durban airport, the community is believed to be at risk for intermittent substantial exposure to ambient air pollutants.

Despite reports of elevated pollution, there have been few scientifically generated data to suggest adverse health outcomes. In a pilot study conducted among students and teachers at the Settlers' Primary School in the SDIB region, Robins *et al.* (2002) reported unusually high prevalence rates for asthma, with ranges of any type of symptoms based asthma from 53.5% to moderate/severe persistent asthma of 16.8% at a school located between two major oil refineries (Engen and Sapref). In addition, approximately 20% of the study sample had marked airway hyperresponsiveness as diagnosed by methacholine challenge testing, a prevalence higher than any other population based reports in scientific literature. This study found statistically significant associations between prior day and prior 48 hour PM_{10}, SO_2, and NO_2 exposures (continuously measured at the school) and increased respiratory, including diminished pulmonary function measures (measured by digital recording peak flow meters) among students with persistent asthma. These effects were observed during a time period when all ambient pollutant measures were well within national and international standards. Based on these results, the South Durban Health Study (SDHS) was funded by the Ethekwini Muncipality (Durban Metro). This cohort study investigated the acute effects of air pollution and other environmental exposures among children in South Durban and a less exposed comparison area in North Durban with the same socioeconomic profile.

The key pollutants that were monitored in the SDIB are SO_2, NO_x, PM_{10} and to a limited extent, CO. Lead, O_3 and volatile organic compounds (VOCs) are not monitored intensively. Most of the monitoring in SDIB was previously done by private consultants contracted to the Durban Metro for management of the Durban Metro Air Quality and Emission Survey, which has been responsible for the Durban South Sulphur Dioxide Monitoring System. An industry-funded South Durban Sulphur Dioxide Management Systems Committee (SDSDMSC) had been continuously monitoring SO_2, at the Settlers' Primary School in Merebank since June 2000.

Table 2. 1: Emissions from point and mobile sources in the South Durban Industrial Basin (Adapted from Ecoserv, 1998).

SOURCE	**SO_2**	**NO_x**	**PM**	**CO**
Oil refineries	27412 (66.1)*	2 863 (15.1)	609 (11.9)	12 474 (11.4)
Other point sources	11977 (28.9)	2 580 (13.6)	2 123 (41.3)	6 408 (5.9)
Vehicles	1224 (3.0)	11 524 (60.8)	2 296 (44.7)	89 718 (82.3)
Other line sources	856 (2.1)	1 975 (10.4)	108 (2.1)	358 (0.4)
Total	**41 469 (100)**	**18 942 (100)**	**5 136 (100)**	**108 989 (100)**

*Tonnes per annum (% of total emission

Continuous monitoring of oxides of nitrogen, carbon monoxide, total reduced sulfates, and PM_{10} at the school commenced in late October 2000. Available data on sulfur dioxide indicated that average and/or maximum exposures at the Settlers School have frequently exceeded World Health organization (WHO), the South African Department of Environmental Affairs (DEAT), and SDSDMSC guidelines. Table 2.1 shows key

pollutants and the primary sources of emission. Point sources account for approximately 94% of the SO_2 emission but only 29% of NO_x emission.

According to this data source, emission sources for PM are almost equally split between point (53%) and mobile (45%). Although a steady decline has been observed for annual SO_2 levels since 1989, some monitoring stations have exceeded the WHO guidelines since about 1995. It is important to note that WHO threshold values are based on exposure to single pollutants. Also, 1-hr and 24-hr exceedances of the DEAT standards occur relatively frequently, e.g., 176 1-hr exceedance episodes in 1999 and 17 24-hr exceedances between 1997 - 1999 (Matooane, 2002). In reality, especially in the SDIB, exposure to many pollutants occurs simultaneously. Combinations of pollutants may possibly have greater effects on airway function than exposure to a single pollutant and may also enhance the patient's reactivity to other stimuli. This synergistic effect has been observed in relation to the acidic gases (SO_2, NO_2 and ozone) and particulate matter (Barnes, 2000).

2.2 Asthma and related phenotypes

Asthma is defined as a chronic inflammatory disorder of the airways with the inflammation leading, in susceptible individuals to episodes of wheezing and other respiratory symptoms that are associated with reversible airflow limitation as well as bronchial hyperresponsiveness to a variety of stimuli[1]. These symptoms are usually associated with widespread but variable airflow limitation and are at least partly

reversible either spontaneously or with treatment (Nadel and Busse, 1998; American Thoracic Society, 2000).

Phenotypes associated with asthma include bronchial hyperresponsiveness (BHR), total IgE (immunoglobulin E), specific IgE directed against different allergens and skin test reactivity against common allergens. Other phenotypes include allergic rhinitis and atopic dermatitis. BHR, defined as an abnormal increase in airflow limitation following a relevant stimulus to the airways, is a major pathophysiological phenomenon of bronchial asthma[2]. The EAACI (European Academy of Allergy and Clinical Immunology) position paper defines atopy as a "personal or familial tendency to produce IgE antibodies in response to low doses of allergens, usually proteins, and to develop typical symptoms such as asthma, rhinoconjunctivitis, or eczema/dermatitis". Several studies have shown a strong association between BHR, atopy and asthma (Sears *et al.,* 1991; Pearce *et al.*, 2000) but it is clear that that not all people with atopy have BHR and also not all people with BHR have asthma. This may indicate that subjects with BHR may have an asthma predisposing gene, yet need a trigger to develop full-blown asthma. Atopy alone is not sufficient to cause asthma, but individuals who are atopic are more likely to have increased airway responsiveness (Fryer *et al.*, 2000) and any assessment of asthma must consider the possible interaction of these two conditions in the expression of the asthma phenotype.

Asthma is a substantial public health burden, particularly for children, both in the number of people affected by the disease, and the related morbidity and cost. Globally as many

300 million people of all ages and ethnic groups suffer from asthma and the disease burden on governments, health care systems, families and patients is increasing worldwide[3]. This burden is both directly attributed to the disease (medical costs) as well as indirect costs to the family and community.

2.2.1 Asthma Prevalence

Asthma is the most common chronic disease during childhood in modern societies. Prevalence rates differ between countries but on average, 10-20% of children in Western Europe and the US are affected (Kabesch, 2006). The International Study of Asthma and Allergies in Childhood (ISAAC) and the European Community Respiratory Health Survey (ECRHS) provided, for the first time, a picture of global patterns of asthma prevalence in childhood and adult life respectively (ECRHS, 1997; ISAAC, 1998). Both studies show a particularly high prevalence of reported asthma symptoms in English-speaking countries i.e. the UK, New Zealand, Australia, the USA and Canada. In the ISAAC, the rest of the world outside the Americas and Western Europe generally showed relatively low asthma prevalence, particularly in developing countries like China and Taiwan. South Africa was recorded as the 15th highest prevalence out of a total of 57 countries (Fig 2.2). The ISAAC study has shown a marked variation in asthma prevalence rates among countries which may be attributed to disparities in hygiene, diet, cigarette smoking, traffic pollution, antenatal exposures and physical activity.

The NHANES III project using a US adult population of 18 393, recorded a prevalence of current asthma of 4.5% and a prevalence of wheezing of 16.4% (Arif *et al.,* 2003). In the US, asthma prevalence, hospitalization and mortality are higher for Black American compared to Caucasians. In Michigan, a cross-sectional study of childhood asthma in an integrated middle class population revealed that the lifetime prevalence of asthma was twice as high for Black American compared with Caucasian children, suggesting that factors such as racial discrimination, differential access to medical care and housing and biological factors are responsible for this disparity (Nelson *et al.,* 1997). Varied prevalences have been found in urban and rural areas, e.g. in Zimbabwe, exercise-induced asthma was associated with urban residence and high living standards (Kaley *et al.,* 1991). In addition, asthma prevalence was shown to be higher in West Germany as compared to East Germany which alludes to asthma being a disease of the Western lifestyle (Von Mutius *et al.,* 1994).

This disparity in prevalence in rural and urban areas may be explained by the hygiene hypothesis. The hygiene hypothesis postulates that hay fever and wheeze are diseases of more affluent urban areas compared to rural farming areas because of the differences in exposure to various allergens in early childhood (Strachan, 2000). Rural children may be exposed to a greater extent that allows for the accumulation of protective immunity. The hypotheses suggests that small families, earlier birth order, less exposure to respiratory infection and reduced exposure to endotoxins, parasites and animal sources of allergens in early childhood as a potential explanation for the increase in asthma prevalence in more westernized urban communities compared to rural communities.

Studies of asthma in SA are scarce with studies conducted in the Transkei, Cape Town, Gauteng, North West province and Durban (Erlich, 2002). In a cross sectional study done in the south central Durban area, approximately 10% of the 367 children and 12% of the 693 adults reported doctor diagnosed asthma (Nriagu *et al.,* 1999). In a Sowetan population of black children, Nagel (1993), in a study investigating the prevalence of childhood asthma in white primary school children in Cape Town, concluded that 52 (4.4%) of the 1 174 children studied had asthma and noted that this prevalence was higher than 3.1% reported in a previous study on African children in Gugulethu. In 1995, Erlich and coworkers evaluated self reported asthma among a population of 1 955 6-10 year old children in Cape Town. These authors reported a relatively high prevalence of wheeze in the past 12 months of 26.8% (as reported by parents) and 10.8% reported an asthma diagnosis. In a later study in Cape Town among young adolescents (13-14 years old), 16% reported wheeze in the previous 12 months and 13.3% reported diagnosis of asthma (Poyser *et al.*, 2002). As compared to these findings the Settlers school study conducted in the South Durban area found 39.1% of reported wheezing in the last 12 months and 13.3% ever having been diagnosed with asthma (Robins *et al.,* 2002).

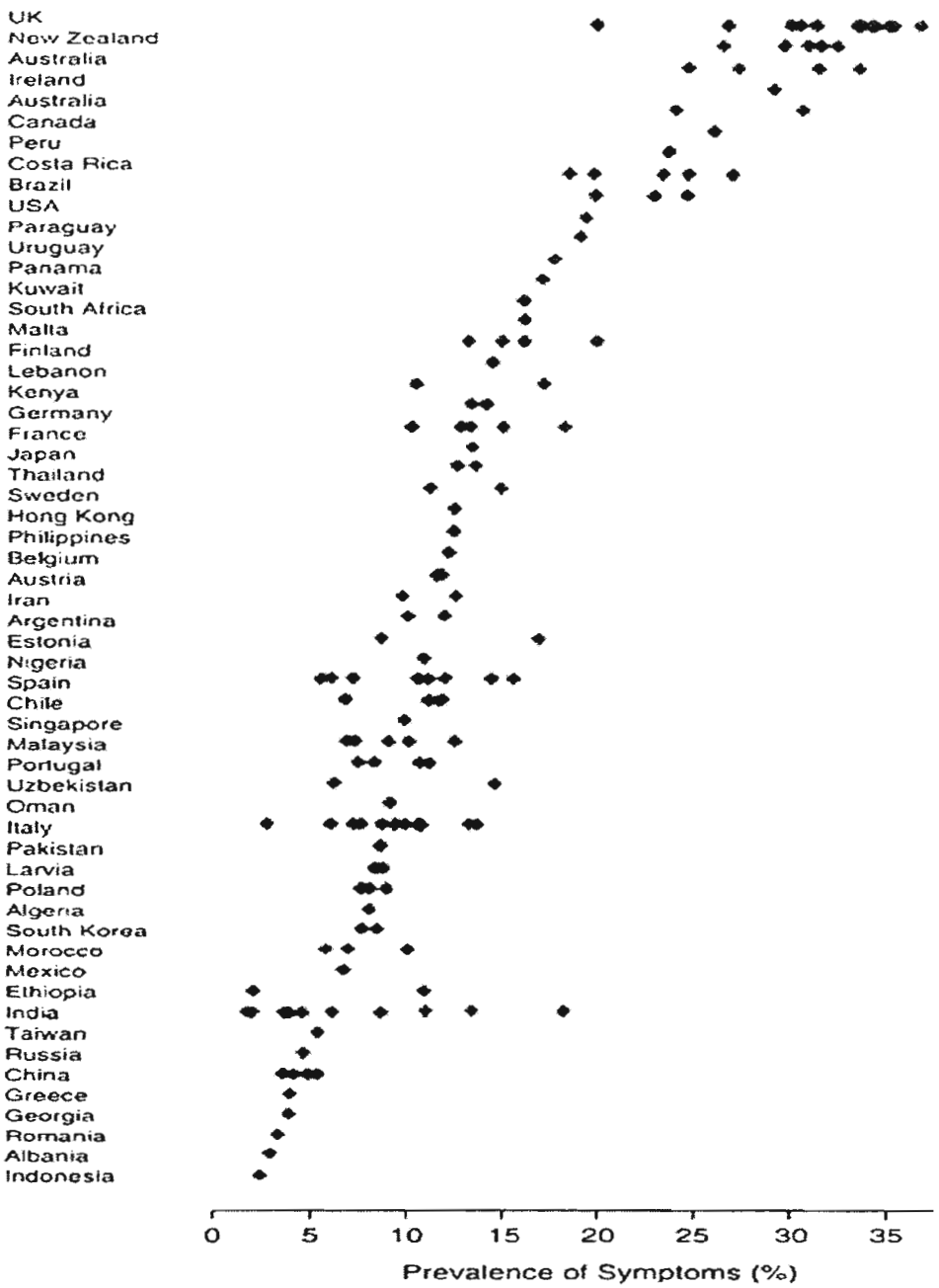


Figure 2.2: Prevalence of asthma symptoms (percentage) from a questionnaire in the ISAAC database (Gold and Wright, 2005)

2.2.2 Risk factors

Increases in asthma prevalence may be attributed to differences in exposure levels to aeroallergens such as house dust mite, smoking behavior, dietary sodium intake, occupation, indoor and outdoor pollution. Indoor air pollutants include biologics (mold, dust mite, cockroach and rodent infestations), dampness and environmental tobacco smoke (ETS) (Strachan, 2000; Arruda *et al.*, 2005). Other factors include psychosocial stress, culture, and access to and quality of medical care (Miller, 1999). Numerous studies have reported an association between environmental tobacco smoke (ETS) exposure and respiratory diseases. Maternal smoking during pregnancy and early childhood is associated with impaired lung growth and diminished lung function, and in asthmatic children, parental smoking increases symptoms and the frequency of asthma attacks (Kabesch, 2006). Other factors implicated in the development of allergy include lower socioeconomic group, sex (during childhood and adolescence, boys are nearly twice as likely as girls to develop asthma), birth order, maternal phenotype and low birth weight (Borish, 1999). One should therefore consider individual and synergistic effects of the above risk factors with regard to asthma.

Aligne *et al.* (2000) argue that urban residence, rather than race, increases the risk of asthma. These authors suggest that asthma is not a racially linked genetic disease. Indeed, indigenous populations living in Africa have very low asthma prevalence, and asthma that does occur is associated with home environmental factors related to urbanization. This view is considered in this study, we have a multiracial population with common

exposure to risk factors, manifestation of respiratory illness should be a result of exposure, rather than race. If asthma is related to some aspect of outdoor pollution and if everyone in a given population is exposed to the same combination of pollutants, then exacerbation of asthma is wholly dependent on individual susceptibility. Comparisons of African populations show that asthma prevalence is not similar among different groups further fortifying the argument that asthma appears to be more related to environment and lifestyle than race (Aligne *et al.,* 2000). However, genetic predisposition to these outcomes, which has been shown to be variable among different race groups, may be the determining factor in susceptibility. The widely accepted paradigm is that environmental factors are important to the development of asthma, but one must be genetically predisposed to respond to environmental triggers such as viruses, the presence and concentration of different allergens, secondary cigarette smoke or environmental pollutants (Los *et al.,* 1999).

2.3 Air pollutant exposure and respiratory outcomes

Over the past two decades, an increasing number of epidemiological studies have linked urban air pollution to increased morbidity and mortality (Samet and White, 2004). A considerable body of literature has focussed on the impacts of ambient pollution on respiratory health, examining a variety of specific outcomes, various pollutants and different population subgroups. Outcomes studied have included acute exacerbation of lung disease associated with exposure to pollutants and the development of chronic lung disease. Studies have considered effects of airway impairment in both healthy and

allergic persons (Peden, 2003; Bernstein *et al.*, 2004). Air pollution has been associated with many signs of asthma exacerbation, e.g. pulmonary function decrements, increased BHR, increased medication use, visits to the emergency departments and hospital admissions (D' Amato *et al.*, 2005).

Despite improvements in air quality in many developed countries, scientific evidence shows that adverse health effects are still associated with current levels of criteria pollutants. This may indicate that current standards are not set low enough to protect us from environmental onslaughts. Alternatively, the presence of non-criteria pollutants at elevated levels in ambient air, locally increased levels of air pollution that are not captured by central monitors, or some combination of these may affect health (Gauderman, 2006).

Outdoor air pollution is a major concern in developing countries. The WHO found that the air quality in large cities in many developing countries is remarkably poor and these people are exposed to ambient concentrations of air pollutants well above the WHO guidelines for air quality (Shannon *et al.*, 2004). Socioeconomic factors such as substandard housing with poor indoor air quality, working in jobs with occupational respiratory risks and limited access to care and medication may enhance susceptibility to pollution. Additionally, genetic and biological risk factors also contribute to susceptibility. Therefore, some groups may be at increased risk of experiencing adverse effects from a given level of ambient air pollution because their baseline risk level may be elevated by other factors (American Thoracic Society, 2000). These groups include

children, the elderly, and those with cardiopulmonary disease (Pediatrics Policy Statement, 2004). Children have increased risk from air pollutants compared with adults because of higher minute ventilation and higher levels of physical activity, especially outdoors.

Asthma has a genetic component that likely works by modifying response to environmental exposures (Gilmour *et al.,* 2006). Disease-susceptibility models postulate that specific genotypes might result in a phenotype only in the presence of a particular exposure or that a specific genotype might result in different phenotypes, depending on environmental exposures (Ober *et al.*, 2005). Although ambient air pollution is considered to be a risk factor for childhood asthma, only some of the children who reside in areas with high pollution have asthma. The gene-environment interaction between childhood asthma and outdoor air pollution has not been extensively investigated.

The association between air pollution and asthma is inconsistent across different studies (Lagoria *et al.,* 2006). For example, in the last severe smog to affect Europe in 1985, there was a stark contrast between the increased rates of mortality from hospital admissions for stroke, cardiovascular disease, and chronic obstructive lung disease and lack of any effect on asthma in the affected areas. Such studies may be insensitive to some changes since they rely on routinely collected information and not on direct observation of symptoms and lung function. The comprehensive Pollution Effects on Asthmatic Children in Europe (PEACE) study did not show any consistent relation between these two variables across 14 centers in Europe (Roemer *et al.*, 1998). These

authors conducted daily PF measurements and took symptom diaries for 2 months during which pollutants were monitored continuously. They did not find any effects of PM_{10}, SO_2, NO_2 or black smoke on PF or respiratory symptoms with 2 010 asthmatic children. This may indicate that the response to pollutants is modified by other factors (Burney, 1999).

Epidemiological studies in a number of industrialized countries throughout the world have reported significant association of acute and chronic lung health effects in the general population with elevated levels of sulphur oxides and carbonaceous particles. PM has been associated repeatedly with asthma aggravation and respiratory symptoms in a variety of settings, including increased symptoms and medication use in asthmatic children (Delfino *et al.,* 1998) and increased cough, phlegm and sore throat (Vedal *et al.,* 1998). Romieu *et al.,* (1996) found that an increase of $20ug/m^3$ PM_{10} was associated with an 8% increase in lower respiratory symptoms in children in Mexico city. Studies have found an association between gaseous pollutants, such as SO_2 and NO_2 and symptoms in children with asthma, including increased risks of developing upper respiratory symptoms in winter months (Von Mutius *et al.,* 1994), associations between both PEF and symptoms in asthmatic children and SO_2 concentrations (Peters *et al.,* 1999) and associations between reduced annual growth in FEV_1 in children and exposure to PM and NO_2 (Gauderman *et al.*, 2004).

In addition to decrements in lung function, these exposures may also increase the risk of respiratory illness (chronic cough, bronchitis, pneumonia) in children and other sensitive

subpopulations (Ohtsuka *et al.,* 2000). Apart from respiratory effects, other important outcomes considered are cardiac diseases, male and female reproductive outcomes, cancers and some haematological disorders. Interestingly, while the epidemiology of the health effects of combustion pollutants is clarified, the physiological and/or toxicological mechanisms of the effects have not yet been clearly elucidated (Li *et al*, 1996; Kelly *et al.,* 1999). The variety of outdoor pollutants and their ability to work synergistically, especially in mixtures of varying composition, confound the identification of one specific mechanism of air pollution toxicity (Ohtsuka *et al.,* 2000).

Studies have shown that both particulate and gaseous pollutants can act both on the upper and lower airways to initiate and exacerbate cellular inflammation. Increased neutrophil, B cell and alveolar macrophage recruitment is seen in bronchoalveolar lavage fluid of both healthy and asthmatic people exposed to diesel exhaust particles (Gilliland *et al.,* 2004). Similar increases in inflammatory cells are found in bronchoalveolar lavage fluid after exposure to O_3, SO_2 or NO_2 which altered lung function and increased airway responsiveness (Saxon and Diaz-Sanchez, 2005). One theory suggests that pollutants such as O_3 and PM can cause pulmonary inflammation directly and might deplete intracellular glutathione, leading to accumulation of oxidized glutathione (Li *et al.,* 2003). Respiratory effects in children from exposure to gaseous air pollutants (O_3, NO_2, acids) and particulates (PM_{10} and $PM_{2.5}$) result from chronically increased oxidative stress, alterations in immune regulation, and repeated pathologic inflammatory responses that overcome lung defenses to disrupt the normal regulatory repair processes (Kelly, 2003). In this theoretical framework, the effects of O_3, NO_2, PM_{10} and $PM_{2.5}$ 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). Therefore genetic studies on the effect of pollutants in asthma have focused on genes that are involved in the inflammation process or antioxidant protection e.g GSTs and NQO1 (Peden, 2005). The variant alleles of these genes result in total absence or a substantial change in enzyme activity, which compromises their biological reaction to environmental pollutants. Furthermore these variants are implicated in allergic diseases and might explain variation in responses to pollutants. This oxidative stress mechanism will be discussed in detail in section 2.6 of this review.

The key exposures that have been considered in the literature are ozone (O_3), sulphur dioxide (SO_2), particulate matter (PM_{10}), nitrogen dioxide (NO_2), carbon monoxide (CO) and lead (Pb) These are the six "criteria air pollutants" regulated by the Clean Air Act of the USA (Ohtsuka *et al*, 2000). Ozone has been implicated in many field studies where decreasing lung function and adverse respiratory effects have been demonstrated in relation to increasing O_3 levels (Romieu *et al.*, 2002; Kelly, 2003; Shannon *et al.*, 2004) In the current study, we have exposure data for oxides of nitrogen (NO and NO_2), PM_{10} and SO_2, therefore only these pollutants are discussed in detail.

2.3.1 Particulate matter (PM_{10})

PM, a major component of air pollution, is a mixture of different solid and liquid particles among which are dust, pollen grains and mould spores. Several studies have observed an association between high atmospheric levels of particulate air pollution and enhanced mortality from respiratory and cardiovascular diseases, exacerbation of allergic asthma, chronic bronchitis, respiratory tract infection, cardiovascular diseases and hospital admissions (Pope *et al.,* 1995; Salvi *et al.,* 1999; Peters *et al.,* 2004). The WHO estimates that inhalation of PM is responsible for 500 000 excess deaths each year worldwide (United Nations Environment Programme and WHO report, 1994). Seaton *et al.,* (1995) hypothesised that fine PM penetrates deep into the airways and is able to induce alveolar inflammation, which is responsible for variations in blood coagubility and release of mediators favouring acute episodes of respiratory and cardiovascular diseases. Ambient particles contain a large number of soluble metals including transition metals that are capable of redox cycling and thus free radical generation. The idea has therefore developed that oxidative stress underlies much of the toxicity of ambient particles. Exposure of phagocytic cells to ambient particles collected from different urban settings causes oxidative stress which correlates with the iron content of the particles (Kelly, 2003).

Epidemiological studies have consistently reported association between particles less than 10μm (PM_{10}) and increasing morbidity and mortality. PM_{10}, typically as low as

30μg/m^3 can produce these health effects (Kelly, 2003). Other studies have estimated that for every 10μg/m^3 increase in PM_{10}, there is an increase in the daily mortality rate between 0.5 and 1.6%. Effects were seen even in cities with mean annual PM_{10} concentrations between 25 and 35 μg/m^3 (Shannon *et al.*, 2004). In a study of 1759 ten-year old children from 12 Southern California cities, Gauderman *et al.* (2004) found that NO_2, acid vapor, $PM_{2.5}$ and elemental carbon exposure were all significantly associated with decreased lung function. These recent studies suggest that the current USA standards for PM_{10} should be lowered to protect public health.

PM_{10} has also been associated with episodes of increased asthma exacerbation. Studies performed in the Utah valley examined the occurrence of respiratory disease symptoms during the year that a steel mill was closed due to a strike and compared it to the years before and after the strike. Respiratory morbidities and the level of particulates were both markedly decreased during the strike year, suggesting disease exacerbation by ambient air particulates (Pope, 1989).

2.3.2 Oxides of Nitrogen

NO_2 is a gaseous pollutant produced by high temperature combustion. Like ozone, it reacts with substrates present in the lung lining fluid compartment, and is therefore unlikely to react directly with the pulmonary epithelium. Instead it is the oxidized species arising from a reaction between NO_2 and the lung lining fluid compartment that is responsible for initiating the signaling cascade which brings inflammatory cells into the

lung (Kelly, 2003). The main outdoor sources of NO_2 include diesel and gasoline powered engines and power plants. Emissions of nitrogen oxides have increased in the past 20 years because of an increase in vehicular emissions. These emissions contribute to ground level ozone (smog) and other environmental problems such as acid rain (Shannon *et al.*, 2004).

NO_2 has been implicated as a risk factor in exacerbating asthma in several studies. The risk of asthma symptoms in Los Angeles was associated with a 1.4 ppb per 8 h NO_2, and the risk of physician diagnosed asthma in the Netherlands was associated with traffic related air pollution measured as NO_2 concentration (Lee *et al.*, 2004). In 2002, van Strein *et al.* used a cohort of infants (with one older sibling with asthma and thus a suggested genetic predisposition) and one time NO_2 measurements to show that infants exposed to more than 17.4 ppb NO_2 had significantly increased risk for respiratory disease compared with those experiencing low level (5.1 ppb) NO_2 exposure. This finding was consistent with a report by McConnell *et al.*, (2006) which showed that outdoor NO_2 is associated with bronchitis symptoms in Southern California. In South Durban, a summary of the NOx emission accounted for approximately 60% of all NOx emissions (CSIR, 2002).

2.3.3 Sulphur dioxide (SO_2)

Sulphur dioxide is released into the atmosphere primarily as a result of industrial combustion of high sulphur containing coal and oil. SO_2 is a respiratory irritant that is

absorbed mostly in the upper airways but increasing ventilation results in deposition in deeper parts of the lung (Koren, 1995). Moreover, SO_2 exposure enhances responses to other environmental agents that exacerbate bronchospasm (D'Amato *et al.,* 2005). Exposure to SO_2 is marked by increased incidence and prevalence of respiratory symptoms, increased hospital visits for respiratory illnesses and impaired lung function (CSIR, 2002). Asthmatics, especially when exercising, may be 10 times more sensitive than non-asthmatics (Carlisle and Sharp, 2001). From a study of SO_2 pollution and asthma exacerbations in the SDIB, it was concluded that sensitive individuals, such as asthmatics, react adversely to higher exposure levels (Matooane and Diab, 2002).

Epidemiological data on whether ambient air pollution contributes to the incidence of asthma are from five studies - three in children and two in adults (Gilmour *et al.*, 2006). These authors cited the following studies as evidence to corroborate this link:

- The PIAMA study (Prevention and Incidence of Asthma and Mite Allergy) with a cohort of more than 4000 Dutch children, found that traffic related air pollution i.e NO_2 and $PM_{2.5,}$ were significantly associated with parental reports of wheeze, doctor-diagnosed asthma, ENT infections and serious colds and flu (relative risk RR, 1.1-1.2) (Brauer *et al.*, 2002).
- An international collaborative study involving the Netherlands, Germany and Sweden and 1,756 infants found association (ORs of 1.3-1.4) between dry cough at night in the first year of life and three pollutants, NO_2, $PM_{2.5}$ and soot (Gehring *et al.*, 2002). No association was found with wheezing, respiratory infections or bronchitis.

- The CHS (Children's Health Study) among 12 Californian communities with >6000 children and exposure data for O_3, PM and NO_2 showed that children who participated in sports activities had an increased risk of asthma when exposed to peak ozone levels (RR=1.8; 95% CI, 1.2-2.8) (McConnell *et al.*, 2002).
- The ASHMOG (Seventh-day Aventists in California) cohort study examined air pollution exposure in nonsmoking adults and reported a relative risk of incident asthma in relation to PM_{10} of 1.30 (95% CI, 0.97-1.73) for 1 000 hr/year exposure to concentrations of PM_{10} that exceeded 100$\mu m/m^3$ (Abbey *et al.*, 1995).
- A study on O_3 that included 115 incident cases of asthma reported an increased risk in men for a 27 ppb (interquartile range) increase in ozone (RR=2.1; CI, 1.0-4.2), but no association in women (McDonnell *et al.*, 1999).

The above studies support a modest increase in risk for air pollution in relation to phenotypes relevant to asthma. Numerous large scale initiatives such as the National Children's Study will more clearly delineate the relationship between environmental exposures and development of diseases such as asthma (Gilmour *et al.*, 2006).

2.3.4 Regulatory control

Protecting populations from the harmful effects of exposure of air pollutants will require effective control measures. Industry (e.g., coal burning, power plants, refineries, and chemical plants) and motor vehicles are major sources of criteria pollutants (Shannon *et al.*, 2004). Sustained air quality improvement depends on a regional commitment and

complementary national policies. In the US, the Clean Air Act of 1970 mandated the EPA to establish the National Ambient Air Quality Standards (Table 2.2). The American Academy of Paediatrics released a policy statement in 2004 that recommends that standards for PM, O_3 and NO_2 be revised in light of recent studies that suggest that children are not adequately protected by current US EPA regulations (Trasande *et al.*, 2005).

South African legislation on air pollution has only recently been revised. The previous Atmospheric Pollution Prevention Act 45 of 1965 had several weaknesses; there was no emphasis on smoke control regulations in residential areas, dust control was not adequately documented, VOCs and fugitive emissions were not controlled and there were no set air quality standards. However the new bill implemented in 2004 aims to address pollution minimization through cleaner production as a sustainable means by which air quality can be improved. It advocates that ambient air quality standards define public air that is not harmful to health and well being while also facilitating and enhancing sustainable development. Air quality management has largely decentralized to the provincial level where local governments are responsible for setting their own guidelines especially with regard to priority areas.[4] Table 2.2 provides a comparison of international air quality guidelines with South African guidelines for common pollutants. South Africa has no promulgated ambient air quality standards but the new guidelines are more or less comparable with US EPA standards. However, the recommended WHO guidelines are far lower than both SA and EPA guidelines for SO_2 and NO_2.

Table 2.2: National and International Air Quality Standards and Guidelines (CSIR, 2002), SA Guidelines and EPA standards [4,5]

Pollutant (units)	Standard/Guidelines	1 hour average	24 hour average	Annual average
SO2 (ppb)	• SA • US EPA • WHO	300 - -	100 140 48	30 30 19
NO2 (ppb)	• SA • US EPA • WHO	200 128 106	100 - -	50 53 21
NO (ppb)	• SA • US EPA • WHO	600	300 - -	150 - -
PM10 (ug/m3)	• SA • US EPA • WHO	- - -	180 150 -	60 50 50

2.4 Asthma genetics

Because the human population is biologically diverse and genetically heterogeneous, it is not surprising that differences in susceptibility to disease among individuals with or without exposure to environmental agents exist. The etiologies of many childhood diseases are due to a combination of factors, including genetic susceptibility and environmental exposures during vulnerable periods of development. Genes regulate cellular growth and development, DNA replication and repair, the metabolism and excretion of endogenous and exogenous (Suk and Collman, 1998). The Human Genome and Environmental Genome projects have generated a long list of genes and their variants. This has helped to identify genes that are implicated in disease pathways. Such genes influence or control cell differentiation, apoptosis, cell kinetics or DNA repair

(Bennett and Waters, 2000). Genetic studies of asthma have focused primarily on genetic alterations associated with BHR and inflammation.

Genetic polymorphisms are defined as variations in DNA that are observed in 1% or more of the population. Genetic polymorphisms may alter the protein structure and function through a single nucleotide base substitution in a gene's coding region, and may decrease or increase gene expression either by affecting mRNA stability when occurring in a gene 3' untranslated region or by altering transcription factor binding when occurring in the 5' promoter region. Alternatively a polymorphism may have no discernable effect on the protein product and may lie within DNA regions that are not involved in gene transcription or translation. Polymorphisms that exist in these regions as variations in repeat sequences throughout the genome have served as the basis for genetic linkage studies (Iannuzzi *et al.*, 2002). The study of genetic polymorphisms promises to help define pathophysiologic mechanisms, to identify individual risk for disease and to suggest novel targets for drug treatment. To identify susceptibility loci, association studies involve typing a genetic polymorphism in unrelated affected and in a group of healthy, matched controls. A given polymorphism is associated with the disease if that allele occurs at a significantly higher frequency among cases compared with controls (Iannuzzi *et al.*, 2002).

A recent review of the literature identified more than 100 reports of genetic variants associated with asthma and asthma-related traits. No more than 8-10 such genes have been replicated in three or more studies, and none of these genes have been consistently associated with same asthma phenotype in studies to date (Yeatts *et al.*, 2006). A major

problem in genetic studies of asthma has been the uncertainty of the phenotype. However, measurable outcomes like BHR can be used objectively. In addition, studies suggest that multiple genes are involved in asthma and the frequencies of these genes may vary in different populations. Progress in elucidating genetic links in the pathogenesis and development of asthma has been impeded by two aspects: genetic variants currently linked to asthma cause relatively small changes in function and it has been difficult to isolate one or two major genes that play a predominant role in asthma. In the face of these challenges, asthma genetic studies have used two major methods: 1) mapping techniques that pinpoint gene loci associated with various mechanisms of asthma, and 2) physiological studies associating genes and polymorphisms that may affect certain aspects of the disease process (Ober and Moffatt, 2000; Illig *et al.*, 2002).

Studies of family aggregation, twins, and linkage analysis provided early proof that genetics plays an important role in the development of asthma. (Cookson *et al.,* 1989; Meyers *et al.,* 1994; Sandford *et al.,* 1996). Substantial evidence exists for linking several chromosomal regions with the development of asthma, including regions 5q, 6p, 11q, 12q, 13q, 14q and 16p (Daniels *et al.,* 1996; Bleecker *et al.*, 1997). Chromosomes 5q and 11q exhibit the most consistent association with BHR and atopy. Candidate genes on these chromosomes include the β_2 adrenoreceptor and interleukin-4 cytokine cluster, the high affinity IgE receptor and Clara cell secretory protein genes (Tamer *et al.,* 2004). A few examples will be discussed below.

One of the earliest studies showing linkage to atopy which has been consistently replicated by other groups has been Cooksons' findings with the *FCER1B* gene which

encodes the ß chain of the high-affinity receptor for IgE (Cookson *et al.*, 1989). Linkage at this locus has been reported for many of the phenotypes associated with asthma, including BHR, total and specific IgE and atopic dermatitis (Barnes *et al.*, 2000). A coding polymorphism in FcεRIβ, an adenine to guanine substitution changes amino acid residue 237 from glutamic acid to glycine (E237G) in the cytoplasmic tail of the protein. E237G is predicted to introduce a hydrophobicity change within the C-terminus of FcεRIβ, this may affect intracellular signaling capacity of FcεRIβ. This variant has been identified in diverse populations and is easily assayed (Moffat and Cookson, 1997). E237G was detected in 53 subjects (5.3%) in an Australian population of 1004 individuals. E237G positive subjects had elevated skin test responses to grass ($p<.005$) and bronchial reactivity to methacholine ($p<.005$). The relative risk of individuals with the E237G having asthma compared with subjects without the variant was 2.3 ($p<.005$) (Hill and Cookson, 1996).

In an Australian family based population of 547 subjects (including cases, siblings and family relatives), the FcεRIβ gene was found to be highly polymorphic, linkage was found to specific IgE responses to common allergens (Palmer *et al.*, 1998). A French-Canadian case-control study of asthma and E237G was also described. There were 100 cases (total IgE > 250 µg/l plus 3 or more positive skin prick tests to allergens) and 100 controls (nonatopic), the prevalence of the E237G polymorphism was 10% ($p< 0.14$) among the cases and 1.5% ($p=0.01$) among the controls. The E237 polymorphism in the FcεRIβ gene was typed in a South African population of 48 black cases, 44 black controls, 41 white cases and 41 white controls. There was a difference in the frequency of E237G between black asthmatics (20%) and white asthmatics (12%) and between

black controls (20%) and white controls (5%)(p<.005). All cases were recruited from an asthmatic clinic and all individuals participating in the study were 6-45 yrs old (Green *et al.,* 1998).

Chromosome 5q is a region with extensive evidence for linkage and association and candidate genes located in this region have been assessed for linkage to asthma. These are interleukins IL3, IL4, IL5, IL9 and IL13, granulocyte-macrophage colony stimulating factor (GM-CSF), B2 adrenergic receptor and CD14. Approximately 60% of studies with chromosome 5q showed association with asthma and related phenotypes (Illig and Wjst, 2002). In a Dutch family based population of 184, the IL13 polymorphism was significantly more prevalent in cases than in controls (24% vs 14%, p<.005) (Howard *et al.,* 2001). In a Californian study on the TNF gene, 236 adult asthma cases from an outpatient clinic and 275 controls were recruited. Cases were more likely than controls to carry one or two copies of the TNFα-308* allelle; 30% of the cases vs 22% of controls had one or more copies of TNFα-308* (p=0.03) (Witte *et al.,* 2002). A case-control study on the association of asthma with the β-adrenergic receptor gene polymorphism and smoking was conducted among 125 asthmatic cases and 136 controls. Smokers with the polymorphism had a significantly increased risk of asthma (OR=7.81,). This association showed a clear dose response relationship with the number of cigarettes smoked, therefore a gene-environment association with smoking and asthma was established (Wang *et al.,* 2001).

IL-4 contributes to the elevated blood level of IgE that is characteristic of asthma and allergy. A polymorphism (IL-4 C-590T) has been identified in a region of the gene that binds transcription factors and influences gene expression (Sandford and Pare, 2000). The IL4 gene is one of the strongest candidate genes for causing atopy, since IL4 is the most important cytokine in the control of IgE production. Location of the mutation in the promoter of this gene is in agreement with an upregulation of IgE responses (LeSouef, 1997). In a study of 157 subjects with fatal or near fatal asthma and 90 subjects with moderate asthma, the IL-4 589T allele was found to be increased in subjects with fatal or near fatal asthma (OR 1.8, p=0.02). A group of 143 non-asthmatic controls were used in this study (Sandford *et al.,* 2000). Among 1120 German schoolchildren, polymorphisms in the IL4 gene were associated with both the development of asthma and the regulation of serum IgE (Kabesch *et al.*, 2004).

Asthma has a strong environmental component and different populations experience different exposures, therefore gene environment interactions have to be considered when determining the genotype/phenotype correlations (Wiesch and Meyers, 2000). This will be discussed in detail in the following section.

2.5 The gene-environment interaction in asthma and allergy

A better understanding of genetic influences on environmental response could lead to more accurate estimates of disease risks and provide a basis for disease prevention and early intervention programs directed at populations at risk, including children (Castro-

Giner *et al.*, 2006). In efforts to understand the relationship between exposure and adverse health effects, scientists are working to develop biomarkers, which are key molecular or cellular markers that link a specific environmental exposure to a health outcome. The challenge is to use biomarkers to establish associations between exposure and human disease in epidemiological studies and then to use the knowledge to design and conduct appropriate preventative interventions in high risk populations (Suk *et al.*, 2003; Scirica *et al.*, 2007).

Gene by environment interactions are often interpreted as an action-reaction mechanism where genes define the potential and limitations of the human body to react to environmental conditions. For e.g, the genetically determined amount and availability of an enzyme may limit the potential of the human organism to metabolize certain foods and amino acids. Thus, the reaction of the organism is determined by its genetic predisposition that is only visible when a certain environment (amino acid) is present. In complex diseases such as asthma, gene by environment interactions are less clear. Multiple genetic and environmental effects overlap and these effects may or may not be independent from each other. Gene by gene interactions will occur in regulatory pathways influencing the disease not by means of single gene interactions but in the context of a biological system (Kabesch, 2006, London, 2007). It is likely that a number of alterations in different genes contribute to the genetic predisposition of an individual to develop atopic diseases and asthma (Peden, 2005).

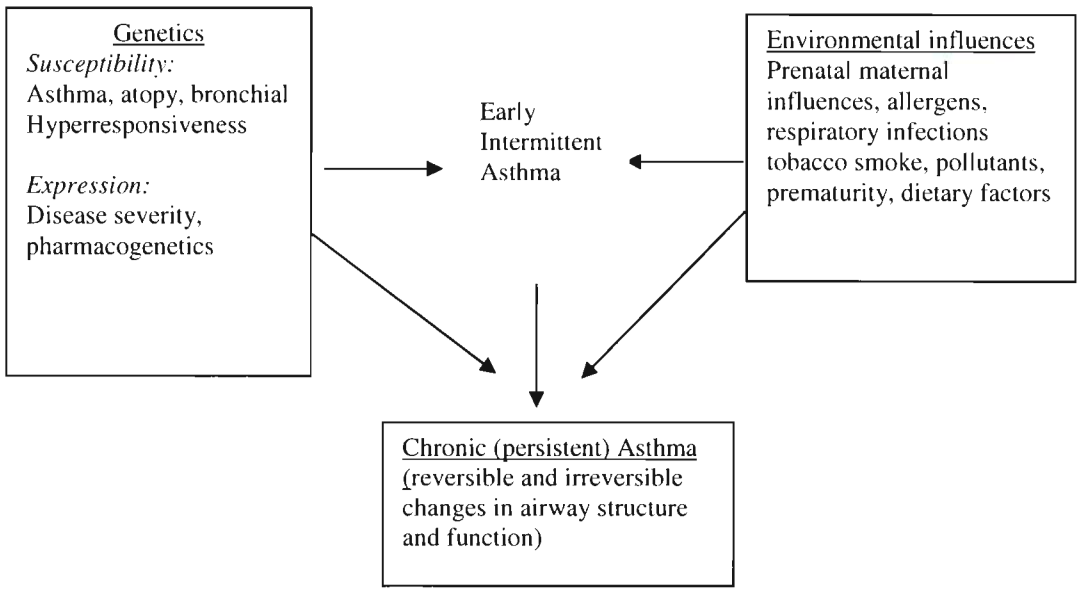


Figure 2.3: Gene environment interactions in the development of asthma (Chipps, 2004)

Figure 2.4 shows a common model of susceptibility to asthma and atopy, which implicates many genes and environmental factors but implies that the effects of genes and environmental factors individually contribute to risk. However it is likely that the interaction at a physiological level is more complex, with genes interacting both with other genes and with environmental risk factors to confer susceptibility (Ober, 2005).

Several models of gene-environment interactions have been suggested:

1. Both the susceptible genotype and the environmental exposure are necessary to produce the disease phenotype.
2. Environmental exposure causes increased risk of disease in everyone but a much greater risk in individuals with the susceptible genotype

3. The environmental exposure will only increase the risk of disease in people with the susceptible genotype.
4. Both the environment and the genotype produce excess risk
5. When there is a protective effect of the genotype, depending on the presence or absence of the exposure, the last 2 models are possible (Wiesch and Meyers, 2000).

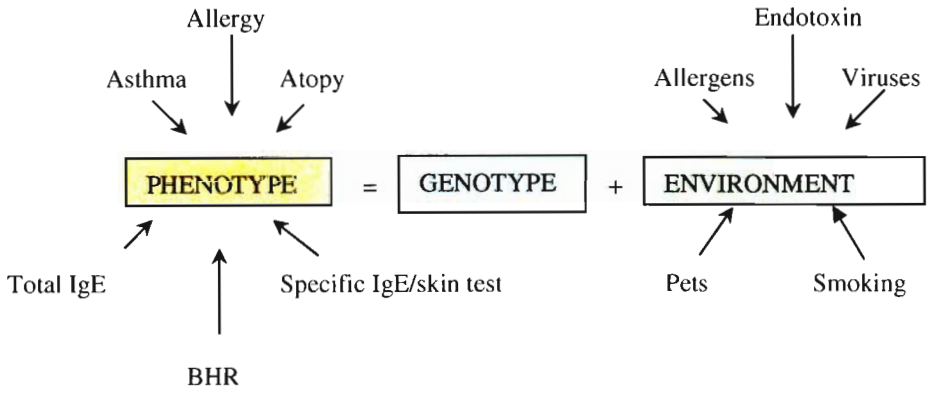


Figure 2.4: Common model of the genetics of complex diseases. Several related and quantitative phenotypes result from the effects of many loci and many environmental factors (Ober, 2005).

2.6 The oxidative stress mechanism

Most genetic studies of asthma have focussed on genes on chromosome 11q and 5q and their association with the key asthma related phenotypes of BHR and atopy. Oxidative stress and the generation of ROS is a critical component of the airway inflammation characteristic of asthma (Fryer *et al*, 2000; Postma *et al.*, 2000). Oxidant stress has

emerged as a mechanism that underlies the toxic effects of most forms of air pollution, including particulate matter. Even though the lung is well equipped to deal with oxidative stress, ambient particles are carried deep into the respiratory tree where they invade and overcome the lungs antioxidant defenses (Kelly and Sandstrom, 2004). Inflammatory cells recruited to the asthmatic airways have an exceptional capability for producing reactive oxygen species (ROS). Activated eosinophils, neutrophils, monocytes and macrophages can generate superoxide (O_2^-) via the membrane associated nicotinamide adenine dinucleotide phosphate (NADPH)-dependent complex. Subsequently, dismutation of O_2^- forms hydrogen peroxide (H_2O_2). Both O_2^- and H_2O_2 are critical for the formation of potent cytotoxic radicals in biological systems through their interaction with other molecules. In addition to recruited inflammatory cells, the constitutive airway cells such as epithelial cells are also potential sources of ROS.

Cells recovered from bronchoalveolar lavage (BAL) fluid and blood of asthmatic subjects have been shown to generate greater amounts of ROS at baseline and after stimulation *ex vivo* than in normal subjects, a feature which is correlated with disease severity. This suggests that the biochemical milieu in asthma contains factors which prime oxidative pathways *in vivo* (Dworski, 2000). Although host antioxidant defenses should detoxify ROS, individuals differ in their ability to deal with an oxidant burden, and such differences are in part genetically determined. Inability to detoxify ROS should perpetuate the inflammatory process, activate bronchoconstrictor mechanisms and precipitate asthma symptoms (Fryer *et al.*, 2000). BHR is also modulated by ROS levels, possibly through the ability to regulate eicosanoid production via stimulation of

arachidonic acid release (Weiss, 1996). It is hypothesized that excess oxidative stress provides a mechanistic framework that unifies inter-relationships between childhood lung function growth, asthma and respiratory infections, environmental exposures such as air pollution and tobacco smoke, and factors such as diet and genetics. Excess oxidative stress is the proximal event leading to inflammation, cell death, and subsequent airway remodeling among individuals with inadequate defenses (Sorensen *et al.,* 2003).

Ozone, PM_{10} and NO_2 have been shown to induce acute inflammatory responses in the airway. Each of these pollutants either acts as an oxidant or induces oxidant responses in the host. Airway or plasma antioxidant status has been associated with protection from the effect of pollutant exposure (Kelly, 2003). Genetic studies to date have focussed on genes thought to play a role in the inflammation or antioxidant protection. The enzymes detoxify metabolites of oxidative stress in two successive phases. Phase I is represented by the cytochrome 450 enzymes, which mediate oxidative metabolism, and by microsomal epoxide hydrolase and other enzymes involved in the detoxification pathway. Phase I chemical reactions may convert harmless compounds into more toxic or carcinogenic metabolites, which require immediate processing in phase II of the detoxification pathway. Phase II enzymes convert toxic metabolites into polar, water-soluble, nontoxic derivatives which can then be excreted from the body (Ivashenko *et al.*, 2002).

Gluthathione-S-Transferases (GSTs) are phase II xenobiotic detoxifying enzymes that are implicated in oxidative defenses. These enzymes function as peroxidases to detoxify

products of oxidative attack and may be determinants of respiratory health (Sorenson *et al.*, 2003). It has been hypothesised that individual ability to detoxify ROS and their products, determined by polymorphisms in genes like GST contributes to the development of BHR and asthma (Hayes and Strange, 1995). GSTs may influence the synthesis of eicosanoids (mediators in the asthma response) via modulation of ROS levels (Fryer *et al.*, 2000). GST enzymes use a wide variety of products of oxidative stress as substrates and thereby have an important role in preventing the build-up of reactive oxygen species. If antioxidant defenses are inadequate, substantial oxidative stress can occur that may interfere with normal lung growth and may contribute to increased incidence, prevalence, and severity of respiratory diseases such as COPD, asthma, and viral infections (Gilliland *et al.*, 2002a). As of 2001, very few studies were done on GSTP1 and GSTM1 in populations of African ancestry (mainly African-American). Indeed only 7 and 1 study was done with GSTM1 and GSTP1 respectively, while 61 and 14 studies were done on Caucasians and Asians (Garte *et al.*, 2001). This reflects a need for such studies on the African continent. NQO1 has also been implicated in the response to oxidative stress (Bergamaschi *et al.*, 2001). The three genes chosen for this study (GSTM1, GSTP1 and NQO1) will be discussed in the following section.

2.7 Gene polymorphisms involved in response to oxidative stress responses

2.7.1 Gluthathione-S-Transferase genes (GSTM1 and GSTP1)

GSTM1 and GSTP1 are important enzymes in the lung that function as antioxidants in xenobiotic, peroxide and hyperperoxides metabolism pathways to reduce oxidative stress

(Zhong *et al.*, 1991; Gilliland *et al.*, 2002b). Several common variants of GSTs are well characterized. The GSTM1 gene is located on chromosome 1p13.3. Depending on ethnic grouping, 20-50% of individuals have the entire gene deleted which is known as the GSTM1null genotype (Zhong *et al.*, 1991; Siedegard *et al.*, 1988). People with this genotype have no protein expression and a decreased antioxidant capability. The frequency of GSTM1null genotypes is higher in Caucasians than in Asians and Africans (Bailey *et al.*, 1998; Roth *et al.*, 2004) and varies from 40-60%.

GSTP1 is strongly expressed in the respiratory epithelium and is the dominant GST in the lung, where it is thought to detoxify lipid and DNA oxidation products. Thus, polymorphism in GSTP1 may influence the development and/or severity of respiratory related phenotypes (Hemmingsen *et al.*, 2001). GSTP1 is located at 11q13.3 and has a common single nucleotide polymorphism at codon (A105G) that results in an amino acid change in the protein from isoleucine (AA) to valine (GG). The allelic frequency of this variant among different populations may vary between 30-35%. GSTP1 (GG) variant has a lower enzyme activity due to the amino acid conversion which affects the hydrophobic binding for electrophilic substrates (Ishii *et al.*, 1999). Children with this genotype may be less able to defend their airways from the adverse effects of excess oxidative stress associated with asthma, they may have a lower attained lung function at maturity and be more susceptible to a spectrum of adverse respiratory outcomes associated with chronic excess oxidative stress (Gilliland *et al.*, 2002b).

Although numerous reports on GST genes have been published, more data is needed from Asian and African populations since these have been relatively underrepresented in gene-environment research. Table 2.3 represents the largest and most recent estimate of these frequencies in healthy populations. Although the effects of GSTM1null and GSTP1 GG are modest in magnitude for individuals, this genetic variation may have public health importance, especially for children with asthma. Evaluating the impact of air pollution at the population level rather than at the individual level of relative risks shows that a small change in the population mean of a quantitative measure, such as lung function, can have considerable impact on the number of subjects with the relevant adverse condition (Kunzli *et al.*, 2000). Children with these genotypes, especially those with asthma, may have lower attained lung function at maturity and be more susceptible to a spectrum of adverse respiratory outcomes associated with chronic excess stress (Gilliland *et al.*, 2002b).

Table 2.3: Studies conducted on metabolic gene polymorphisms by race group (Garte *et al.*, 2001)

Gene	**Ethnicity**	**No of studies**	**No of subjects**	**Variant homozygous allele frequency**
GSTM1	Caucasian Asian African	50 11 7	10514 1511 479	0.5 (0.4-0.6) 0.5 (0.4-0.5) 0.3 (0.2-0.4)
GSTP1	Caucasian Asian African	13 1 1	2282 243 82	0.4

There is geographic and ethnic variation in genotype frequencies for GSTM1null and GSTP1AG/GG. Higher frequencies have been found in Caucasians and Asians while lower frequencies have been found in Black Americans. A meta-analysis revealed that

people from Great Britian had the highest frequency of GSTM1null compared to all other Caucasian populations (Garte *et al.*, 2001). There has been only one study conducted in SA among Africans where the frequency of the GSTM1 null frequency was low (20%), which is in agreement with the range of between 16% and 36% that has been reported for other African populations (Adams *et al.*, 2003). This, however, is in contrast to the high frequency GSTM1 null of between 40 and 60% reported for Caucasians and Chinese (Chen *et al.*, 1996). With Indians living in India, Buch *et al.*, (2002) found a prevalence of the null polymorphism to be 49%.

The GSTP1 Val105 variant has been associated with low substrate affinity and thus reduced enzyme activity. The allelic frequency of the GSTP1 AG/GG variant was significantly higher for a SA Xhosa population (79%) than that reported for other African ethnic groups i.e. 23% in Tanzanians, 23% in Vendas and 42% in Zimbabweans). This wide range may be attributed to small sample sizes (all 4 studies had sample sizes less than 102 participants). This high GSTP1 AG/GG frequency was similar to that observed for African Americans, but low compared to Asians (Adams *et al.*, 2003).

GSTM1 and GSTP1 genotypes were associated with statistically significant deficits in annual lung function growth in a cohort of 3 135 children (Gilliland *et al.*, 2002a). The GSTM null allele was associated with an annual deficit in FVC growth (-0.21%, 95% CI, -0.50, -0.40) and 0.27% (95% CI, -0.05, -0.04) annual deficit in FEV_1 growth, which translates to lower attained lung volume and air flow. People with the GSTP1GG genotype had a 0.35% (95% CI,-0.62, -0.07) deficit per year in FVC growth and a 0.34%

(95% CI, -0.68, p<.005) reduction in growth for FEV_1 (Gilliland *et al.*, 2002a). It has been reported that children with GSTM1null genotype exposed to tobacco smoke *in utero* have an increased prevalence to early onset asthma and a range of other respiratory conditions and that the GSTP1 genotype influences the risk or severity of respiratory infection in school-aged children. Fryer *et al.* (2000) reported that the frequency of the GSTP1GG was significantly lower in asthmatic than in control subjects. Indeed the presence of this genotype conferred a sixfold lower risk of asthma than GSTP1AA.

Parallel effects of the GSTM1 null and GSTP1AA genotypes were seen with histamine release enhanced by diesel exhaust particles. People carrying these genotypes showed higher histamine concentrations after diesel exhaust plus allergen challenge. It has been suggested that GSTs affect synthesis of eicosanoids such as leucotrienes that modulate allergic responses. These authors suggest that GSTs play a part in controlling the response to diesel exhaust particles by detoxifying reactive oxygen species derived from diesel exhaust. These results have obvious clinical and public health relevance especially for sensitized individuals living in urban environments (Gilliland *et al.*, 2004).

2.7.2 Nicotinamide adenine dinucleotide quinone oxidoreductase (NQO1)

NQO1 is an important phase II enzyme which catalyses the detoxification of reactive quinines that can produce ROS through redox cycling. This enzyme is therefore an important part of oxidative defenses (Sorenson *et al.*, 2003). A point mutation in codon 187, causing a proline to serine change in amino acid (CC to TT) results in complete loss

of enzyme activity in homozygous subjects, whereas those with 2 wild type alleles (CC) have normal activity (Traver *et al.*, 1992). The frequency of the NQO1 TT genotype varies across ethnic groups from 2% in Caucasians, 2% in African Americans, 4% in Mexican Hispanics to 5% in Chinese populations (Ross *et al.*, 2000). This gene has not been as extensively studied as the GST enzymes and frequency of the NQO1 TT allele in a South African population has not been determined.

NQO1 catalyzes the two-electron reduction of quinones to hydroquinones, thus bypassing the potentially toxic semiquinone radical intermediate. NQO1 is thought to play a detoxifying role limiting redox cycling of labile semiquinone radicals both to quinines and hydroquinone, thus reducing the production of the superoxide anion, hydrogen peroxide, and ultimately of the hydroxyl radical (OH). NQO1 generated hydroquinones are targets of O_3 which oxidizes them to semiquinones and gives rise to the hydroxyl radical. In GSTM1 positive subjects, such an increased production of hydroquinones can be neutralized by GSH conjugation. People carrying the NQO wt genotype, but lacking the GSTM1 are less able to conjugate hydroquinones, a condition that could favor their responsiveness to O_3. Bergamaschi and colleagues (2001) reported that airway inflammation was increased only among individuals with a combination of NQO1 Pro/Pro and the GSTM null genotypes. Similarly, in a highly exposed Mexico City population, David *et al.* (2003) found that children carrying one or two copies of the NQO1 Pro allele who were GSTM1 null were at decreased risk of asthma. These findings suggest a protective effect for the NQO1 Ser allele in GSTM1null subjects. This may be explained by the alternate mechanism by which NQO1 works. In addition to its

detoxifying role, NQO1 can also catalyze the bioactivation of some quinones to more reactive hydroquinones that, in turn, auto-oxidize to produce ROS or undergo rearrangement to generate alkylating species (Bergamaschi *et al.*, 2001). .

Because the homozygous NQO1 TT is essentially a null phenotype, it provides a convenient molecular tool with which to assess the potential chemoprotective role of NQO1 *in vivo*. Previous work on the null polymorphism in NQO1 has almost exclusively been examined from the perspective of the susceptibility to cancer. The NQO1 TT allele has been associated with an increased risk of urothelial tumors, acute myeloid leukemia, cutaneous basal cell carcinomas and paediatric leukemias and it was also found that it is a significant risk factor for benzene induced hematoxicity in exposed workers. This polymorphism may also be important for chemotherapy using antitumor quinines. Mitomycin C is currently the only quinone used extensively in chemotherapeutic regimens. NQO1 is one of the reductases that are involved in the bioactivation of mitomycin C and it has been found that the effectiveness of mitomycin therapy in individuals carrying the NQO1 polymorphism is diminished (Traver *et al.*, 1992; Ross *et al.*, 2000).

2.8 Genetic epidemiological studies

A number of reports on GSTM1 and GSTP1 have shown a relationship with asthma or a related phenotype. Table 2.4 provides a snapshot of selected studies that have been conducted in recent years. As with the candidate gene studies, these results were often

contradictory, especially with respect to GSTP1 and its variants. Most studies with the GSTM1 gene have reached similar conclusions.

In a case-control study conducted by Tamer and coworkers (2004), it was found that asthma patients had a higher prevalence of the GSTM1null genotype (63.4%) than the control group (40.8%) OR=2.3. (CI 1.3, 4.2). Additionally, the GSTP1 GG genotype and the combined GSTM1null/GSTP1GG genotype were more common in the asthma group than the control group. A case-control study conducted in Russia among 109 asthma patients and 90 controls showed that people with the GSTM1null genotype were found to be at approximately 3.5 fold higher risk of developing asthma (Ivashenko *et al.,* 2000). Similarly, Gilliland and coauthors who investigated 1183 grade 4 schoolchildren in California, USA, using incident respiratory illnesses as determined by monitoring school absences as an outcome, found that children with the GSTM1null genotype had slightly higher rates of respiratory illnesses than those with the GSTM1 genotype (RR= 1.1, CI: 0.9, 1.3). In this study, homozygous GSTP1GG was found to be protective against acute respiratory illnesses and was associated with a lower risk compared to the AA genotype (RR=0.71 (CI 0.54, 0.93)(Gilliland *et al.,* 2002b). Further, these authors demonstrated a modest but significant association between decreased lung function (FEV_1) and the GSTM1null and GSTP1GG variants. Children with these genotypes, especially those with asthma may have lower attained lung function at maturity and be more susceptible to a wide spectrum of adverse respiratory effects associated with chronic oxidative stress (Gilliland *et al.,* 2002a).

Contrary to the above reports, Carroll *et al.* (2005) found that GSTM1null and GSTP1GG genotypes were associated with increases in FEV_1 and FVC, however these authors had a comparatively lower sample size of 222 children compared to the study by Gilliland *et al.*, (2002b). Lee *et al.*, (2005) found that homozygous GSTP1 AA was significantly associated with physician diagnosed asthma (OR =2.0, CI 1.1, 3.6, p=0.02) among 236 Taiwanese schoolchildren. The risk with GSTM1 null genotype was positive (OR=1.3, CI 0.8, 2.3), but failed to reach statistical significance. Although a protective role for GSTP1GG genotype in the development of asthma has been demonstrated in two independent studies (Fryer *et al.*, 2000; Anynacioglu *et al.*, 2003), others have failed to replicate this polymorphism (Brasch-Anderson *et al.*, 2004) or even found opposite effects of the polymorphism (Tamer *et al.*, 2004).

The use of antioxidants has been proposed as a way to boost the capability of individuals to address the effects of oxidative stress. Romieu and coworkers (2005) found that asthmatic children with the GSTM1null genotype were more susceptible to the impact of ozone exposure to small airways function. They found that supplementation with the antioxidants Vitamins C and E above the minimum daily requirement may compensate for this genetic susceptibility. The beneficial effect was seen primarily in the GSTM1 null individuals between the placebo and supplement groups, than in the GSTM1 positive children. Among GSTM1 null children with moderate and severe asthma, the effect of supplementation was enhanced. This is an important finding and relates directly to changing dietary intake to improve resistance to disease, especially in people who have a diminished capacity to deal with an oxidative burden due to the mutations they carry.

Very few studies have been done with these above genes in the context of gene-environment interactions. Gilliland *et al.,* (2004) used a human inhalation challenge model and found that people with both the GSTM1 null and the GSTP1 AA genotypes had significantly higher allergic responses to diesel exhaust particles than those with other combination of genotypes. Lee *et al.* (2004) examined the relationship between the GSTP1 polymorphism, outdoor air pollution (designated high and moderate areas) and childhood asthma using 61 asthmatic schoolchildren and 95 controls in Taiwan. There was a significant interaction between the GSTP1 AA genotype and risk of asthma in high pollution districts. In the low pollution district, the frequency of GSTP1AA was not related to increased risk of childhood asthma (OR=1.4, 95% CI: 0.3,6.3). The odds ratio for risk of asthma in the moderate pollution district for the GSTP1AG/GG genotype was 1.5 (95% CI: 0.4,5.9) and in the high air pollution district, 3.8 (95%CI: 1.0,17.1).

Table 2.4 includes a brief summary of selected reports that have been published on the GSTM1, GSTP1 and NQO1 genes. Note that these studies often used varied study designs, definition of phenotype and sample sizes. Since gene frequencies are different among various racial groups, this may account for the different associations between gene polymorphisms and asthma in the different studies. Additionally, each locus may be in linkage disequilibrium with an unknown causal gene(s), which is a fundamental limitation of the candidate gene approach (Gilliland *et al.*, 2002a).

In essence, GSTM1, GSTP1 and NQO1 genes may contribute to the variation in lung functions growth and are implicated in the pathobiology of respiratory diseases based on their roles in perpetuating oxidative stress. This genetic variation may have public health importance especially for children with asthma. Since these variants are common in the general population, the number of sensitised individuals living in urban populations may be significant. Certainly, these are not the only genes involved in protection against oxidative stress, nor are they unique in their action, but they provide a good model to investigate the gene-environment interaction with complex diseases such as asthma. It is worth the effort to either challenge or corroborate the association of these genes to respiratory disease in different populations. Since genetic studies are usually costly and ethically challenging to conduct, a future meta analysis of this and other studies may provide conclusive evidence of the roles of these genes and their variants in respiratory linked disease.

Table 2.4: Summary of studies conducted with GSTM1, GSTP1 and NQO1 polymorphisms

Outcome	Population	Genes	Results	Reference
BHR and asthma	Caucasians 202 subjects, UK	GSTP1	GSTP1 GG was significantly lower in asthmatic children than in controls: 6x lower risk of asthma than GSTP1AA (OR=0.16, 95%CI 0.05-0.55) p<.005. GSTP1GG was significantly lower in patients with BHR than in controls (OR=0.23, 95%CI 0.06-0.88) p=0.031 GSTP1GG was significantly less common in subjects with positive skin tests than in those with negative skin tests (p=0.01).	Fryer *et al.*, 2000
BHR	145 children with asthma, UK	GSTP	GSTP1 GG was associated with 3X reduction in risk of BHR	Child *et al.*, 2003
Asthma	436 children from different districts with high and low air pollution exposures Asian, Taiwan	GSTP1	Significant gene environmental interaction between GSTP1 alleles and air pollution. GSTP1 AA was at increased risk of asthma in the high pollution district.	Lee *et al.*, 2004
Asthma	103 controls 101 asthma Caucasian, Turkey	GSTP1	Asthma patients had a higher prevalence of the GSTM1 null genotype than the controls (63.4% vs 40.8%) GSTP1 GG had a 3.5 fold increased risk to asthma Asthma patients had higher prevalence of GSTP1GG allele (45%) than the control group (27.3%)	Tamer *et al.*, 2004
Asthma	145 Caucasian probands (nuclear families) , children were all asthmatics, UK	GSTP1 maternal	GSTP1 GG and GSTP1 AA genotypes were associated with non significant increases in lung function. Maternal GSTP1 GG was significantly associated with offspring lung function and strongly predictive of FEV1/FVC (GG 105.2%, AG+AA =97.9%) Associations bet maternal GSTP1 GG and FEV1/FVC was significant (p<.005).	Carroll *et al*, 2005

Outcome	Population	Genes	Results	Reference
Asthma	182 Caucasian, US	GSTM1 GSTP1	GSTM1 null was associated with 1.89 fold risk of diisocynate induced asthma. GSTP1 GG related to high total IgE levels	Pirrila *et al.*, 2001
Decline of lung function in smokers	299 subjects (286 Caucasian and 13 African American) and 322 controls (308 Caucasian and 13 African American), US	GSTM1 and GSTP1	None of the GSTM1 and GSTP1 genotypes separately had any significant effect on lung function decline. But there was an association between lung function and all three GSTs simultaneously.	He *et al.* 2002
Respiratory illness related school absences	Cohort of 1183 Californian schoolchildren (9-11 years) Caucasian/Hispanic, African American and Asian, US	GSTM1 GSTP1	GSTM 1null 43% prevalence GSTP1 AA 39% AG 45.2% GG 13.7% GSTM1 null was associated with higher rates of respiratory illness Children with at least one varaint GSTP1 allele were protected from respiratory illnesses (RR=0.80, 95% CI 0.65-0.99)	Gilliland *et al.*, 2002b
Childhood lung function growth	1940 children, 8-11 yrs, California, USA	GSTM1 GSTP1	GSTM1 null associated with deficits in annual growth rates for FVC and FEV1 GSTP1 GG : slower lung function growth than children with GSTP1 AG or GSTP1AA	Gilliland *et al.*, 2002a
Smoking and asthma	German schoolchildren n=3054	GSTM1 and GSTP1	GSTM1null and ETS: risk of asthma, wheeze and shortness of breath was higher than GSTM positive without ETS. GSTM1 null =51.6% No major effect of GSTM1 null genotypes on the prevalence of asthma or wheeze.. Combined analysis of smoke exposure and GSTM null +ETS the increased risk was statistically significant for all wheeze outcomes and asthma.	Kabesch *et al.*, 2004

Outcome	Population	Genes	Results	Reference
Allergic responses	19 subjects of mixed ethnic population, Caucasian, Hispanic, African American and Asian, Los Angeles, USA	GSTM1 GSTP1	GSTM1 null or the GSTP105 showed enhanced nasal allergic responses in the presence of diesel exhaust particles. Effect was largest in cases with both GSTM1 null and GSTP1 AA genotypes. None of the GSTs modified the allergic response to allergen challenge alone. Combinations GSTM1 null + GSTP1 AA had higher allergic responses to diesel exhaust particles than other genotypes combined.	Gilliland *et al.*, 2004
Asthma	2950 children, grades 4,7 and 11. California, USA	GSTM1	Effects of in utero exposure to maternal smoking on asthma and wheezing was restricted to children with the GSTM1null genotype.	Gilliland *et al*, 2002c
Atopic bronchial asthma	109 Russian patients, 90 controls, both adults and children	GSTM1	GSTM1null was associated with increased rsik to asthma (OR=3.49, 95% CI=1.93-6.37) 76.1% asthmatic patientshad GSTM1null genotype compared to 47.8% controls (p<.005)	Ivashenko *et al.*, 2002
Asthma	Asian, Taiwan 236 children	GSTP1 GSTM1	Homozygous GSTP1 AA had increased risk of asthma (OR=1.94, 95% CI 1.08-3.59). An increased risk for childhood asthma was also noted with the GSTM1 null genotype but did not reach statistical significance (OR=1.37, 95% CI 0.8-2.38).	Lee *et al.*, 2005
Lung function	316 Caucasian parents 418 children, UK	GSTM1 GSTP1	Children with GSTP1GG and GSTM1 null were associated with increased FEV1 and FVC GSTM1 null was assoc with a 6.7% higher FEV1. In the siblings, the GSTP1GG genotype was assocaited with a 9.6% increase in FEV1 (p<.005) and a 10.7% increase in FVC (p<.0051). GSTM1null genotype was associated with a 6.7% increase in FEV1 (p<.005) and a 4.1% increase in FVC (p=0.063).	Carroll *et al.*, 2005.

Outcome	Population	Genes	Results	Reference
Asthma	Czech population of 1006 adults	GSTM1 and GSTT1	GSTM null genotype did not differ significantly between patients with allergic diseases/asthma and healthy controls However when compared with GSTM present, asthmatics with the null allele had worse lung function	Holla *et al.*, 2006
Lung Function variability in response to ozone exposure	24 healthy adult subjects, Europe	NQO1 GSTM1	Subjects with the NQO1wt and GSTM1null genotypes showed significant decrements in FEV1(p=0.02) and PEF (p=0.03).	Bergamaschi *et al.*, 2001
Asthma	218 case parent triads, Mexico	NQO1 GSTM1	No association with NQO1 alone and asthma risk But GSTM null, NQO ser were at reduced risk of asthma compared with Pro/Pro homozygotes (p=0.01) Protective effect of the NQO1 Ser allele in a pop of GSTM null children with increased ozone exposure.	David *et al.*, 2003

2.9 Public health impacts of gene-exposure-disease investigations

Public health has evolved on the premise that genes could not be modified, effectively disqualifying them as targets for intervention. However, for complex diseases, genetic information may be used either pre-symptomatically or after disease onset, for targeted interventions including diet, medication, and lifestyle modifications. Genetic information may motivate people to improve their health behaviour, or at the other extreme, it may lead to a fatalistic view of genetic risk with people shunning preventative behaviours or treatments. Genetically susceptible population subgroups may be identified, marginalized or discriminated against in various ways- the creation of a genetic underclass. Family relationships, insurance (life, travel, and health), employment, finance, adoption, migration, forensic, and legal settings are all examples of where genetic discrimination may occur. Multi-disciplinary education programs for health professionals are needed on the scientific, ethical, legal, and social issues related to public health genetics, as are programs on bioinformatics and statistical genetics, cultural anthropology and health behavior. Debates about the future of epidemiology and public health often depict high risk and population approaches to prevention as controversial (Halliday *et al.,* 2004).

Genetics has been heralded by some as a revolution, but it is more likely an evolution, a progression whereby advances in genetics are integrated into medicine and public health, in a considered and gradual way, accompanied by the necessary social and ethical debate. The convergence of public health and genetics holds the possibility of improved understanding of the etiology, prevention and management of complex diseases such as asthma. The evolution of public health genetics has already begun as shown by the

plethora of peer reviewed papers from both the public health and genetics communities, as well as many public consultation documents. The literature consists not only of the basic research, but many authors are grappling with issues ranging from the methodological to the health applications of genetic research.

Research focused on gene-environment interactions hold great promise in treating and managing asthma. However there are few studies that examine the relationship between genetic risk factors and environmental exposures in the exacerbation of asthma. Most of these studies were conducted mainly in the Northern hemisphere with Caucasian, Hispanic and Asian populations. The South Durban Health Study (SDHS) was designed in response to the Multipoint Plan which was proposed at governmental level to understand the state of pollution in the South Durban Area, to develop a system to monitor fluctuations in pollution levels and to determine to extent to which pollution adversely impact on the health of the community (Naidoo *et al.*, 2006). This longitudinal study, the first on the African continent, involved repeated measures of pollutant exposures, across different seasons, among a cohort of schoolchildren. In order to measure the health effects of exposure, daily lung function measures, FEV_1 and PF were taken daily for a period of 3 weeks in each of 4 intensive phases. This study had several important advantages. First, the study population of children exposed to ambient pollutants was confined to defined areas, each area with its own monitoring sites allowing a more precise estimation of exposure. Second, the 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. Thirdly, the sample

deliberately selected persistent asthmatics, which provided additional power to identify specific impacts on susceptible groups.

In this study the frequency of polymorphic variants of GSTM1, GSTP1 and NQO1 in a multiracial South African population was determined and these variants were evaluated as susceptibility markers for asthma and related phenotypes such as BHR and atopy. Further, a potential gene-environment interaction with specific pollutants was assessed. We investigated whether polymorphisms of enzymes known to modulate or protect from epithelial oxidative damage e.g. GSTs and NQO1 account for the variation in response to environmental exposures. We focused on GSTM1 and GSTP1 genotypes since these genes are expressed in the lung, are involved in antioxidant defense pathways and have common functional variant alleles. Changes in FEV_1 and PF in response to pollutants such as SO_2, NO, NO_2 and PM_{10} was assessed. We hypothesized that children with polymorphic genotypes might have greater susceptibility to reductions in FEV_1 and PEF associated with air pollution exposure.

2.10 Aims of study

i) To assess the frequency of gene variants involved in the oxidative stress response, i.e. GSTM1 (present vs null genotype), GSTP1 (Ile105Val; AA→AG/GG) and the NQO1 (Pro/Ser; CC →CT/TT) among a cohort of schoolchildren from south Durban (highly industrialised) and north Durban (no industry).

ii) To evaluate the association of GSTM1 (present vs null genotype), GSTP1 (Ile105Val; AA→AG/GG) and the NQO1 (Pro/Ser; CC →CT/TT) genotypes and respiratory outcomes such as two grades of asthma, doctor diagnosed asthma, BHR and atopy among these children.

iii) To investigate fluctuations in intraday variability and nadir FEV_1 and PF in relation to daily averages in ambient air pollutants (SO_2, NO_2, NO, and PM_{10}) using genotype as an effect modifier among the entire cohort of schoolchildren.

iv) Aims (i), (ii) and (iii) will be investigated with 12 possible genotype combinations of the three genes under study.

CHAPTER 3:

METHODOLOGY

3.1 Overview of methodology

This study investigated three genetic polymorphisms which are known to contribute to differing susceptibilities to respiratory outcomes when exposed to varying environmental conditions. The project adds a genetic epidemiology component to the SDHS study which was carried out between May 2004 and February 2005. The SDHS study characterized the health status of a representative sample of children and adults from communities in South Durban, with comparison communities in North Durban. The prevalence of various respiratory outcomes was established using validated methodology among children in seven schools from both study regions. Acute respiratory outcomes was correlated with daily changes in levels of air pollution. Detailed monitoring of PM, NO_x, SO_2, temperature & relative humidity and various other pollutants was carried out near the schools (Appendix 3.1 and 3.2).

The SDHS determined responses to FEV_1, FVC, and PEF in relation to daily fluctuations in SO_2, NO_2, and PM_{10}. We investigated genetic modifiers to environmental exposures by evaluating oxidative stress genes i.e GSTM1, GSTP1 and NQO1. For each of these polymorphisms, we examined whether the relationship between the pollutants and respiratory outcomes (BHR, atopy, persistent asthma and all asthma) differ by genotype.

3.2 Selection of Communities/Sampling strategy

In order to properly characterise both exposure and health outcomes, a broad geographical coverage of the Durban South basin was necessary. The following residential areas were selected in the Durban South: (a) Merebank, (b) Wentworth/Austerville; (c) Bluff and (d) Lamontville. Comparison communities in the northern residential areas of the Metropolitan boundaries selected were: (a) Newlands East; (b) Newlands West and (c) KwaMashu. The latter communities were selected because of: their proximity to each other; having a similar socio-economic profile as communities in the Durban South and having relatively little industrial exposure.

3.3 School Selection

To ensure that the study sample was representative of the immediate geographic location of the monitoring station, only schools at which the bussing in of students from surrounding communities was minimal (<15%) were selected. Meteorological factors and location of nearby industries were also considered in school selection. None of the schools were selected on the basis of information about the health status of the students at the school, anecdotal or otherwise. Each school in the selected communities was visited by the research team to assess school location, geography and potential sources of exposure. Among those schools meeting the specified criteria, one school was chosen at

random in each of the seven participating communities. Schools included Nizam Road, Assagai, Dirkie Uys and Ethuthukweni in the South of Durban and Ngazana, Ferndale and Briardale in the north of Durban.

3.4 Ethical Considerations

3.4.1 Ethics Approval

This research project was approved by the Ethics Committee of the University of KwaZulu-Natal (Ref H099/04).

3.4.2 Individual informed consent

Individual informed consent was necessary for all participants. In the case of children, this was obtained from their parent or guardian (Appendix 3.5). The children themselves were given an informed assent form (Appendix 3.6). In this instance each participant was given a comprehensive explanation in the language of their choice (Appendix 3.7). The content of these forms included the aims of the research, the purpose of the interview, the tests that would be conducted on them, use of their data and the confidentiality of all results. It was emphasised that participation was voluntary and withdrawal at any time was permitted without penalty. No financial incentives were provided for participation in the study.

3.4.3 Participants' confidentiality

All information obtained during the study from interviews and genetic assessments were treated in a strictly confidential manner and were only accessible to the research team. These results would be released to any clinician/guardian/agency if this was desired by the individual participant.

3.4.4 Reporting and publication of results and reports

In the publication of research results and reports, all data will be treated as grouped, thus no individual will be identified from such documentation. As per the required guidelines of the University of KwaZulu-Natal, the final content of the articles submitted to peer review scientific journals will be the responsibility of the researchers

3.5 Student Recruitment

At each of the seven schools the following sampling strategy was adopted: (1) two 4^{th} grade classes were randomly prioritized as classroom 1 and classroom 2; (2) all students in these classrooms were asked to complete a screening questionnaire, which included questions related to diagnosed asthma, frequency of symptoms and details of household adult membership; (3) the prevalence of known or probable persistent asthma among the two selected classrooms was reviewed on the basis of the screening questionnaires (Annexures 3.3 and 3.4); (4) it was intended that all the students in these two selected classrooms form the study sample provided that there were at least 20 cases of persistent asthma in the two combined classrooms, but because these numbers were not achieved in

the selected classrooms, (5) students from grades 3-6 (if available) in the school completed the screening questionnaire. The numbers of students that formed the study sample consisted of a total of 317 children from the "randomly selected classrooms" (referred to as "Type A classrooms" in this thesis). A total of 52 children with persistent asthma (based on a screening questionnaire) were "selected or invited" participants and will be referred to the "Type B classrooms" in this thesis.

The choice of these grades (3 to 6) was driven by the following considerations: the expectation that the learners in grades lower than 3 would have difficulties completing the research instruments and correctly performing respiratory testing and the likelihood that the overall prevalence of asthma would be likely be greater among younger children. The dual pronged study design (a randomly selected group of students and a group selected on the basis of known health outcomes) was intended to ensure the inclusion of an adequate number of students with known or probable persistent asthma. This was done to provide the statistical power to address whether such students are at particularly increased risk for any measurable adverse health effects of exposure to ambient air pollution. The students in the pre-selected classrooms represented a random, population based sample which permitted description of community based prevalence of disease outcomes among children. For the genetic study, the sample group comprised 369 students, of whom 317 made up the population based random sample and 52 formed the non-randomly selected persistent asthmatics. This sample group was a subset of the total SDHS group which had 423 children enrolled in the study, 342 from the random sample (Type A classrooms) and 81 selected persistent asthmatics (Type B classrooms). The

loss in sample size in the genetic study was attributed to parents' refusal to grant ethical consent for genetic testing. This loss in sample size was mainly among the white population, which accounts for the very low numbers of whites in our total sample.

The conduct of the various health assessments (questionnaires, spirometry, methacholine challenge testing and serial peak flow recording) on the study sample of the SDHS is detailed in appendix 3.1

3.6 Collection of blood samples

In order to reduce the degree of contamination during the blood sampling, the skin was carefully scrubbed with soap and water, followed by an alcohol swab. The cleaned surface was rinsed with distilled water and dried with metal-free tissue paper (Kleenex) before puncture. Immediately after the cleaning, a puncture was made using a Minilancet. About 2.5 ml of blood was collected in a lead-free plastic vacuutainer containing EDTA powder (Ram Scientific Inc., North Carolina). The vacuutainers were capped and stored for analysis in temperature controlled storage boxes. The extractions were conducted by trained phlebotomist.

3.7 DNA Extraction

Blood samples were stored at 4°C until they were ready to be processed. Genomic DNA was extracted using a PUREGENE DNA isolation kit (cat #D5000; GENTRA,

Minneapolis, MN). DNA was quantified using the Nanodrop ND 1000 Spectrophotometer. DNA samples were aliquoted and stored at –70°C. Working stocks were kept at 4°C at a concentration of 100 ng/µl. All genotyping assays were conducted by a researcher who was blind to child ID and status.

3.8 GSTM1 Polymorphism

The presence or absence of the GSTM1 gene was determined by using a differential PCR method (Bell *et al.*, 1993). DNA (100 ng) was added to a PCR mix containing 30pmol of each GSTM1 primer (G5-5' GAA CTC CCT GAA AAG CTA AAG C; G6-5'GTT GGG CTC AAA TAT ACG GTG G, Roche Diagnostics), 10 pmol of β-globin gene primers (PC04-5' CAA CTT CAT CCA CGT TCA CC and GH20-5' GAA GAG CCA AGG ACA GGT AC, Roche Diagnostics), 200µmol deoxynucleoside triphosphates, 1 U Taq polymerase (Roche Diagnostics), 1 X $(NH_4)_2SO_4$ PCR buffer [16,6 mM $(NH_4)_2SO_4$, 50 mM β-mercaptoethanol, 6,8 µM Tris (pH 8.8), 80µg/ml BSA, 1,6 mM $MgCl_2$] and 3,3 mM $MgCl_2$ in a final volume of 30µl.

The reaction mixture was placed in a GeneAmp® PCR system 9700 thermal cycler for 3 minutes at 94°C, and then subjected to 24 cycles of 94°C for 10 seconds, 57°C for 20 seconds, and 72°C for 45 seconds. Final elongation step at 72°C for 5 minutes was included. PCR products from coamplification were resolved on 4% ethidium bromide stained agarose gel (Whitehead Scientific, Pty, Ltd). The β-globin gene, which was used

as a positive control produced a band at 268 bp and the GSTM1 positive genotype was identified by a band at 215 bp. The absence of this 215 bp band confirmed the GSTM null genotype.

3.9 GSTP1 and NQO1 polymorphisms

The GSTP1 and NQO1 genotypes were determined by TaqMan assays (Applied Biosystems, Foster City, CA). The NQO1P187S (rs1800566) was done using Assay-by-Design (Taqman® SNP Genotyping Assays) assay mix, whereas GSTP1 (rs 947894) was done using Assay-on-Demand (Taqman® SNP Genotyping Assays) assay mix. All PCR amplifications were performed using the 5'-nuclease assay on Gene-Amp PCR Systems 9700 (Applied Biosystems).

3.9.1 GSTP1

Context Sequence (Chr11q12, 11S4191-D11S4162)

CGTGGAGGACCTCCGCTGCAAATAC[G/A]TCTCCCTCATCTACACCAACTATGT

NCBI SNP Reference : rs947894; SNP Type: INTRON

Reporter 1: Dye: VIC; Quencher: NFQ

Reporter 2: Dye : FAM; Quencher: NFQ

3.9.2 NQO1

NQO1P187S-CTF: Forward Primer Seq : TGCATTTCTGTGGCTTCCAAGT

NQO1P187S-CTR : Reverse Primer Seq : TGGAGTGTGCCCAATGCTATATG

NCBI SNP Reference : rs 1800566

Reporter 1 : NQO1P187S-CTV2; Dye: VIC; Quencher: NFQ;

Sequence : TCAGTTGAGGTTCTAAG

Reporter 2 : NQO1P187S-CTM2; Dye: FAM; Quencher: NFQ;

Sequence: TCAGTTGAGATTCTAAG

Design Strand : Reverse

DNA was spun down and air dried for 20 min. The PCR master mix was made up as below and 5ul was added to the air dried DNA in each well. Essentially, 10ng genomic DNA was amplified in a 5ul reaction containing 900 nmol/L each primer, 200 nmol/L each probe, and Taqman Universal PCR Master Mix (ABI).

GSTP1 PCR reaction preparation

Genomic DNA (dried in plate)	10 ng
2X Universal PCR master mix (ABI 4304437)	2.5 µl
20X Assay mix (probe/primer mix)	0.25 µl
dH2O	2.25 µl.
Total	5 µl

NQO1 PCR reaction preparation

Genomic DNA	10 ng
2X Universal PCR master mix (ABI 4304437)	2.5 µl
40X Assay mix (probe/primer mix)	0.125 µl
dH2O	2.375 µl
Total	5 µl

Plates were covered with an optical film (ABI) and then spun down in an Eppendorf centrifuge 5810 R. The reaction mixture was incubated at 50°C for 2 mins for optimal AmpErase UNG activity to prevent any carryover contamination, followed by AmpliTaq Gold enzyme activation at 95°C for 10 min. This was followed by 40 amplification cycles consisting of denaturation at 95°C for 15 secs followed by annealing and primer extension at 60°C for 1 min. The fluorescence of PCR products were detected by the ABI Prism 7900HT sequence detection system and was analyzed by SDS software (Applied Biosystems). Allelic discrimination plots were used to determine major and minor bands. The notation used was homozygous = major (1/1), homozygous= minor (2/2) and heterozygote = (1/2). Initially control plates were looked at to distinguish the major and minor alleles. The barcodes for NQO1 and GSTP 1 polymorphisms were run, detectors VIC (red, 1/1) and FAM (blue, 2/2). The quencher was non fluorescent.

In addition to the 369 DNA samples, the 384 well plate included genotype controls (known genotypes) and NTC wells (no template controls). Controls were run first. Sixteen quality control samples were used per 384-well plate along with 24 samples of known genotype. An additional 6 blind replicate samples were included in the analyses.

All the data submitted for analyses had to pass quality controls, with 100% matching for quality control samples and blind replicates and at least 95% plate efficiency.

3.10 Environmental Air Quality Monitoring

In the SDHS detailed outdoor environmental monitoring was done on site at the schools and at other relevant areas for comparison. Continuous monitoring over the study time period was done for PM_{10}, NOx, SO_2 (results of which are used in this study) and various other pollutants (Appendix 3.2 for detailed methodology used in SDHS for the air quality monitoring).

3.11 Genotype Models

GSTM1 was dichotomized into the null genotype and the present genotype, whereas the GSTP1 and NQO1 polymorphisms were categorised into two groups, based on the absence or presence of the polymorphic allele (wild type homozygous versus the combined heterozygous plus the variant homozygous genotype). The dominant model (where the polymorphic allele included both the heterozygote and homozygote genotypes) was used because of the expected increased statistical power. Gene-gene interactions between the three genotypes were also evaluated as outlined in Table 3.1.

3.12 Data Analysis Strategies

After completion of the data collection, data entry and data verification phases, descriptive analyses were conducted. Frequency distributions of categorical variables and means, standard deviation and ranges of continuous variables were calculated. Continuous variables included age, weight, height and levels of environmental pollutants. Categorical variables included genotype, age, race, sex, region (north/south Durban), disease outcomes (asthma, BHR and atopy).

Table 3.1 : Single gene (List 1)and gene-gene (List 2) interaction models

List 1 :	**List 2**
GSTM1 Positive* Null	**a) GSTM1 with NQO1** GSTM1 pos NQO1 CC* GTSM1pos NQO1 CT+TT GSTM1 null NQO1 CC GSTM1 null NQO1 CT+TT
GSTP1 Ala/Val Dominant model AA* AG + GG	**b) GSTM1 with GSTP1** GSTM1 pos GSTP1 AA* GSTM1 pos GSTP1 AG + GG GSTM1 null GSTP1 AA GTSM1 null GSTP1 AG + GG
NQO1 Pro/Ser Dominant model CC* CT + TT	**c) GSTP1 with NQO1** GSTP1 AA NQO1 CC* GSTP1 AA NQO1 CT+TT GSTP1 AG+ GG NQO1 CC GSTP1 AG + GG NQO1 CT+TT

*represents the reference genotype

After completion of the data collection, data entry and data verification phases, descriptive analyses were conducted. Frequency distributions of categorical variables and means, standard deviation and ranges of continuous variables were calculated. Continuous variables included age, weight, height and levels of environmental pollutants.

Categorical variables included genotype, age, race, sex, region (north/south Durban) and disease outcomes (asthma, BHR and atopy).

A systematic approach to sampling of the study population was undertaken to ensure that (1) a population based sample (n=317) was randomly selected to ensure some generalisability of results, referred to as Type A classrooms and (2) that children with preexisting asthma are present in sufficient numbers to allow for efficient analysis (Type B classrooms); n = 52. The entire sample (both types A and B; n=369) was used for demographic data and in the GEE regression models to evaluate the gene*pollutant effect. Inclusion of both types (random and selectively sampled children) was intended to ensure an adequate number of students with known or probable persistent asthma in order to provide adequate statistical power to address the association between health effect and exposure to environmental pollutants, while adjusting for the various covariates, including the polymorphisms. The type A or population based sample was used in all the gene frequency analysis, bivariate analysis, and multiple regression models to determine associations between genotype and respiratory linked outcomes.

These analyses were also conducted for the exposed (south Durban) and comparison (north Durban) communities separately, using Pearson's chi-squared test to determine any statistically significant differences between the groups. Genotype was investigated as a predictor of outcome/response and as a modifier of the main effects of environmental variables such as environmental tobacco smoke (ETS) and air pollution. Logistic models were developed for binary outcome variables and adjusted for relevant covariates. Effect

modifications were examined by including interaction terms in the models. Odds ratios were calculated for binary outcome variables. Confidence intervals of 95% were calculated and p values < 0.05 were considered statistically significant.

All data was initially captured using Microsoft Excel Software, with double entry, examination of data ranges for implausible values, logic checks for ensuring answer validity and consistency and automated skip patterns incorporated. All analyses were done using STATA (version 9, College Station, TX, USA).

Outcome variables:

1. Bronchial hyperresponsiveness (BHR)
2. Asthma (all asthma and persistent asthma)
3. Atopic status (skin test allergic status)
4. Pulmonary function measures (including nadir and intraday variability of peak expiratory flow (PF) and forced expiratory volume in one second (FEV_1)

   Nadir FEV1/PEF was the daily lowest valid value (i.e the minimum best of all the lung function values taken for a particular day).

   Intraday or "within-day" variability for FEV1 (or PEF in the same manner) was defined by $100\left(\frac{\text{maximum best FEV1 - minimum best FEV1}}{\text{maximum best FEV1}}\right)$

   where the "best FEV1" is the highest valid value for the specific time of day (08h00, 09h45, and 11h30,13h20) i.e. a single summary lung function measurement per child, per day.

Exposure/Independent Variables:

1. Genotype (specifically polymorphic variants of GSTM1, GSTP1 and NQO1), (coded as shown in table 3.1).
2. Environmental pollution exposure i.e SO_2, NO, NO_2, and $PM_{10.}$ were examined in relation to changes in pulmonary function. There were treated as continuous variables.

Covariates:

The following covariates were considered for potential confounding and/or effect modification:

- Race/Ethnicity (categorical)
- Age (continuous)
- Sex
- Exposure to environmental tobacco smoke
- Region (North or South Schools)

In the analyses, respiratory outcomes were considered as the dependent variable and genotype as independent. Models were fitted to estimate odds ratios of binary respiratory outcomes associated with each genotype using the dominant genetic model for the variant allele where the heterozygote and the homozygote variants are combined in the analysis. Odds ratio (OR) and 95% confidence intervals (CI) were calculated. P-values less than 0.05 were considered significant. We conducted additional analyses examining the interaction between genes on the risk of respiratory linked outcomes.

We then ran multiple logistic regression adjusting for potential covariates. We decided *a priori* to include age, sex and race in our final models and tested a range of other covariates including region (north or south schools) and caregiver smoking. The effect of each covariate on the genotype-outcome relationship was tested using nested models and the likelihood ratio test. We did not find any interaction between genotype and race, although this may be attributed to low power. Age, sex, race and region were included as covariates in the final models.

Data Analysis Strategies for Specific Aim 1:

To evaluate the relationship between bronchial hyperresponsiveness (BHR), atopy and genotype.

Bronchial Hyperresponsiveness

BHR was defined as follows:

1 = Marked BHR: dose <= 4 and 20% or more drop on FEV_1 (compared to maximum (saline) or 20% increase on bronchodilator (compared to baseline)

2= Probable BHR : 4< dose <= 8 and 20% or more drop on FEV_1 (compared to maximum (saline)) or 20% increase on bronchodilator (compared to baseline)

3 = Borderline/ Possible BHR : 8 < dose <= 16 and 20% or more drop on FEV_1 (compared to maximum saline) or 20% increase on bronchodilator (compared to baseline)

4 = Normal Airway reactivity : dose > 16 and drop less than 20% with any other dose.

Categories 1, 2 and 3 were collapsed to define the variable "positive evidence of airway hyperreactivity" while category 4 defined "normal airway reactivity"

Atopy

Atopy was defined as having a positive reaction to the skin prick test greater than that of the response to the histamine for any of the following allergens: house dust mite, cat, dog cockroach, cladosporium, grass and mold. A greater than 3mm difference in mean diameter between allergen and control wheal was considered positive. Atopy was considered as a dichotomous variable i.e. atopic or non-atopic.

Environmental Tobacco Smoke

The Caregiver Questionnaire (Appendix 3.4) was used to assess whether the child was exposed to environmental tobacco smoke. The child was considered to have significant exposure to tobacco smoke if G413 >0 or G414 was answered "YES".

G413. How many people who live in [child's] home smoke?	_____ people ☐1 None
G414. Do you smoke cigarettes, even occasionally?	☐1 Yes ☐2 No

Data Analysis Strategies for Specific Aim 2:

To investigate the effect of genotype in relation to the prevalence of asthma of any severity and persistent asthma

Asthma Severity

In this study, asthma severity was categorized in two ways: probable (or known) asthma of any severity (designated "Any Asthma"); and probable (or known) persistent asthma, including mild and moderate to severe persistent asthma (designated "Persistent Asthma"). These categories were determined by the responses from the screening questionnaire (which was completed by either the caregiver or head of household) modelled on the ATS and BRMC questionnaires.

Any Asthma

A child was considered **to have probable (or known) asthma (of any severity)** if any of the following were true:

(a) Three or more of the six non-exercise related symptoms (i.e., questions S22, S23, S24, S25, S28 and S29) were reported (at any frequency or level greater than "never").

S22. In the past 12 months, how often has your child had a **cough that won't go away**?	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never
S23. In the past 12 months, how often has your child had **wheezing** (a whistling sound from the chest) **with a cold**?	☐1 more than 1 time per month ☐2 3 to 12 times in the whole year ☐3 1 or 2 times in the whole year ☐4 never
S24. In the past 12 months, how often has your child had **wheezing** (a whistling sound from the chest) ***without* a cold**?	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never

S25. In the past 12 months, how often has your child had an attack of **wheezing** that made it **hard to breathe or catch his or her breath**?	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never
S28. In the past 12 months, how often has your child complained that his or her **chest felt tight or heavy**?	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never
S29. In the past 12 months, how often has your child's **sleep been disturbed due to wheezing, coughing, chest tightness or shortness of breath**?	☐1 most nights ☐2 more than 1 time per week ☐3 more than 2 times per month ☐4 more than 1 time per month ☐5 3 to 12 times in the whole year ☐6 1 or 2 times in the whole year ☐7 never

Either exercise symptoms (i.e., S26 and S27) was reported with frequency of three times or more in the past year i.e. S26 (1, 2, 3, or 4); S27 (1, 2, 3, or 4)

S26. In the past 12 months, how often has your child **wheezed while exercising, running or playing?**	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never
S27. In the past 12 months, how often has your child **coughed while exercising, running or playing**?	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never

(b) There is a diagnosis of asthma (i.e., "asthma" (S30 (1)), "reactive airway disease" (S30 (3)), and/or "asthmatic bronchitis"(S30(5)) were checked on question S30) with

any symptoms (questions S22 through S29) or doctor-prescribed medication (i.e., "yes" on question S31) in the past year.

S30. Has a doctor or nurse **EVER** said that your child had: (check ALL that apply)	☐1 Asthma ☐2 Bronchitis or Bronchiolitis ☐3 Reactive Airway Disease (RAD) ☐4 Pneumonia ☐5 Asthmatic Bronchitis
S31. In the past 12 months has your child **taken any medications, nebulisers, or inhalers (pumps) prescribed by a doctor** for any of the conditions listed above?	☐1 Yes ☐2 No

Persistent Asthma

A child was considered to have **probable (or known) mild persistent asthma** if, firstly, the child meets the diagnostic criteria for asthma above, and secondly, any of the following are true:

a) any daytime symptom (i.e., questions S22 through S28) is reported as being present "every day"

S22. In the past 12 months, how often has your child had a **cough that won't go away**?	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never
S23. In the past 12 months, how often has your child had **wheezing** (a whistling sound from the chest) **with a cold**?	☐1 more than 1 time per month ☐2 3 to 12 times in the whole year ☐3 1 or 2 times in the whole year ☐4 never
S24. In the past 12 months, how often has your child had **wheezing** (a whistling sound from the chest) ***without* a cold**?	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month

	☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never
S25. In the past 12 months, how often has your child had an attack of **wheezing** that made it **hard to breathe or catch his or her breath**?	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never
S26. In the past 12 months, how often has your child **wheezed while exercising, running or playing?**	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never
S27. In the past 12 months, how often has your child **coughed while exercising, running or playing**?	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never
S28. In the past 12 months, how often has your child complained that his or her **chest felt tight or heavy**?	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never

b) sleep disturbance (question S29) is reported "more than one time per week" or "most nights" i.e 1 or 2

S29. In the past 12 months, how often has your child's **sleep been disturbed due to wheezing, coughing, chest tightness or shortness of breath**?	☐1 most nights ☐2 more than 1 time per week ☐3 more than 2 times per month ☐4 more than 1 time per month ☐5 3 to 12 times in the whole year ☐6 1 or 2 times in the whole year ☐7 never

c) Daily use of doctor-prescribed medicine (i.e., "yes" on question S32) with any daytime symptom reported as being present "more than two times per week" i.e. option 2 for Questions S22-S28 above.

S32. Does your child take **any of these doctor-prescribed medications every day**, even when he/she is **not** having trouble breathing?	☐1 Yes ☐2 No ☐8 Does not apply

d) one or more daytime symptoms are reported as being present "more than 2 times per week" i.e. option 2 for Questions S22-S28 above.

e) Sleep disturbance reported is reported "more than 2 times per month" i.e option 2 on S29.

f) Daily use of doctor-prescribed medicine (i.e., "yes" on question S32).

Data Analysis Strategies for Specific Aim 3:

To investigate fluctuations in FEV_1 and PEF in relation to daily averages in ambient air pollutants (SO_2, NO_2, NO, and PM_{10}) using genotype as effect modifiers

The longitudinal design of this study allowed the investigation of how daily and bihourly fluctuations in outdoor contaminant levels affect fluctuations in symptoms and pulmonary function measures. Linear regression models were fitted using generalized estimating equations (GEEs) to accommodate correlation structure arising from repeated measurements on the same individual. Our intensive phase constituted a total of 60 days of lung measurements. On each day, 4 lung tests were done on each student at different

times of the morning and the most valid blows were used to calculate the PFT values used as depicted below. In total, 240 measurements were performed on each child during the 2004-2005 study period. GEE models were introduced by Liang and Zeger in 1986 as an estimation technique that may be applied in any generalized linear model setting and allows robustness against heteroskedasticity and correlation of the error distribution. An exchangeable correlation working structure was used.

Well-standardized and validated prediction equations for pulmonary function or several of the racial/ethnic groupings of children present in the study are not available. For this reason, an assessment of pulmonary function measures as percents of predicted was not attempted. An FEV_1 result obtained from the Airwatch was only considered valid if the result was between 30-120% of each child's personal best as defined by that child's highest recorded FEV_1 during baseline spirometry, methacholine challenge testing and (when indicated) post-bronchodilator spirometry. From these data, the best valid blow was selected from each of the four sessions to represent the child's pulmonary function at that point in time. Additionally only blows that were recorded by the Airwatch device as "error-free" were included in the analysis. Additional measures of lung function taken from the Airwatch included within-day variability of FEV_1 and PF and the daily lowest valid value ("minimum best" or "nadir") for FEV_1 and PF. Within-day variability for FEV_1 is defined as:

$$100\left(\frac{\text{maximum best FEV1 - minimum best FEV1}}{\text{maximum best FEV1}}\right)$$

where the "best FEV_1" is the highest valid value for the specific time of day (08h00, 09h45, and 11h30,13h20) i.e. a single summary lung function measurement per child, per

day. Within-day variability for PF is defined analogously to within-day variability for FEV_1. Increased intraday variability and lower nadir values are markers of worsening of asthma.

Covariates used in the GEE models included race, school, caregiver smoking, asthma severity and interactions between asthma severity and exposure. Effect modification was examined by including genotype (List 1 and 2) as an interaction term in the models. Possible lag effects (days) were modeled to account for possible prior exposure effects. For the GEE regression models, the percent change in within day variability in FEV_1 and PF, and in nadir FEV_1 and PF, are for an interquartile increase in a particular pollutant. Scaling the percents in this manner makes them directly relevant to the exposures experienced by the study participants and makes the percents for different pollutants directly comparable to each other (Naidoo *et al.*, 2006).

CHAPTER 4:

RESULTS

Demographic and genotypic characterization

We genotyped a cohort of South African children for polymorphic variants of GSTM1, GSTP1 and NQO1. Demographic, phenotypic and genotypic characteristics of study subjects are summarized in Table 4.1. Of the 423 children recruited for the SDHS study, only 369 children provided informed consent to participate in the genetics study. Most of the White parents were unwilling to give consent for the genetics aspect of the study, we therefore had a relatively small White population of 20 children. Coloureds are indigenous to South Africa and have a mixed ancestry. The average age was 10.1 yrs and both males and females were well represented with the females outnumbering the males (42.6% and 57.4% respectively). Education levels among the caregivers was high with 42.8% having matriculated from high school, however there was a significant disparity with income. Approximately 20% of all caregivers earned R 10 000 or less per annum, while 48% earned between R 10 000-R75 000 per annum which indicates a relatively poor socioeconomic group.

Seven primary schools were sampled, 4 in South Durban (Assegai, Dirkie Uys, Nizam and Enthukweni) and 3 in North Durban (Briardale, Ferndale and Ngazana) as comparison sites. The northern communities were selected because of their proximity to

Table 4.1 : Demographic and phenotypic and genotypic characteristics of study population (n=369)

Categories	South Durban N=185 (%)	North Durban N=184 (%)
Age, yr[1]	10.5 (1.1)	10.4 (0.7)
Sex		
Male	81(43.8)	76 (41.3)
Female	104 (56.2)	108 (58.7)
Race		
African	82 (45.0)	97 (53.6)
Indian	48 (26.4)	45 (24.9)
Coloured	32 (17.6)	39 (21.6)
White	20 (11.0)	
Participation from schools	185 (50.1)	184 (49.9)
Caregiver Education (N=292, %)		
Standard 9 or less	58 (31.4)	55(29.9)
High School Matriculant	51 (27.6)	74 (40.2)
Some tertiary education	28 (15.1)	26 (14.1)
Annual Household Income (N=178, %)		
R10 000 or less	17 (9.2)	20 (10.9)
R10 000-R75 000	33 (17.9)	52 (28.3)
R 75 000 or more	26 (14.1)	30 (16.3)
Caregiver smokes N= 93	39 (50.6)	54 (50.0)
Exposure to environmental tobacco smoke[2] N=194	82 (44.3)	112 (60.9)
Genotype		
GSTM1[3]		
GSTM positive	138 (74.6)	124 (67.4)
GSTM null	47(25.4)	60 (32.6)
GSTP1[4]		
Unrestricted: Ile-Ile (AA)	65 (38.2)	56 (32.0)
Ile-Val (AG)	78 (45.9)	80(45.7)
Val-Val (GG)	27(15.9)	39 (22.3)
Dominant: Ile-Val (AG)+Val-Val (GG)	105 (61.8)	119(68.0)
NQO1[5]		
Unrestricted: Pro (CC)	116 (67.4)	106 (60.6)
Pro/Ser (CT)	44 (25.6)	66 (37.7)
Ser (TT)	12 (7.0)	3 (1.7)
Dominant Pro/Ser (CT)/Ser (TT)	56 (32.6)	69(39.4)

1: Mean and SD at study entry
2: Exposure to at least one smoker in household
3. GSTM1 (positive or null genotype),
4. GSTP1; A allele codes for isoleucine, G allele codes for valine.
AA is the major allele while AG and GG represent polymorphic alleles.
5. NQO1; C allele codes for Proline, T allele codes for serine.
CC is the major allele, while CT and CC are polymorphic alleles

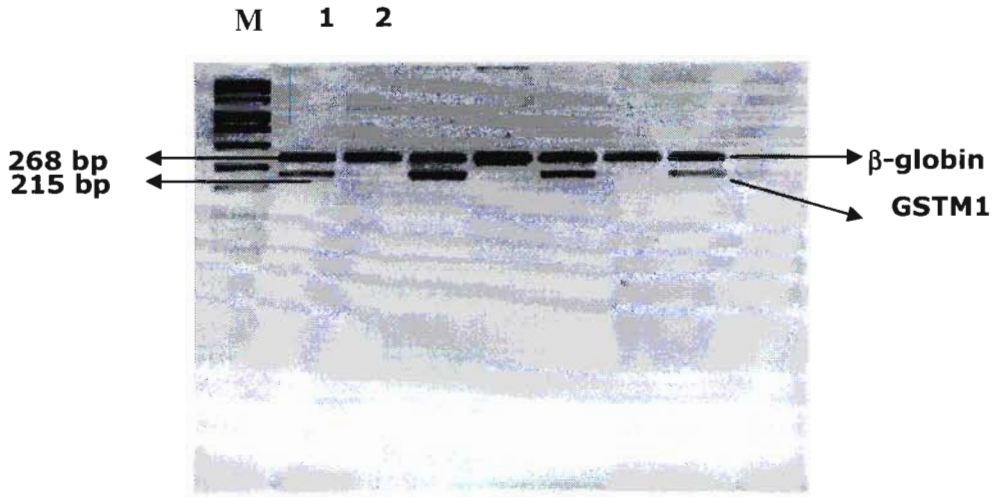


Figure 4.1: PCR products from co-amplification of GSTM1 (215 bp) with β-globin (268 bp) as the positive control.
Lane M = molecular weight marker VIII (Roche Diagnostics)
Lane 1 = GSTM1 positive genotype (+/+), 215 bp.
Lane 2 = GSTM null genotype (0/0)

each other, similar socioeconomic profiles as communities in the Durban South, and distance from major industry with anticipated low exposure to industrial emissions.

Multiplex PCR allowed us to distinguish between GSTM1null (0/0) from +/+ and +/0 subjects. However, it did not allow us to distinguish between heterozygotes (+/0) and homozygotes (+/+) genotypes (Figure 4.1). The GSTP1 and NQO1 genotypes were determined by real time PCR using TaqMan assays (Applied Biosystems, Foster City, CA). Allelic discrimination plots (Figure 4.2) were used to determine major and minor alleles. The notation used was homozygous = major allele (1/1), homozygous= minor allele (2/2) and heterozygote = (1/2). For GSTP1 and NQO1, we were unable to assign a genotype to about 6% of DNA samples. Since these samples were different for each gene, poor DNA quality could not be a reason for nonamplification.

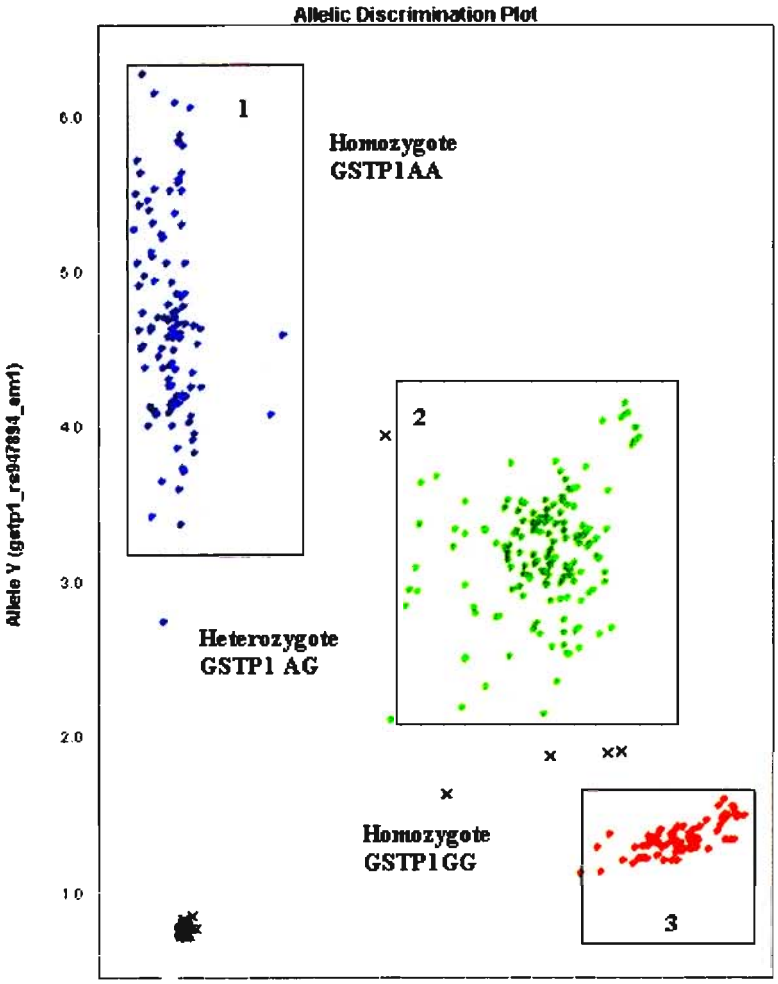


Figure 4.2: Allelic discrimination plot, GSTP1. ● 1= Allele X=homozygote GSTPGG (2/2), ● 2 =Both =heterozygote AG (1/2), ●3= Allele Y: homozygote AA(1/1), ■ NTC= non template control, X=Undetermined

A relatively low genotypic frequency of the GSTM1 null genotype was found (29%) in this South African sample, while a comparatively higher frequency of the polymorphic GSTP1AG/GG (65%) and NQO1CC/CT genotypes (36%) were found in this population. The wild type homozygotes GSTP1 AA and NQO1 CC were present at about 35% and 64% while the GSTP1 GG (valine) and NQO1 TT (serine) frequencies were 19.1% and 4.3% respectively. Frequencies for each gene achieved Hardy-Weinberg equilibrium. The

frequency was 0.29 for GSTM1null, the minor Val allele and the minor Ser allele frequencies were 0.42 and 0.24 respectively. In order to evaluate the frequencies and effects of gene-gene combinations on respiratory outcomes, we stratified our population into 12 possible gene-gene permutations and genotypic frequencies are shown in Table 4.2.

The Type A classrooms represents a random population based sample. In the Type A classrooms, polymorphic genotypes were present at the following frequencies: GSTM1 null (30%), GSTP1 AG/GG (65%) and NQO1 CT/TT (36%). The numbers for the White population was very low (n=20) so frequencies should be interpreted judiciously (Table 4.3). A varied distribution of the GSTP1 AG/GG genotype was evident among different race groups with the African and Coloured populations having the highest frequencies (78.6% and 69.0% respectively). The GSTM1null frequency varied among race groups with the lowest frequency (21%) recorded for the African population.

As expected, there were no significant differences in genotype frequencies by sex. However, there were significant differences among race groups. Africans had a lower odds of the GSTM1null genotype, but had a greater chance of carrying the polymorphic GSTP1AG/GG genotype (OR=2.02, CI 1.00-4.07). Indians showed a greater odds of carrying the NQO1CT/TT genotype (OR=3.19, CI 0.12-0.52) and a lower odds of carrying the GSTP1AG/GG genotype (OR=0.25, CI 0.12-0.52) (Table 4.3, Table 4.4).

Table 4.2 : Genotypic frequency of wild type and polymorphic alleles in Type A classrooms

Genotype	**Type A[4] classroom** n (%)
GSTM1	
GSTM positive	221 (69.7)
GSTM null	96 (30.4)
GSTP1	
Unrestricted: Ile-Ile (AA)	105 (35.6)
Ile-Val (AG)	137 (46.3)
Val-Val (GG)	54 (18.2)
Dominant: Ile-Val (AG)+Val-Val (GG)	191 (64.5)
NQO1	
Unrestricted: Pro (CC)	191 (64.3)
Pro/Ser (CT)	93 (31.3)
Ser (TT)	13 (4.2)
Dominant Pro/Ser (CT)/Ser (TT)	106 (35.7)
GSTM1 with NQO1[1]	
GSTM1 pos NQO1 CC	143 (48.2)
GSTM1pos NQO1 CT+TT	66 (22.2)
GSTM1 null NQO1 CC	48 (16.2)
GSTM1 null NQO1 CT+TT	40 (13.5)
GSTM1 with GSTP1[2]	
GSTM1 pos GSTP1 AA	72 (24.3)
GSTM1 pos GSTP1 AG + GG	134 (45.3)
GSTM1 null GSTP1 AA	33 (11.2)
GSTM1 null GSTP1 AG + GG	57 (19.3)
GSTP1 with NQO1[3]	
GSTP1 AA NQO1 CC	53 (18.8)
GSTP1 AA NQO1 CT+TT	43 (15.3)
GSTP1 AG+ GG NQO1 CC	126 (44.7)
GSTP1 AG + GG NQO1 CT+TT	60 (21.3)

1. NQO1 CC = Pro, NQO1 CT+TT = Pro/Ser + Ser/Ser, 22 undetermined samples
2. GSTP1 AA= Ile/Ile, GSTP1 AG + GG = Ile/Val + Val/Val, 24 undetermined samples
3. 22 undetermined samples
4.. Type A = population based random sample

Table 4.3: Genotype distribution stratified by race and sex (Type A)

GENOTYPE (n=317)		RACE (%)				SEX (%)	
		African (n=148)%	**Indian (n=82)%**	**Coloured (n=67)%**	**White (n=20)%**	**Male (n=131)%**	**Female (n=186)%**
GSTM1	Present	115(77.7)	51(62.2)	43(64.2)	12(60.0)	82(62.6)	139(74.7)
	Null	33(22.3)	31(37.8)	24(35.8)	8(40.0)	49(37.4)	47(25.3)
GSTP1	AA	29(21.2)	49(62.8)	20(31.2)	7(41.2)	47(38.8)	58(33.1)
	AG	73(53.3)	24(30.8)	30(46.9)	10(58.8)	44(36.7)	49(27.7)
	GG	35(25.6)	5(6.4)	14(21.9)	0 (0.0)	7(5.8)	6(3.4)
	AG/GG	108(78.8)	29(37.2)	44(68.8)	10(58.8)	74(61.2)	117(66.9)
NQO1	CC	104(73.8)	30(40.0)	43(68.3)	14(77.8)	69(57.5)	122(68.9)
	CT	33(23.4)	36(48.0)	20(31.8)	4(22.2)	53(43.8)	84(48.0)
	TT	4(2.8)	9(12.0)	0 (0.0)	0(0.0)	21(17.4)	33(19.9)
	CT/TT	37(26.2)	45(60.0)	20(31.8)	4(22.2)	51(42.5)	55(31.1)

4.2 Prevalence of asthma and related phenotypes

We were unable to obtain a full dataset because some subjects were unwilling to undertake a methacholine challenge test or did not wish to donate a blood sample. Loss of information occurred in a random manner, and was not concentrated in any of the subgroups. Of the 369 students, 98 (26.6%) did not submit to a methacholine challenge test. When we evaluated if these children differed by health status, the students with missing methacholine challenge data had the following health status: 66 (71.7%) had "any asthma", 18 (26.9%) had doctor diagnosed asthma, 40 (43.5%) had persistent asthma and 30 (38%) were atopic compared with children who took the methacholine test: 116(47.5%) had "any asthma", 28 (12.6%) had doctor diagnosed asthma, 64 (26.2%) had persistent asthma and 105 (43.4%) were atopic. Therefore subjects with preexisting respiratory symptoms may have been concerned about the implications of the methacholine challenge test and declined to participate. This loss of information may

have introduced bias into our statistical models for BHR, however this is most likely to be in the direction of the null.

Table 4.4: Effect of stratification of race on genotype and respiratory outcome

	African		Indian		White	
	OR	**CI**	**OR**	**CI**	**OR**	**CI**
Genotype						
GSTM1 null	0.51	0.27-0.98*	1.07	0.54-2.11	1.31	0.46-3.68
GSTP1 AG/GG	2.02	1.00-4.07*	0.25	0.12-0.52*	0.63	0.21-1.92
NQO1 CT/TT	0.79	0.41-1.54	3.19	1.57-6.48*	0.65	0.18-2.26
Respiratory outcome						
Any asthma	0.70	0.33-1.44	0.73	0.33-1.59	1.89	0.58-6.16
Persistent asthma	0.64	0.25-1.61	0.32	0.10-1.03	0.52	0.10-1.03
BHR	0.47	0.20-1.12	0.51	0.20-1.27	0.47	0.10-2.08
Atopy	1.14	0.51-2.53	3.31	1.41-7.82**	12.20	2.26-65.73**

*p-value < 0.05
**p-value <.005
Models adjusted for age and sex and region

There was a relatively large prevalence of "any asthma" which was based on a number of different symptoms such as cough, wheeze etc. This broad definition may account for the high prevalence of "asthma of any severity" among the population based sample (46.1%), with 20.4% reporting moderate to severe persistent asthma. However, doctor diagnosed asthma was comparatively low at 10.9%. Marked airway reactivity ($PC_{20} \leq 2$ mg/ml) was found in 10.3% of children from Type A classrooms, with an additional 7.0% of children with probable BHR and 10.7% with possible BHR (Table 4.4). Atopy, defined as at least 1 positive skin test to seven different allergens (house dust mite, cockroach, cat, dog, mould mix, *Cladosporium*, grass mix) was present in 40.4% of the children (Figure 4.3).

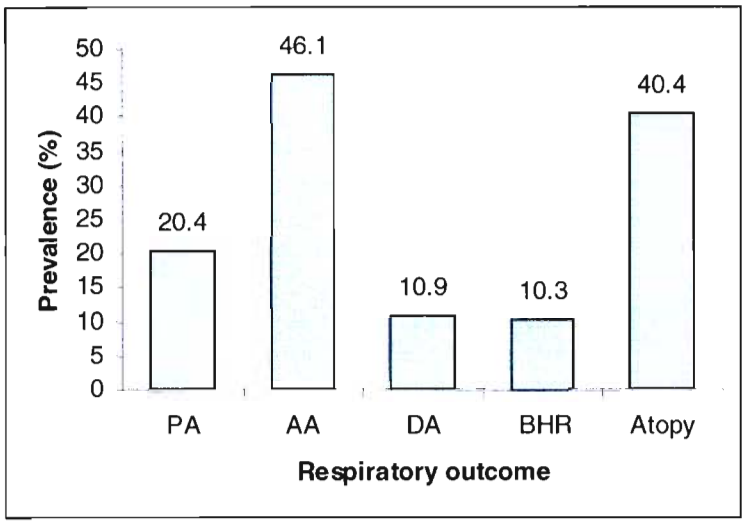


Figure 4.3 Prevalence of asthma and related phenotypes from Type A Classrooms. PA= persistent asthma (n=58), AA= any asthma (n=131), DA = doctor diagnosed asthma (n=27), marked BHR (n=28) and Atopy (n=109).

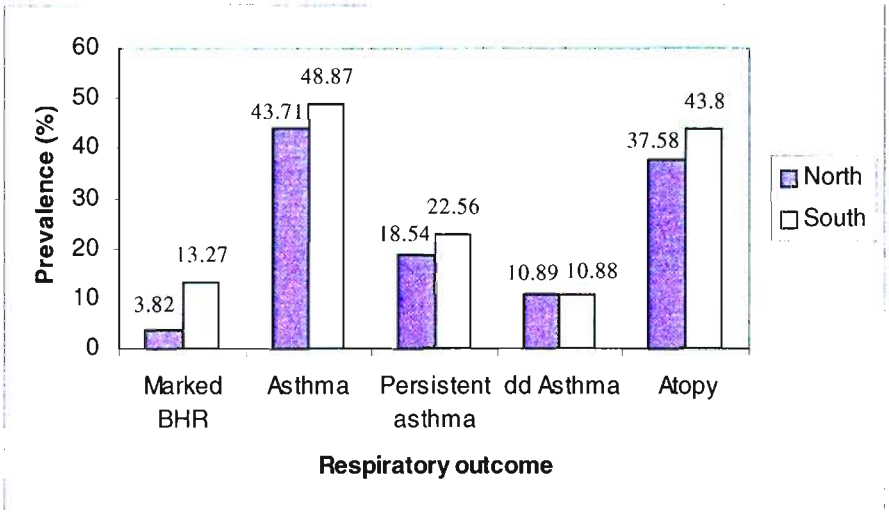
Table 4.5 Prevalence of Bronchial Hyperresponsiveness (BHR) among children from the Type A classroom.

Level of BHR	**n (%)**
Marked ($PC_{20} \leq 2$ mg/ml)	28 (10.3)
Probable ($2< PC_{20} \leq 8$ mg/ml)	19 (7.0)
Possible ($8< PC_{20} \leq 16$ mg/ml)	29 (10.7)
No evidence of airway reactivity ($PC_{20} > 16$ mg/ml)	195 (72.0)

PC_{20} = dose of methacholine causing a 20% fall in baseline FEV_1

In summary, among the population based random sample, prevalence of "any asthma" and persistent asthma, determined by symptoms, was high, with one-fifth reporting persistent asthma and slightly less than half the population reporting asthma of any severity. One tenth of our population reported doctor diagnosed asthma and displayed marked BHR from methacholine challenge tests. When respiratory outcome was stratified by race, only Indians and Whites showed a greater propensity to being atopic than Africans and Coloureds (Table 4.4 and Table 4.8). Although statistically significant,

the wide confidence interval for whites should be considered judiciously since our sample had a very low number of white students.



Asthma: "Any Asthma, dd asthma: doctor diagnosed asthma

Figure 4.4 Prevalence of respiratory outcomes (based on Type A classrooms) stratified by region (South Durban vs North Durban).

The prevalence of asthma of any grade and persistent asthma were both approximately 5% higher in the south while doctor diagnosed asthma was almost identical in both areas. The differences in respiratory health between north and south schools were highlighted by tests of airway responsiveness: a greater number of students in the south schools had marked BHR (13.3%) compared to those from the northern schools (3.8%). When we collapsed the categories of probable, possible and marked BHR as positive evidence of airway reactivity, we found 30.9% of children in the south presented with any evidence of airway reactivity compared to 20.6% of children in the north in the type A classrooms. More students from the south were atopic (43.8% vs 37.9%) (Figure 4.4)

4.3 Bivariate analysis of genotype and respiratory outcome

In this section we used the Pearsons chi squared test to evaluate whether a particular respiratory outcome was dependent on genotype. Table 4.6 shows GST and NQO1 frequencies in relation to persistent asthma (which included all persistent asthmatics from both the Type A and Type B classrooms), any asthma, doctor diagnosed asthma, atopy and BHR. Within the sample, 74.2% of all children with 'any asthma' have the GSTM1 pos while 27.5% of them have the null genotype. 26.9% of persistent asthmatics to have a GSTM1 deficiency. The frequency of GSTP1 AG/GG was significantly higher in subjects with persistent asthma (p= 0.03), and it was approximately 3 times higher than the AA genotype for children presenting with persistent asthma. No significant associations were identified for NQO1 and any of the respiratory outcomes. Using the unrestricted model in Table 4.7, in which the GSTP1GG homozygote was considered independent of the GSTP1 AG heterozygote, we found a significant association between persistent asthma (p<.005) and GSTP1, as well as between atopy and NQO1 (p = 0.05)

Table 4.6: Frequencies of GSTM1, GSTP1 and NQO1 genotypes stratified by respiratory outcomes.

GENOTYPE		ANY ASTHMA N=336		PERSISTENT ASTHMA N=336		DOCTOR DIAGNOSED ASTHMA N=228		BHR N=271		ATOPY N=321	
		Case	Non case	Case	Non case	Case	Non case	Case	Non case	Case	Non case
GSTM1	Present	135 (74.2)	106(68.8)	76 (73.1)	165(71.1)	32 (69.6)	171(70.8)	22 (78.6)	172(70.8)	92 (68.2)	134(72.0)
	Null	47 (25.8)	48 (31.2)	28 (26.9)	67(28.9)	14 (30.4)	71(29.2)	6 (21.4)	71(29.2)	43 (31.8)	52 (28)
p-value[1]		0.28		0.71		0.91		0.38		0.45	
GSTP1	AA	58 (34.3)	53(36.3)	26 (26.8)	85(39)	18 (42.9)	76 (33.6)	10 (38.5)	79(35)	48 (37.8)	54(30.9)
	AG/GG	111 (65.7)	93 (63.7)	71 (73.2)	133(61)	24 (57.1)	150(66.4)	16 (61.5)	147(65)	79 (62.2)	121(69.1)
p-value		0.71		**0.03**		0.25		0.72		0.21	
NQO1	CC	106 (61.3)	96(67.6)	67 (68.4)	135(62.2)	27 (61.4)	153(67.1)	21 (75.0)	143(63.3)	79 (59.8)	111(64.5)
	CT/TT	67 (38.7)	46(32.4)	31 (31.6)	75(32.9)	17 (38.6)	75(32.9)	7 (25.0)	83(36.7)	53 (40.2)	61(35.5)
p-value		0.24		0.29		0.46		0.21		0.40	

[1]p value from Pearsons chi squared test

Table 4.7: Frequencies of GSTM1, GSTP1 and NQO1 genotypes stratified by respiratory outcomes.

GENOTYPE		ANY ASTHMA N=315		PERSISTENT ASTHMA N=315		DOCTOR DIAGNOSED ASTHMA N=268		BHR N=252		ATOPY N=307	
		Case	Non case	Case	Non case	Case	Non case	Case	Non case	Case	Non case
GSTP1	AA	58 (34.3)	53(36.3)	26 (26.8)	85(39)	18 (42.9)	76(33.6)	10 (38.5)	79(35.0)	79 (59.9)	54(30.9)
	AG	77 (45.6)	68(46.9)	43 (44.3)	102(46.8)	16 (38.1)	107(47.3)	11 (42.3)	105(46.5)	47 (35.6)	78(44.6)
	GG	34 (20.1)	25(17.1)	28 (28.9)	31(14.2)	8 (19.1)	43(19.0)	5 (19.2)	42(18.6)	6 (4.6)	43(24.6)
p-value		0.78		**P<.005**		0.47		0.92		0.05	
NQO1	CC	106 (61.3)	96(65.8)	67 (68.4)	135(62.2)	27 (61.4)	153(67.1)	21 (75.0)	143(63.30	48 (37.8)	111(64.5)
	CT	62 (35.8)	39(27.5)	28 (28.6)	73(33.6)	16 (38.1)	67(29.40	7 (25.0)	73(32.3)	62 (48.8)	53(30.8)
	TT	5 (2.9)	7(4.9)	3 (3.0)	9(4.1)	8 (19.1)	8(3.5)	0	10(4.4)	17 (13.4)	8(4.7)
p-value		0.218		0.56		0.62		0.33		0.68	

[1]p value from Pearsons chi squared test

Table 4.8: Frequencies of GSTM1, GSTP1 and NQO1 genotypes stratified by respiratory outcome (case), race and region (north and south)

Race	Genotype	Any asthma N=131 (%)		Persistent asthma N=58(%)		Doctor Diagnosed asthma n=27(%)		BHR N=62 (%)		Atopy N=109 (%)	
		North	South	North	South	North	South	North	South	North	South
African	GSTM1pos	22(75.9)	18(78.3)	8(66.7)	12 (80.0)	3(75.0)	4(100)	11(100)	9(64.3)	19(79.2)	11(73.3)
	GSTM1null	7(24.1)	5(21.7)	4(33.3)	3(20.0)	1(25.0)	0	0	5(35.7)	5(20.8)	4(26.7)
Indian	GSTM1pos	12(70.6)	11(84.6)	1(33.3)	3(100)	4(100.0)	3(100)	4(66.6)	9(90.0)	9(42.9)	15(68.2)
	GSTM1null	5(29.4)	2(15.4)	2(66.6)	0	0	0	2(33.3)	1(10.0)	12(57.1)	7(31.8)
Coloured	GSTM1pos	11(55.0)	15(88.2)	5(38.4)	10(100)	4(50.0)	0	8(80.0)	6(54.5)	6(54.5)	5(83.3)
	GSTM1null	9(45.0)	2(11.8)	8(61.6)	0	4(50.0)	1(100)	2(20.0)	0	5(45.5)	1(16.7)
White	GSTM1pos	0	6(50.0)	0	1(50)	0	2(66.7)	19(79.2)	3(60.0)	0	6(60.0)
	GSTM1null	0	6(50.0)	0	1(50)	0	1(33.3)	5(20.8)	2(40.0)	0	4(40.0)
African	GSTP1AA	4(16.0)	5(23.8)	1(10.0)	4(30.7)	0	1(25.0)	3(33.3)	2(15.4)	9(40.9)	3(23.1)
	GSTP1 AG/GG	21(84.0)	16(76.2)	9(90.0)	9(69.2)	3(100)	3(75.0)	6(66.7)	11(84.6)	13(59.1)	10(76.9)
Indian	GSTP1AA	10(58.8)	7(53.8)	1(33.3)	1(33.3)	3(75.0)	2(66.7)	3(60.0)	7(70.0)	14(66.7)	11(52.4)
	GSTP1 AG/GG	7(41.2)	6(46.2)	2(66.7)	2(66.7)	1(25.0)	1(33.3)	2(40.0)	3(30.0)	7(33.3)	10(47.6)
Coloured	GSTP1AA	4(20.0)	7(46.7)	1(7.69)	4(40.0)	2(25.0)	0	1(10.0)	1(20.0)	1(9.1)	0
	GSTP1 AG/GG	16(80.0)	8(53.3)	12(92.3)	6(60.0)	6(75.0)	0	9(90.0)	4(80.0)	10(90.9)	6(100)
White	GSTP1AA	0	5(50.0)	0	1(100)	0	1(50.0)	0	3(60.0)	0	3(33.3)
	GSTP1 AG/GG	0	5(50.0)	0	0	0	1(50.0)	0	2(40.0)	0	6(66.7)
African	NQO1 CC	21(75.0)	15(71.4)	11(100)	11(84.6)	2(50)	3(75.0)	7(63.6)	2(15.4)	19(79.2)	11(78.6)
	NQO1 CT/TT	7(25.0)	6(28.6)	0	2(15.4)	2(50)	1(25.0)	4(36.4)	11(84.6)	5(20.8)	3(21.4)
Indian	NQO1 CC	3(18.8)	7(53.9)	1(33.3)	3(10.0)	2(50)	2(66.7)	3(60.0)	7(70.0)	6(31.6)	9(40.9)
	NQO1 CT/TT	13(81.2)	6(46.1)	2(66.7)	0	2(50)	1(33.3)	2(40.0)	3(30.0)	13(68.4)	13(59.1)
Coloured	NQO1 CC	10(50.0)	10(66.7)	6(46.2)	7(70.0)	3(37.5)	13(76.5)	6(60.0)	1(20.0)	7(63.6)	5(83.3)
	NQO1 CT/TT	10(50.0)	5(33.3)	7(53.8)	3(30.0)	5(62.5)	4(23.5)	4(40.0)	4(80.0)	4(36.4)	1(16.7)
White	NQO1 CC	0	9(81.8)	0	1(100)	0	1(50.0)	0	3(60.0)	0	8(80.0)
	NQO1 CT/TT	0	2(18.2)	0	0	0	1(50.0)	0	2(40.0)	0	2(20.0)

Table 4.8 depicts the frequency distribution of genotype versus respiratory outcome which is stratified by both race and region (North and South). It was not feasible to perform any statistical comparisons since the number of subjects was radically decreased in each 2 x 2 table. Although population stratification is an important issue in genetic epidemiology, our low numbers preclude the option of stratifying by race in the subsequent logistic analysis. Table 4.9 shows the distribution of combination gene-gene frequencies according to different respiratory outcomes. Generally, the genotype combinations considered "at risk", i.e. GSTM1 null + NQO1 CT/TT; GSTM1null GSTP1 AG/GG; and GSTP1 AG/ GG + NQO1 CT/TT were more frequent among the children presenting with any asthma and atopy.

Table 4.9: Frequencies of GSTM1, GSTP1 and NQO1 gene-gene combinations among participants presenting with respiratory linked outcomes

GENOTYPE INTERACTION		ANY ASTHMA	PERSISTENT ASTHMA	DOCTOR DIAGNOSED ASTHMA	BHR	ATOPY
GSTM1 pos	NQO1 CC	81 (46.8)	49 (50.0)	19 (43.2)	16 (57.1)	53 (40.2)
GSTM1pos	NQO1 CT+TT	47 (27.2)	22 (22.5)	12 (27.3)	6 (21.4)	38 (28.8)
GSTM1 null	NQO1 CC	25 (14.5)	18 (18.4)	8 (18.2)	5 (17.9)	26 (19.7)
GSTM1 null	NQO1 CT+TT	20 (11.5)	9 (9.2)	5 (11.4)	1 (3.6)	15 (11.4)
GSTM1 pos	GSTP1 AA	40 (23.7)	15 (15.2)	13 (31.0)	8 (30.8)	29(22.8)
GSTM1 pos	GSTP1 AG + GG	84 (49.7)	54 (55.7)	16 (38.1)	12 (46.2)	56 (44.1)
GSTM1 null	GSTP1 AA	18 (10.7)	11 (11.3)	5 (11.9)	2 (7.7)	19 (15.0)
GSTM1 null	GSTP1 AG + GG	27 (16.0)	17 (17.5)	8 (19.1)	4 (15.4)	23 (18.1)
GSTP1 AA	NQO1 CC	32 (19.6)	16 (17.4)	10 (23.8)	8 (30.8)	23 (18.6)
GSTP1 AA	NQO1 CT+TT	23 (14.1)	8 (8.7)	8 (19.1)	2 (7.7)	22 (17.8)
GSTP1 AG+ GG	NQO1 CC	66 (40.5)	46 (50.0)	16 (38.1)	11 (42.3)	51 (41.1)
GSTP1 AG + GG	NQO1 CT+TT	42 (25.8)	22 (23.9)	8 (19.1)	5 (19.2)	28 (22.6)

4.4. Multiple logistic regression models

The random based population sample (Type A) was included in the regression models to examine predictors for adverse health outcomes. Associations of genetic variables with respiratory outcomes of interest (atopy, airway hyperresponsiveness and asthma) were examined using multivariate logistic regression models. Stepwise regression showed that race and region (north and south Durban) were significant covariates in the logistic models, and based on literature, it was decided *a priori* to include other covariates such as age and sex. Tests for genotype*race interactions yielded no significant results. Multiple logistic models using each of respiratory outcomes as the dependent variable and region (north or south schools) as the independent variable showed that children in the south schools were more likely to present with marked BHR than those in the north (adj OR= 3.5, CI: 1.4-8.4, p= p<.005) (not shown in tables). They also had a 2-fold greater risk of having persistent asthma (adj OR=2.0; CI: 1.2-3.2; p<.005) compared to children in the north.

Subjects with the GSTP1 AG/GG genotype were significantly associated with persistent asthma (unadj OR=1.7; CI:1.0-2.9; p=0.03), however after adjustment for age, sex, race and region, this association was not significant (OR=1.6, CI: 0.9-2.9 (Table 4.10). Individuals with both GSTM1pos and the GSTP1 AG/GG genotypes were more likely to have persistent asthma (adj OR=2.4, CI 1.2-4.9, p=0.01). Additionally, GSTP1 GG was significantly associated with persistent asthma (adj OR = 2.7; CI=1.3-5.8, p<.005). Neither the GSTM1 nor the NQO1 genotypes were significant predictors of persistent

asthma. Neither ETS nor caregiver smoking modified the respiratory effects of genotype (results not shown). With respect to atopy and BHR, no significant associations were detected with any of the three genotypes examined.

Most of the 12 genotype combinations showed decreased risk for outcomes such as any asthma, doctor diagnosed asthma, marked BHR and atopy, which may be attributed to a competitive effect of one genotype on the other. When these logistic models were stratified by north and south regions, generally, the odds ratios decreased slightly while confidence intervals increased, which may be attributed to the decreased sample size in each strata.

Table 4.10: Association of genotypes with "any asthma", persistent asthma and doctor diagnosed ddasthma

Genotypes	ANY ASTHMA N=172			PERSISTENT ASTHMA N=104			DOCTOR DIAGNOSED ASTHMA N=46		
	Adj OR	**95%CI**	**P-value**	**Adj OR**	**95%CI**	**P-value**	**Adj OR**	**95%CI**	**P-value**
GSTM1									
Present	1.00			1.00			1.00		
Null	0.75	0.45-1.23	0.26	1.06	0.61-1.86	0.82	0.92	0.45-1.88	0.83
GSTP1									
AA	1.00			1.00			1.00		
AG/GG	1.08	0.64-1.81	0.76	1.65	0.92-2.97	0.09	0.77	0.37-1.60	0.49
GG	1.27	0.63-2.57	0.49	**2.74**	**1.29-5.84**	**<.005**	1.05	0.38-2.87	0.93
NQO1									
CC	1.00			1.00			1.00		
CT/TT	1.39	0.84-2.28	0.19	0.83	0.48-1.43	0.49	1.12	0.56-2.27	0.74
TT	0.59	0.16-2.07	0.14	0.69	0.16-1.48	0.56	0.49	0.59-2.48	0.59
GSTM1 with NQO1									
GSTM1 pos NQO1CC	1.00			1.00			1.00		
GSTM1 pos NQO1CT/TT	1.55	0.86-2.81	0.13	0.99	0.52-1.89	0.99	1.18	0.52-2.71	0.68
GSTM1 null NQO1CC	0.89	0.46-1.73	0.73	1.47	0.72-3.04	0.28	1.02	0.40-2.56	0.97
GSTM1 null NQO1CT/TT	0.97	0.46-2.11	0.95	0.74	0.31-1.81	0.51	1.00	0.32-3.16	0.99
GSTM1 with GSTP1									
GSTM1 pos GSTP1 AA	1.00			1.00			1.00		
GSTM1 pos GSTP1 AG/GG	1.22	0.66-2.23	0.52	**2.40**	**1.16-4.95**	**0.01**	0.72	0.30-1.69	0.45
GSTM1 null GSTP1 AA	0.96	0.42-2.18	0.92	2.57	0.96-6.91	0.06	0.82	0.25-2.62	0.73
GSTM1 null GSTP1 AG/GG	0.79	0.38-1.65	0.52	1.89	0.79-4.50	0.15	0.75	0.27-2.08	0.59
GSTP1 with NQO1									
GSTP1 AA NQO1 CC	1.00			1.00			1.00		
GSTP1 AA NQO1 CT/TT	1.07	0.46-2.47	0.87	0.74	0.26-2.07	0.57	1.38	0.46-4.15	0.56
GSTP1 AA/AG NQO1 CC	0.93	0.48-1.79	0.83	1.67	0.79-3.52	0.17	0.81	0.32-2.05	0.66
GSTP1 AA/AG NQO1 CT/TT	1.43	0.68-2.98	0.34	1.39	0.61-3.15	0.43	0.72	0.25-2.06	0.54

Logistic regression models adjusted for age, sex, race and region (north and south).

Table 4.11: Association of genotypes with BHR and atopy

Genotypes	BHR N=28			ATOPY N=135		
	Adj OR	**95%CI**	**P-value**	**Adj OR**	**95%CI**	**P-value**
GSTM1						
Present	1.00			1.00		
Null	0.69	0.26-1.83	0.47	0.98	0.58-1.67	0.96
GSTP1						
AA	1.00			1.00		
AG/GG	0.81	0.33-1.98	0.65	1.34	0.74-2.39	0.33
GG	0.93	0.29-1.99	0.59	0.94	0.43-2.04	0.88
NQO1						
CC	1.00			1.00		
CT/TT	0.64	0.25-1.59	0.34	0.95	0.56-1.59	0.85
TT	0.76	0.31-1.92	0.56	0.44	0.12-1.48	0.19
GSTM1 with NQO1						
GSTM1 pos NQO1CC	1.00			1.00		
GSTM1 pos NQO1CT/TT	0.79	0.29-2.17	0.65	1.27	0.69-2.35	0.43
GSTM1 null NQO1CC	0.89	0.30-2.64	0.84	1.37	0.69-2.71	0.37
GSTM1 null NQO1CT/TT	0.26	0.03-2.11	0.21	0.65	0.28-1.50	0.31
GSTM1 with GSTP1						
GSTM1 pos GSTP1 AA	1.00			1.00		
GSTM1 pos GSTP1 AA/AG	0.75	0.27-2.03	0.57	1.32	0.67-2.63	0.43
GSTM1 null GSTP1 AA	0.59	0.11-3.05	0.53	0.98	0.41-2.34	0.96
GSTM1 null GSTP1 AA/GG	0.66	0.18-2.41	0.53	1.34	0.59-3.03	0.48
GSTP1 with NQO1						
GSTP1 AA NQO1 CC	1.00			1.00		
GSTP1 AA NQO1 CT/TT	0.36	0.07-1.86	0.22	0.87	0.35-2.14	0.76
GSTP1 AA/AG NQO1 CC	0.57	0.20-1.59	0.28	1.30	0.61-2.79	0.49
GSTP1 AA/AG NQO1 CT/TT	0.59	0.17-1.99	0.39	1.15	0.49-2.65	0.74

Logistic regression models adjusted for age, sex, race and region (north and south)

4.5. Regression Models of ambient exposure and lung function measures using generalised estimating equations

The gene-environment-interaction was assessed by including a product term (genotype X pollutant) in the linear regression models. These models assessed the relationship between SO_2, NO, NO_2 and PM_{10} exposure and changes in lung function tests (FEV_1 and PF) using GSTM1, GSTP1 and NQO1 genotypes as effect modifiers (Tables 4.12 to 4.29). In these models, the estimate is the expected change in lung function associated with an increase in one interquartile range in ambient pollutant. There were a few significant gene*environment interactions with GSTP1 and NQO1 variants and selected pollutants ($p_{int} < 0.05$).

The SDHS produced substantial repeated measures of PFTs on each child i.e each child provided 4 PFTs during a single school day, this was done every day for three consecutive weeks, once every season. Simultaneously, daily and hourly measurements of the criteria pollutants mentioned above were collected continuously for the duration of the study. The latter data was made available for the purposes of this study. The estimates for FEV_1 and PF variability increased with increasing pollutant exposure in GSTM1null children (Table 4.12). This was evident for NO and NO_2 at lag 5, while increased estimates for GSTM1null subjects were observed at all lags 1-5 for intraday variability in PF. The corresponding pollutant effect in GSTM1 positive children was lower than the GSTM1null estimate and not statistically significant. There was a single statistically significant effect for SO_2, while both FEV_1 and PF variability increased for PM_{10} exposure at all lags (Table 4.13). No significant interactions were observed between

variants of GSTM1 and exposure to any of the pollutants tested using the four lung function tests.

Evaluation of the gene-environment interaction with the GSTP1 genotype revealed that the pollutant slope for children with the GSTP1 AA genotype was often higher (i.e FEV_1 and PF variability increased with increased NO and NO2 pollutant levels) than the slope for the polymorphic GSTP1 AG/GG genotype. Among the children with the GSTP1AA genotype, statistically significant effect modification was observed for NO and NO_2 at lags 4 and 5 ($p<0.05$) (Table 4.16). There was no effect modification with GSTP1 and SO_2 or PM_{10} except for lag 4 where FEV_1 variability among GSTP1AA subjects exposed to SO_2 was greater than GSTP1 AG/GG children ($p<.005$) (Table 4.17). There was no significant effect modification with either NO or NO_2 and the GSTP1 genotype (Table 4.18) using nadir FEV_1 and PF as lung function outcomes. However, an increase in SO_2 exposure produced significant interactions with GSTP1AA ($p_{int} < 0.05$) using nadir PF as an outcome at lags 4, 5 and the 5 day average. (Table 4.19). An increase in the estimate or slope of the GSTP1 AG/GG genotype showed an improved nadir lung function measure with increased SO_2 and PM_{10} exposure. GSTP1AG/GG was therefore protective compared to the GSTP1AA genotype.

There was significant effect modification with NQO1, NO_2 and NO for intraday variability in FEV_1 and PF across lags 1-5 including the 5 day average (Table 4.20). The estimates imply that FEV_1 and PF variability increases with increased exposure to NO and NO_2 in subjects with the NQO1CC wildtype. These effects were significant ($p<0.05$)

while the pollutant*NQO1CT/TT interaction effect was not. We found a significant gene*environment interaction for NQO1 and exposure to NO and NO_2, both at lag 2 using the two lung function outcomes, intraday variability in PF and nadir PF as outcome measures. With increased exposure to PM_{10} children with the NQO1CC genotype had significant effect modification with intraday variability in PF (Table 4.21). There were significant gene*environment interactions for NQO1 CC and increased exposure to PM10 at lags 1,3,4 and the 5 day average with intraday PF. Similarly decrements in nadir PF values among children with the NQO1 CC genotype were statistically significant for NO and NO_2 (Table 4.22) Conversely Table 4.23 showed a slight increase in the estimate for NQO1 CC but these effects, even though significant, were very small compared to the NQO1 CT/TT genotype.

After examining the main effects of each SNP, our goal was to examine potential interactions between these SNPs. We also assessed the association of the four pollutants (NO, NO_2, SO_2 and PM_{10}) and combined genotypes as effect modifiers with intraday variability in FEV_1 and PF (Tables 24-29). The 5 day averages for pollutant exposure were used for the GEE models. There was little significant modification of the effect on intraday variability by any of the 12 genotype combinations tested with pollutant exposure. Only 3 of the 12 gene-gene combinations showed a trend. Children with the GSTM1null GSTP1AG/GG genotype combination showed increased variability in PF for NO and NO_2, and for nadir FEV_1 for PM_{10}. Those children who had the GSTP1AG/GG, NQO1CC and GSTM1pos NQO1CC genotype combinations showed a significant effect modification of lung function measures with increased exposure to NO, NO_2 and PM_{10}.

Table 4.12 Gene -environment interactions with the GSTM1 genotype. Percent change[1] in intraday variability[2] of FEV_1 and peak flow (PF) associated with ambient levels[3] of NO_2, and NO from single pollutant linear regression models using generalized estimating equations (GEE).

Lung Function Outcome	Genotype	Lags	NO_2			NO		
			EST	P-Val	CI	EST	P-Val	CI
Intraday FEV_1	GSTM1 positive	Lag 1	0.39	0.06[5]	-0.05, 0.78	**0.84**	**<.005**	**0.42, 1.25**
	GSTM1 null		0.35	0.24[5]	-0.24, 0.94	0.52	0.09	-0.09, 1.14
				0.91[4]	-0.74,0.66		0.41	-1.04,0.42
	GSTM1 positive	Lag 2	0.27	0.15	-0.11, 0.66	0.28	0.21	-0.16, 0.73
	GSTM1 null		0.24	0.39	-0.31, 0.79	0.00	0.99	-0.68, 0.68
				0.91	-0.71,0.62		0.49	-1.10,0.52
	GSTM1 positive	Lag 3	0.13	0.48	-0.22, 0.47	0.41	0.05	-0.07, 0.82
	GSTM1 null		0.18	0.45	-0.29, 0.67	0.04	0.40	-0.54, 0.63
				0.84	-0.53,0.66		0.32	-1.08,0.35
	GSTM1 positive	Lag 4	0.33	0.07	-0.03, 0.68	0.14	0.69	-0.56, 0.84
	GSTM1 null		0.43	0.08	-0.06, 0.92	0.44	0.13	-0.12, 1.00
				0.73	-0.50,0.72		0.69	-0.56,0.85
	GSTM1 positive	Lag 5	0.34	0.08	-0.04, 0.72	**0.68**	**<.005**	**0.25, 1.10**
	GSTM1 null		**0.68**	**0.02**	**0.12, 1.24**	**0.79**	**0.02**	**0.15, 1.44**
				0.32	-0.33,1.01		0.76	-0.65,0.88
	GSTM1 positive	5 days	0.31	0.12	-0.09, 0.71	**0.55**	**0.02**	**0.09, 1.01**
	GSTM1 null	average	0.36	0.21	-0.21, 0.92	0.41	0.20	-0.22, 1.05
				0.89	-0.64,0.73		0.72	-0.92,0.64
Intraday PF	GSTM1 positive	Lag 1	**0.33**	**0.09**	**-0.05, 0.71**	**0.71**	**<.005**	**0.30, 1.11**
	GSTM1 null		**0.69**	**0.02**	**0.11, 1.27**	**0.72**	**0.02**	**0.13, 1.31**
				0.33	-0.34,1.02		0.97	-0.69,0.72
	GSTM1 positive	Lag 2	0.19	0.31	-0.18, 0.57	0.25	0.27	-0.19, 0.71
	GSTM1 null		**0.83**	**<.005**	**0.28, 1.37**	**0.99**	**<.005**	**0.26, 1.73**
				0.06	-0.01,1.29		0.09	-0.12,1.59
	GSTM1 positive	Lag 3	0.17	0.31	-0.16, 0.51	0.27	0.18	-0.13, 0.67
	GSTM1 null		**0.69**	**<.005**	**0.22, 1.18**	**0.77**	**0.02**	**0.13, 1.41**
				0.07	-0.05,1.10		0.19	-0.25,1.24
	GSTM1 positive	Lag 4	**0.41**	**0.03**	**0.05, 0.77**	0.29	0.20	-0.15, 0.72
	GSTM1 null		**0.72**	**<.005**	**0.20, 1.23**	**0.73**	**0.02**	**0.11, 1.35**
				0.33	-0.32,0.93		0.24	-0.30,1.19
	GSTM1 positive	Lag 5	**0.43**	**0.04**	**0.03, 0.82**	**0.53**	**0.02**	**0.07, 0.97**
	GSTM1 null		**0.76**	**<.005**	**0.19, 1.33**	**0.85**	**0.01**	**0.17, 0.53**
				0.34	-0.34,1.02		0.42	-0.47,1.12
	GSTM1 positive	5 days	0.33	0.10	-0.06, 0.72	**0.53**	**0.02**	**0.08, 0.98**
	GSTM1 null	average	**0.80**	**<.005**	**0.24, 1.37**	**0.95**	**<.005**	**0.28, 1.62** --
				0.16	-0.06,0.72		0.30	-0.37,1.21

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: NO_2: 8.19 ppb and NO: 29.7 ppb.

[2] 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 (08h00, 09h45, 11h30, 13h20).
Intraday variability for PF is defined analogously to within-day variability for FEV_1.

[3] pollution levels used in regression models combine measured and imputed values
Covariates in each model: race, school, caregiver smokes, asthma severity, interactions between asthma severity and exposure, interaction between gene and pollutants

[4] Interaction p-value (gene-pollutant interaction)

[5] p-value for the change in estimate
An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

Table 4.13 Gene-environment interactions with the GSTM1 genotype. Percent change[1] in intraday variability[2] of FEV_1 and peak flow (PF) associated with ambient levels[3] of SO_2 and PM_{10} from single pollutant linear regression models using generalized estimating equations (GEE)

Lung Function Outcome	Genotype	Lags	SO_2			PM_{10}		
			EST	**P-Val**	**CI**	**EST**	**P-Val**	**CI**
Intraday FEV_1	GSTM1 positive	Lag 1	**0.38**	**0.05**[5]	**0.00, 0.76**	-0.07	0.65	-0.40,0.23
	GSTM1 null		0.32	0.28[5]	-0.26, 0.89	-0.16	0.54	-0.77,0.41
				0.85[4]	-0.72,0.59		0.75	-0.78,0.56
	GSTM1 positive	Lag 2	-0.11	0.56	-0.48, 0.26	-0.00	0.94	-0.2,0.27
	GSTM1 null		-0.15	0.58	-0.67, 0.38	0.12	0.63	-0.37,0.62
				0.90	-0.66,0.59		0.65	-0.43,0.70
	GSTM1 positive	Lag 3	-0.05	0.76	-0.40, 0.29	-0.04	0.80	-0.32,0.25
	GSTM1 null		-0.34	-0.19	-0.86, 0.17	0.15	0.57	-0.35,0.65
				0.34	-0.88,0.31		0.53	-0.39,0.76
	GSTM1 positive	Lag 4	-0.11	0.54	-0.46, 0.24	0.04	0.78	-0.27,0.36
	GSTM1 null		0.18	0.41	-0.25, 0.62	0.32	0.26	-0.24,0.87
				0.28	-0.24,0.83		0.40	-0.36,0.91
	GSTM1 positive	Lag 5	0.11	0.49	-0.21, 0.43	0.23	0.48	-0.41,0.87
	GSTM1 null		**0.56**	**0.04**	**0.02, 1.11**	0.32	0.25	-0.23,0.87
				0.14	-0.21,0.43		0.49	-0.41,0.87
	GSTM1 positive	5 days	0.02	0.96	-0.74, 0.78	0.02	0.89	-0.29,0.34
	GSTM1 null	average	0.15	0.77	-0.82, 1.12	0.13	0.66	-0.44,0.70
				0.82	-0.97,1.22		0.75	-0.54,0.75
Intraday PF	GSTM1 positive	Lag 1	0.19	0.22	-0.12, 0.52	0.00	0.95	-0.29,0.31
	GSTM1 null		0.59	0.09	-0.09, 1.28	0.38	0.25	-0.26,1.03
				0.28	-0.33,1.12		0.31	-0.34,1.08
	GSTM1 positive	Lag 2	-0.17	0.34	-0.51-0.17	0.09	0.48	-0.18,0.38
	GSTM1 null		0.18	0.55	-0.41-0.77	**0.67**	**0.02**	**0.11,1.23**
				0.29	-0.29,0.99		0.07	-0.05,1.19
	GSTM1 positive	Lag 3	-0.16	0.35	-0.51-0.18	0.11	0.44	-0.16,0.39
	GSTM1 null		0.08	0.80	-0.57-0.74	**0.59**	**0.02**	**0.06,1.12**
				0.49	-0.45,0.95		0.12	-0.12,1.07
	GSTM1 positive	Lag 4	0.16	0.39	-0.21-0.01	0.25	0.13	-0.07,0.56
	GSTM1 null		0.29	0.34	-0.29-0.87	**0.74**	**0.01**	**0.15,1.33**
				0.71	-0.53,0.77		0.15	-0.17,1.16
	GSTM1 positive	Lag 5	0.08	0.63	-0.24-0.40	0.25	0.13	-0.07,0.58
	GSTM1 null		0.21	0.42	-0.31-0.73	**0.62**	**0.04**	**0.04,1.20**
				0.66	-0.45,0.72		0.28	-0.30,1.04
	GSTM1 positive	5 days	0.02	0.96	-0.69-0.73	0.15	0.31	-0.15,0.46
	GSTM1 null	average	0.81	0.20	-0.43-2.03	**0.64**	**0.04**	**0.03,1.25**
				0.23	-0.48,2.05		0.17	-0.20,1.16

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: PM_{10}: 29.4 ug m^{-3} and SO_2: 9.8 ppb.

[2] 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 (08h00, 09h45, 11h30, 13h20).
Intraday variability for PF is defined analogously to within-day variability for FEV_1.

[3] pollution levels used in regression models combine measured and imputed values

Covariates in each model: race, school, caregiver smokes, asthma severity, interactions between asthma severity and exposure, interaction between gene and pollutants

[4] Interaction p-value (gene-pollutant interaction)

[5] p-value for the change in estimate

An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

Table 4.14 Gene-environment interactions with the GSTM1 genotype. Percent change[1] in nadir[2] FEV_1 and PF associated with ambient levels[3] of NO_2, and NO from single pollutant linear regression models using generalized estimating equations (GEE).

Lung Function Outcome	Genotype	Lags	NO_2			NO		
			EST	P-Val	CI	EST	P-Val	CI
Nadir FEV_1	GSTM1 positive	Lag 1	0.00	0.92[5]	0.09,0.11	-0.00	0.72	-0.02,0.01
	GSTM1 null		0.01	0.22[5]	-0.01,0.03	0.00	0.55	-0.01,0.03
				0.42[4]	-0.01,0.04		0.49	-0.02,0.03
	GSTM1 positive	Lag 2	0.00	0.78	-0.01,0.02	0.00	0.76	-0.01,0.02
	GSTM1 null		0.01	0.21	-0.01,0.03	0.01	0.24	-0.00,0.03
				0.43	-0.01,0.03		0.43	-0.02,004
	GSTM1 positive	Lag 3	0.00	0.48	-0.01,0.02	0.00	0.68	-0.01,0.02
	GSTM1 null		0.01	0.11	-0.00,0.03	0.02	0.07	-0.00,0,03
				0.68	-0.02,0.03		0.24	-0.01,0.04
	GSTM1 positive	Lag 4	0.00	0.45	-0.01; 0.02	0.00	0.55	-0.01,0.02
	GSTM1 null		0.01	0.24	-0.01,0.03	0.01	0.23	-0.01,0.03
				0.64	-0.02,0.03		0.54	-0.02,0.03
	GSTM1 positive	Lag 5	0.00	0.66	-0.01,0.01	0.00	0.75	-0.01;0.01
	GSTM1 null		0.00	0.38	-0.01,0.27	0.01	0.32	-0.01, 0.03
				0.66	-0.02,0.03		0.52	-0.02,0.03
	GSTM1 positive	5 days	0.00	0.43	-0.01,0.02	0.00	0.70	-0.01;0.02
	GSTM1 null	average	0.01	0.15	-0.00,0.03	0.01	0.21	-0.00;0.03
				0.52	-0.02,0.03		0.44	-0.02,0.04
Nadir PF	GSTM1 positive	Lag 1	-1.75	0.12	-3.95,0.46	-1.73	0.11	-3.87, 0.42
	GSTM1 null		-1.96	0.27	-5.46,1.54	-2.81	0.13	-6.47, 0.86
				0.92	-4.35,3.92		0.62	-5.32,3.16
	GSTM1 positive	Lag 2	-1.46	0.17	-3.56,0.64	**-2.59**	**0.02**	**-4.75, -0.43**
	GSTM1 null		-1.82	0.28	-5.08,1.45	-2.46	0.18	-6.09, 1.17
				0.86	-4.23,3.53		0.95	-4.09,4.35
	GSTM1 positive	Lag 3	-1.46	0.17	-3.56,0.63	-1.93	0.06	-3.94, 0.08
	GSTM1 null		-1.54	0.31	-4.56,1.48	-2.05	0.26	-5.62, 1.52
				0.78	-4.02.3.04		0.96	-4.21,3.97
	GSTM1 positive	Lag 4	-0.84	0.41	-2.86, 1.17	-0.97	0.38	-3.13, 1.19
	GSTM1 null		-1.28	0.42	-4.39,1.83	-1.04	0.58	-4.71, 2.64
				0.82	-4.15,3.27		0.98	-4.32,4.19
	GSTM1 positive	Lag 5	-1.20	0.25	-3.25, 0.86	-0.78	0.48	-2.96, 1.40
	GSTM1 null		-2.20	0.22	-5.68, 1.28	-0.99	0.66	-5.36, 3.38
				0.63	-5.04,3.03		0.93	-5.08,4.67
	GSTM1 positive	5 days	-1.43	0.22	-3.71, 0.86	-1.97	0.12	-4.44, 0.49
	GSTM1 null	average	-1.86	0.31	-5.46, 1.74	-2.43	0.26	-6.65, 1.79
				0.84	-4.69, 3.83		0.86	-5.33,4.42

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: NO_2: 8.19 ppb and NO: 29.7 ppb.

[2] Nadir FEV1 is defined as the minimum of the (up to 4) best FEV1s on a given day, Nadir PF is defined analogously

[3] pollution levels used in regression models combine measured and imputed values

Covariates in each model: race, school, caregiver smokes, asthma severity, interactions between asthma severity and exposure, interaction between gene and pollutants

[4] Interaction p-value (gene-pollutant interaction)

[5] p-value for the change in estimate

An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

Table 4.15 Gene-environment interactions with the GSTM1 genotype. Percent change[1] in nadir[2] FEV_1 and PF associated with ambient levels[3] of SO_2, and PM_{10} from single pollutant linear regression models using generalized estimating equations (GEE).

Lung Function Outcome	Genotype	Lags	SO_2			PM_{10}		
			EST	P-Val	CI	EST	P-Val	CI
Nadir FEV_1	GSTM1 positive	Lag 1	0.00	0.74[5]	-0.07,0.11	**0.01**	**0.03**	**0.00,0.02**
	GSTM1 null		0.00	0.64[5]	-0.02,0.00	**0.02**	**0.03**	**0.00,0.03**
				0.70[4]	-0.02,0.01		0.62	-0.02,0.03
	GSTM1 positive	Lag 2	0.00	0.19	-0.00,0.01	**0.01**	**0.01**	**0.00,0.02**
	GSTM1 null		0.00	0.19	-0.00,0.02	**0.02**	**0.01**	**0.00,0.03**
				0.82	-0.02,0.02		0.69	-0.01,0.02
	GSTM1 positive	Lag 3	**0.01**	**0.04**	**0.00,0.02**	**0.01**	**0.02**	**0.00,0.02**
	GSTM1 null		**0.01**	**0.03**	**0.00,0.03**	**0.02**	**0.02**	**0.00,0.03**
				0.53	-0.01, 0.02		0.68	-0.02,0.02
	GSTM1 positive	Lag 4	0.01	0.06	-0.00,0.02	0.01	0.07	0.00,0.02
	GSTM1 null		0.00	0.42	-0.00,0.02	0.01	0.06	0.00,0.03
				0.50	-0.02,0.01		0.70	-0.02,0.02
	GSTM1 positive	Lag 5	0.00	0.67	-0.00,0.01	0.01	0.20	0.00,0.02
	GSTM1 null		0.00	0.42	-0.02,0.00	0.01	0.07	0.00,0.03
				0.38	-0.02,0.01		0.55	-0.01,0.03
	GSTM1 positive	5 days	0.01	0.17	-0.00,0.04	**0.01**	**0.05**	**0.00,0.03**
	GSTM1 null	average	0.01	0.36	-0.01,0.04	**0.02**	**0.03**	**0.00,0.03**
				0.86	-0.05,0.04		0.61	-0.02,0.03
Nadir PF	GSTM1 positive	Lag 1	-0.52	0.54	-2.18, 1.14	-0.08	0.93	-1.86, 1.70
	GSTM1 null		-1.25	0.58	-5.66, 3.16	-0.18	0.91	-3.29, 2.93
				0.76	-5.42,3.97		0.96	-3.68,3.48
	GSTM1 positive	Lag 2	0.58	0.51	-1.15, 2.30	-0.23	0.77	-1.80, 1.33
	GSTM1 null		0.86	0.66	-3.01, 4.74	-0.09	0.95	-2.80, 2.62
				0.89	-3.93,4.51		0.93	-2.98,3.27
	GSTM1 positive	Lag 3	0.91	0.26	-0.67, 2.49	-0.26	0.76	-1.95, 1.43
	GSTM1 null		1.46	0.53	-3.06, 5.98	-0.19	0.89	-2.99, 2.61
				0.82	-4.21,5.32		0.97	-3.19,3.34
	GSTM1 positive	Lag 4	0.41	0.67	-1.46, 2.27	-0.28	0.76	-2.07, 1.51
	GSTM1 null		0.79	0.73	-3.63, 5.20	-0.79	0.60	-3.76, 2.18
				0.88	-4.38,5.14		0.77	-3.98,2.96
	GSTM1 positive	Lag 5	-0.33	0.67	-1.85, 1.20	-0.95	0.29	-2.70, 0.80
	GSTM1 null		-0.07	0.97	-3.61, 3.48	-1.62	0.26	-4.44, 1.20
				0.89	-3.58,4.10		0.69	-3.99,2.64
	GSTM1 positive	5 days	0.74	0.71	-3.19, 4.67	-0.61	0.53	-2.51, 1.29
	GSTM1 null	average	0.95	0.87	-10.36, 12.3	-0.69	0.68	-3.93, 2.55
				0.97	-11.65, 12.1		0.97	-3.83,3.67

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: PM_{10}: 29.4 ug m^{-3} and SO_2: 9.8 ppb.

[2]Nadir FEV1 is defined as the minimum of the (up to 4) best FEV1s on a given day, Nadir PF is defined analogously

[3] pollution levels used in regression models combine measured and imputed values

Covariates in each model: race, school, caregiver smokes, asthma severity, interactions between asthma severity and exposure, interaction between gene and pollutants

[4]Interaction p-value (gene-pollutant interaction)

[5]p-value for the change in estimate

An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

Table 4.16 Gene-environment interactions with the GSTP1 genotype. Percent change[1] in intraday variability[2] of FEV_1 and peak flow (PF) associated with ambient levels[3] of NO_2, and NO from single pollutant linear regression models using generalized estimating equations (GEE).

Lung Function Outcome	Genotype	Lags	NO_2			NO		
			EST	P-Val	CI	EST	P-Val	CI
Intraday FEV1	GSTP1AA	Lag 1	**0.67**	**0.01**[5]	**0.14, 1.20**	**0.94**	**<.005**	**0.41, 1.47**
	GSTP1 AG/GG		0.26	0.26[5]	-0.19, 0.71	**0.62**	**0.01**	**0.15, 1.09**
				0.25[4]	-1.11,0.28		0.37	-1.03,0.38
	GSTP1AA	Lag 2	0.36	0.19	-0.18, 0.91	0.22	0.69	-0.42, 0.85
	GSTP1 AG/GG		0.27	0.21	-0.16, 0.69	0.11	0.50	-0.42, 0.64
				0.78	-0.78,0.59		0.80	-0.93,0.79
	GSTP1AA	Lag 3	0.29	0.23	-0.19, 0.76	0.45	0.10	-0.08, 0.99
	GSTP1 AG/GG		0.11	0.58	-0.27, 0.49	0.28	0.25	-0.19, 0.76
				0.56	-0.79,0.43		0.64	0.89,0.54
	GSTP1AA	Lag 4	**0.48**	**0.05**	**0.00, 0.97**	**0.67**	**0.02**	**0.10, 1.25**
	GSTP1 AG/GG		0.34	0.09	-0.05, 0.72	0.21	0.38	-0.25, 0.67
				0.64	-0.77,0.48		0.22	-1.20,0.27
	GSTP1AA	Lag 5	**0.54**	**0.03**	**0.05, 1.02**	**0.72**	**0.01**	**0.14, 1.30**
	GSTP1 AG/GG		**0.46**	**0.04**	**0.03, 0.88**	**0.76**	**<.005**	**0.27, 1.24**
				0.80	-0.73,0.56		0.93	-0.71,0.78
	GSTP1AA	5 days	0.51	0.06	-0.03, 1.05	**0.75**	**0.01**	**0.19, 1.30**
	GSTP1 AG/GG	average	0.27	0.24	-0.18, 0.71	0.38	0.16	-0.15, 0.92
				0.49	-0.94,0.45		0.35	-1.13,0.40
Intraday PF	GSTP1AA	Lag 1	0.37	0.15	-0.13, 0.86	0.47	0.10	-0.08, 1.02
	GSTP1 AG/GG		0.32	0.16	-0.13, 0.76	**0.71**	**<.005**	**0.25, 1.17**
				0.88	-0.71,0.61		0.50	-0.47,0.95
	GSTP1AA	Lag 2	0.25	0.32	-0.24, 0.74	0.21	0.52	-0.42, 0.83
	GSTP1 AG/GG		0.39	0.08	-0.04, 0.83	0.42	0.13	-0.12, 0.97
				0.67	-0.51,0.79		0.60	-0.61,1.04
	GSTP1AA	Lag 3	**0.45**	**0.05**	**0.00, 0.89**	0.37	0.17	-0.16, 0.91
	GSTP1 AG/GG		0.20	0.30	-0.18, 0.58	0.35	0.14	-0.11, 0.82
				0.41	-0.82,0.34		0.96	-0.72,0.69
	GSTP1AA	Lag 4	**0.65**	**0.01**	**0.13, 1.16**	**0.70**	**0.02**	**0.12, 1.28**
	GSTP1 AG/GG		0.35	0.09	-0.06, 0.76	0.17	0.51	-0.33, 0.67
				0.37	-0.94,0.35		0.17	-1.29,0.22
	GSTP1AA	Lag 5	**0.51**	**0.05**	**-0.01, 1.02**	**0.69**	**0.03**	**0.06, 1.32**
	GSTP1 AG/GG		0.44	0.06	-0.01, 0.88	0.44	0.09	-0.07, 0.94
				0.84	-0.74,0.60		0.53	-1.04,0.54
	GSTP1AA	5 days	0.49	0.06	-0.02, 1.00	0.57	0.06	-0.02, 1.16
	GSTP1 AG/GG	average	0.37	0.11	-0.09, 0.82	**0.54**	**0.05**	**0.01, 1.07**
				0.72	-0.79,0.55		0.93	-0.81,0.74

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: NO_2: 8.19 ppb and NO: 29.7 ppb.

[2] 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 (08h00, 09h45, 11h30, 13h20).
Intraday variability for PF is defined analogously to within-day variability for FEV_1.

[3] pollution levels used in regression models combine measured and imputed values

Covariates in each model: race, school, caregiver smokes, asthma severity, interactions between asthma severity and exposure, interaction between gene and pollutants

[4] Interaction p-value (gene-pollutant interaction)

[5] p-value for the change in estimate

An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

Table 4.17 Gene-environment interactions with the GSTP1 genotype. Percent change[1] in intraday variability[2] of FEV_1 and peak flow (PF) associated with ambient levels[3] of SO_2 and PM_{10} from single pollutant linear regression models using generalized estimating equations (GEE)

Lung Function Outcome	Genotype	Lags	SO_2			PM_{10}		
			EST	P-Val	CI	EST	P-Val	CI
Intraday FEV1	GSTP1AA	Lag 1	0.20	0.44[5]	-0.31, 0.72	0.08	0.77	-0.45, 0.62
	GSTP1 AG/GG		**0.51**	**0.02**[5]	**0.09, 0.92**	-0.09	0.65	-0.47, 0.29
				0.36[4]	-0.34,0.94		0.61	-0.82,0.48
	GSTP1AA	Lag 2	-0.23	0.31	-0.67, 0.22	0.27	0.23	-0.17, 0.70
	GSTP1 AG/GG		0.04	0.87	-0.41, 0.48	-0.03	0.85	-0.37, 0.30
				0.40	-0.35,0.88		0.28	-085,0.24
	GSTP1AA	Lag 3	-0.18	0.35	-0.55, 0.20	0.19	0.41	-0.26, 0.64
	GSTP1 AG/GG		-0.05	0.83	-0.50, 0.40	0.01	0.97	-0.33, 0.35
				0.66	-0.44,0.69		0.53	-0.74,0.38
	GSTP1AA	Lag 4	0.10	0.64	-0.31, 0.50	0.39	0.10	-0.07, 0.85
	GSTP1 AG/GG		0.02	0.94	-0.38, 0.41	0.10	0.61	-0.29, 0.49
				0.77	-0.62,0.46		0.35	-0.89,0.31
	GSTP1AA	Lag 5	0.18	0.39	-0.22, 0.57	0.26	0.27	-0.21, 0.73
	GSTP1 AG/GG		0.34	0.11	-0.08, 0.75	0.25	0.22	-0.15, 0.65
				0.57	-0.39,0.72		0.96	-0.63,0.60
	GSTP1AA	5 days	-0.06	0.90	-0.90, 0.79	0.28	0.26	-0.20, 0.75
	GSTP1 AG/GG	average	0.34	0.44	-0.52, 1.21	0.02	0.92	-0.37, 0.40
				0.47	-0.70,1.50		0.41	-0.87,1.35
Intraday PF	GSTP1AA	Lag 1	0.29	0.16	-0.12, 0.70	0.12	0.61	-0.36, 0.60
	GSTP1 AG/GG		0.28	0.21	-0.15, 0.71	0.06	0.77	-0.34, 0.45
				0.96	-0.57,0.54		0.84	-0.68,0.55
	GSTP1AA	Lag 2	-0.08	0.68	-0.46, 0.30	0.40	0.07	-0.03, 0.82
	GSTP1 AG/GG		-0.08	0.71	-0.54, 0.37	0.09	0.61	-0.26, 0.44
				0.99	-0.55,0.55		0.27	-0.85,0.24
	GSTP1AA	Lag 3	-0.24	0.29	-0.67, 0.20	0.27	0.24	-0.18, 0.73
	GSTP1 AG/GG		-0.08	0.74	-0.53, 0.38	0.17	0.32	-0.17,0.52
				0.60	-0.43,0.74		0.73	-0.67,0.47
	GSTP1AA	Lag 4	**0.50**	**0.00**	**0.16, 0.85**	0.36	0.13	-0.11, 0.83
	GSTP1 AG/GG		-0.03	0.91	-0.51, 0.45	0.35	0.09	-0.05,0.74
				0.06	-1.08,0.02		0.96	-0.62,0.59
	GSTP1AA	Lag 5	0.18	0.33	-0.18, 0.54	0.32	0.17	-0.13, 0.78
	GSTP1 AG/GG		0.05	0.81	-0.35, 0.45	0.31	0.14	-0.10, 0.72
				0.63	-0.64,0.39		0.97	-0.64,0.59
	GSTP1AA	5 days	0.31	0.39	-0.39, 1.00	0.22	0.34	-0.24, 0.68
	GSTP1 AG/GG	average	0.04	0.93	-0.90, 0.98	0.23	0.24	-0.16, 0.62
				0.60	-1.26,0.73		0.97	-0.58,0.60

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: PM_{10}: 29.4 ug m^{-3} and SO_2: 9.8 ppb.

[2] 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 (08h00, 09h45, 11h30, 13h20).
Intraday variability for PF is defined analogously to within-day variability for FEV_1.

[3] pollution levels used in regression models combine measured and imputed values
Covariates in each model: race, school, caregiver smokes, asthma severity, interactions between asthma severity and exposure, interaction between gene and pollutants

[4] Interaction p-value (gene-pollutant interaction)

[5] p-value for the change in estimate
An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

Table 4.18 Gene-environment interactions with the GSTP1 genotype. Percent change[1] in nadir[2] FEV_1 and PF associated with ambient levels[3] of NO_2, and NO from single pollutant linear regression models using generalized estimating equations (GEE).

Lung Function Outcome	Genotype	Lags	NO_2			NO		
			EST	P-Val	CI	EST	P-Val	CI
Nadir FEV1	GSTP1AA	Lag 1	-0.01	0.42[5]	-0.03, 0.01	-0.01	0.14	-0.03, 0.00
	GSTP1 AG/GG		0.01	0.27[5]	-0.01, 0.03	0.00	0.53	-0.01, 0.02
				0.19[4]	-0.01,0.04		0.12	-0.01,0.04
	GSTP1AA	Lag 2	0.00	0.78	-0.02, 0.02	-0.01	0.35	-0.03, 0.01
	GSTP1 AG/GG		0.01	0.16	0.00, 0.03	0.01	0.14	0.00, 0.03
				0.27	-0.01,0.04		0.10	-0.00,0.05
	GSTP1AA	Lag 3	0.00	0.94	-0.02, 0.02	-0.01	0.55	-0.02, 0.01
	GSTP1 AG/GG		0.01	0.06	0.00, 0.03	0.01	0.09	0.00, 0.03
				0.21	-0.01,0.04		0.12	-0.00,0.04
	GSTP1AA	Lag 4	-0.01	0.56	-0.02, 0.01	-0.01	0.39	-0.03, 0.01
	GSTP1 AG/GG		0.01	0.11	0.00, 0.03	0.01	0.11	0.00, 0.03
				0.14	-0.01,0.04		0.09	-0.00,0.04
	GSTP1AA	Lag 5	-0.01	0.55	-0.02, 0.01	-0.01	0.49	-0.03, 0.01
	GSTP1 AG/GG		0.01	0.28	-0.01, 0.02	0.01	0.24	-0.01, 0.03
				0.25	-0.01,0.04		0.20	-0.01,0.04
	GSTP1AA	5 days	0.00	0.68	-0.03, 0.02	-0.01	0.35	-0.03, 0.01
	GSTP1 AG/GG	average	0.01	0.12	0.00, 0.03	0.01	0.14	0.00, 0.03
				0.20	-0.01,0.04		0.09	-0.00,0.05
Nadir PF	GSTP1AA	Lag 1	-2.78	0.12	-6.30, 0.74	-3.08	0.09	-6.63, 0.47
	GSTP1 AG/GG		-0.93	0.46	-3.38, 1.51	-1.20	0.33	-3.58, 1.19
				0.40	-2.44,6.13		0.39	-2.39,6.16
	GSTP1AA	Lag 2	-2.51	0.13	-5.73, 0.70	-3.08	0.07	-6.36, 0.19
	GSTP1 AG/GG		-0.63	0.60	-3.01, 1.74	-1.36	0.29	-3.90, 1.17
				0.36	-2.12,5.88		0.42	-2.42,5.86
	GSTP1AA	Lag 3	-2.58	0.08	-5.48, 0.32	-2.84	0.08	-6.02, 0.35
	GSTP1 AG/GG		-0.16	0.88	-2.24, 1.92	-0.74	0.55	-3.15, 1.67
				0.18	-1.15,5.99		0.30	-1.89,6.09
	GSTP1AA	Lag 4	-2.45	0.12	-5.52, 0.62	-2.94	0.08	-6.26, 0.38
	GSTP1 AG/GG		0.00	1.00	-2.26, 2.25	0.42	0.74	-2.10, 2.94
				0.21	-1.36,6.25		0.11	-0.80,7.52
	GSTP1AA	Lag 5	-3.04	0.08	-6.41, 0.33	-3.42	0.08	-7.28, 0.43
	GSTP1 AG/GG		-0.42	0.72	-2.69, 1.85	0.97	0.46	-1.58, 3.51
				0.21	-1.44,6.68		0.06	-0.22, 9.01
	GSTP1AA	5 days	-2.79	0.13	-6.38, 0.80	-3.31	0.09	-7.12, 0.50
	GSTP1 AG/GG	average	-0.54	0.68	-3.11, 2.03	-0.80	0.59	-3.70, 2.09
				0.32	-2.16, 6.66		0.31	-2.28, 7.29

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: NO_2: 8.19 ppb and NO: 29.7 ppb.

[2] Nadir FEV1 is defined as the minimum of the (up to 4) best FEV1s on a given day. Nadir PF is defined analogously

[3] pollution levels used in regression models combine measured and imputed values

Covariates in each model: race, school, caregiver smokes, asthma severity, interactions between asthma severity and exposure, interaction between gene and pollutants

[4] Interaction p-value (gene-pollutant interaction)

[5] p-value for the change in estimate

An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

Table 4.19 Gene-environment interactions with the GSTP1 genotype. Percent change[1] in nadir[2] FEV_1 and PF associated with ambient levels[3] of SO_2, and PM_{10} from single pollutant linear regression models using generalized estimating equations (GEE).

Lung Function Outcome	Genotype	Lags	SO_2			PM_{10}		
			EST	P-Val	CI	EST	P-Val	CI
Nadir FEV1	GSTP1AA	Lag 1	-0.01	0.18[5]	-0.02,0.00	0.01	0.42	-0.01, 0.03
	GSTP1 AG/GG		0.00	0.65[5]	-0.01,0.01	**0.02**	**0.01**	**0.00, 0.03**
				0.20[4]	-0.01,0.03		0.47	-0.02,0.03
	GSTP1AA	Lag 2	0.00	0.70	-0.02,0.01	0.01	0.41	-0.01, 0.03
	GSTP1 AG/GG		**0.01**	**0.03**	**0.00, 0.03**	**0.02**	**0.01**	**0.01,0.03**
				0.08	-0.00,0.03		0.37	-0.01,0.03
	GSTP1AA	Lag 3	0.00	0.49	-0.01, 0.02	0.01	0.53	-0.01, 0.02
	GSTP1 AG/GG		**0.02**	**0.01**	**0.00, 0.03**	**0.02**	**0.01**	**0.00, 0.03**
				0.10	-0.01,0.03		0.43	-0.01,0.03
	GSTP1AA	Lag 4	0.00	0.73	-0.02, 0.01	0.00	0.87	-0.02,0.02
	GSTP1 AG/GG		**0.01**	**0.02**	**0.00, 0.03**	**0.02**	**0.02**	**0.00,0.03**
				0.06	-0.00,0.04		0.25	-0.01,0.04
	GSTP1AA	Lag 5	-0.01	0.19	-0.02, 0.00	0.00	0.80	-0.02, 0.02
	GSTP1 AG/GG		0.00	0.47	-0.01, 0.01	0.01	0.08	0.00,0.02
				0.15	-0.01,0.03		0.43	-0.01,0.03
	GSTP1AA	5 days	-0.01	0.63	-0.04, 0.02	0.01	0.56	-0.02, 0.03
	GSTP1 AG/GG	average	0.03	0.06	0.00, 0.06	**0.02**	**0.02**	**0.00, 0.03**
				0.11	-0.01,0.04		0.46	-0.02,0.04
Nadir PF	GSTP1AA	Lag 1	**-2.39**	**0.04**	**-4.71, 0.07**	-1.36	0.36	-4.26, 1.53
	GSTP1 AG/GG		0.52	0.67	-1.85, 2.89	0.88	0.41	-1.22, 2.97
				0.08	-0.39,6.21		0.22	-1.33,5.81
	GSTP1AA	Lag 2	-0.70	0.55	-3.02, 1.61	-1.23	0.35	-3.80, 1.33
	GSTP1 AG/GG		1.98	0.10	-0.38, 4.33	0.89	0.34	-0.94, 2.73
				0.11	-0.60,5.96		0.19	-1.02,5.28
	GSTP1AA	Lag 3	-0.43	0.69	-2.57, 1.71	-1.56	0.30	-4.49, 1.36
	GSTP1 AG/GG		**2.67**	**0.04**	**0.12, 5.23**	0.66	0.50	-1.26, 2.58
				0.07	-0.20,6.42		0.21	-1.27,5.72
	GSTP1AA	Lag 4	-2.35	0.06	-4.80, 0.09	-1.90	0.21	-4.88, 1.08
	GSTP1 AG/GG		**2.83**	**0.04**	**0.20, 5.45**	0.53	0.61	-1.49,2.54
				0.00	1.62,8.74		0.19	-1.17,6.02
	GSTP1AA	Lag 5	-1.92	0.03	-3.70, -0.14	-2.26	0.11	-5.07,0.54
	GSTP1 AG/GG		1.20	0.30	-1.07, 3.48	-0.15	0.88	-2.15, 1.85
				0.03	0.26,5.99		0.23	-1.33,5.55
	GSTP1AA	5 days	-4.18	0.13	-9.64, 1.28	-1.51	0.36	-4.76, 1.74
	GSTP1 AG/GG	average	4.82	0.11	-1.08, 10.72	0.25	0.82	-1.94, 2.45
				0.03	1.08,16.93		0.38	-2.16,5.68

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: PM_{10}: 29.4 ug m^{-3} and SO_2: 9.8 ppb.
[2]Nadir FEV1 is defined as the minimum of the (up to 4) best FEV1s on a given day, Nadir PF is defined analogously
[3] pollution levels used in regression models combine measured and imputed values
Covariates in each model: race, school, caregiver smokes, asthma severity, interactions between asthma severity and exposure, interaction between gene and pollutants
[4]Interaction p-value (gene-pollutant interaction)
[5]p-value for the change in estimate
An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

Table 4.20 Gene-environment interactions with the NQO1 genotype. Percent change[1] in intraday variability[2] of FEV_1 and peak flow (PF) associated with ambient levels[3] of NO_2, and NO from single pollutant linear regression models using generalized estimating equations (GEE).

Lung Function Outcome	Genotype	Lags	NO_2			NO		
			EST	P-Val	CI	EST	P-Val	CI
Intraday FEV1	NQO1 CC	Lag 1	**0.45**	**0.05**[5]	**0.00, 0.91**	**0.90**	**<.005**	**0.43, 1.37**
	NQO1 CT/TT		0.40	0.14[5]	-0.13, 0.92	**0.67**	**0.02**	**0.13, 1.21**
				0.88[4]	0.74, 0.64		0.54	-0.94,0.49
	NQO1 CC	Lag 2	0.37	0.09	-0.06, 0.80	0.38	0.14	-0.12, 0.87
	NQO1 CT/TT		0.17	0.50	-0.33, 0.68	-0.04	0.89	-0.70, 0.61
				0.55	-0.86,0.46		0.31	-1.24, 0.39
	NQO1 CC	Lag 3	0.22	0.25	-0.16, 0.60	0.36	0.11	-0.08, 0.81
	NQO1 CT/TT		0.09	0.73	-0.39, 0.56	0.17	0.57	-0.42, 0.77
				0.66	-0.74,0.47		0.61	-0.93,0.55
	NQO1 CC	Lag 4	**0.48**	**0.02**	**0.09, 0.87**	**0.45**	**0.05**	**0.00, 0.90**
	NQO1 CT/TT		0.32	0.18	-0.15, 0.79	0.23	0.46	-0.37, 0.84
				0.61	-0.77, 0.45		0.57	-0.97, 0.53
	NQO1 CC	Lag 5	**0.60**	**0.01**	**0.16, 1.03**	**0.81**	**<.005**	**0.34, 1.27**
	NQO1 CT/TT		0.39	0.12	-0.10, 0.87	**0.80**	**0.01**	**0.20, 1.41**
				0.53	-0.86,0.44		0.99	-0.76, 0.76
	NQO1 CC	5 days	0.42	0.07	-0.03, 0.87	**0.64**	**0.01**	**0.13, 1.14**
	NQO1 CT/TT	average	0.29	0.28	-0.24, 0.81	0.40	0.21	-0.23, 1.03
				0.71	-0.82,0.55		0.57	-1.03,0.57
Intraday PF	NQO1 CC	Lag 1	**0.66**	**<.005**	**0.23, 1.08**	**0.97**	**0.01**	**0.54, 1.41**
	NQO1 CT/TT		0.08	0.75	-0.43, 0.59	0.36	0.21	-0.19, 0.91
				0.09	-1.23,0.09		0.08	-1.31,0.08
	NQO1 CC	Lag 2	**0.67**	**<.005**	**0.26, 1.08**	**0.82**	**<.005**	**0.33, 1.31**
	NQO1 CT/TT		-0.05	0.83	-0.55, 0.44	-0.03	0.92	-0.70, 0.63
				0.03	-1.36,-0.08		***0.04***	-1.68,-0.04
	NQO1 CC	Lag 3	**0.49**	**0.01**	**0.12, 0.85**	**0.51**	**0.02**	**0.08, 0.93**
	NQO1 CT/TT		0.09	0.70	-0.37, 0.55	0.18	0.54	-0.39, 0.75
				0.18	-0.98,0.18		0.36	-1.03,0.38
	NQO1 CC	Lag 4	**0.69**	**<.005**	**0.30, 1.08**	**0.57**	**0.02**	**0.10, 1.04**
	NQO1 CT/TT		0.27	0.29	-0.24, 0.79	0.20	0.53	-0.41, 0.80
				0.20	-1.05,0.22		0.34	-1.13,0.39
	NQO1 CC	Lag 5	**0.78**	**<.005**	**0.34, 1.22**	**0.86**	**<.005**	**0.37, 1.35**
	NQO1 CT/TT		0.21	0.42	-0.30, 0.73	0.30	0.35	-0.33, 0.93
				0.10	-1.24,0.11		0.16	-1.35,0.22
	NQO1 CC	5 days	**0.71**	**<.005**	**0.28, 1.14**	**0.89**	**<.005**	**0.41, 1.38**
	NQO1 CT/TT	average	0.14	0.61	-0.38, 0.66	0.29	0.36	-0.34, 0.92
				0.09	-1.24,0.09		0.13	-1.38,0.18

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: NO_2: 8.19 ppb and NO: 29.7 ppb.

[2] 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 (08h00, 09h45, 11h30, 13h20).

Intraday variability for PF is defined analogously to within-day variability for FEV_1.

[3] pollution levels used in regression models combine measured and imputed values

Covariates in each model: race, school, caregiver smokes, asthma severity, interactions between asthma severity and exposure, interaction between gene and pollutants

[4] Interaction p-value (gene-pollutant interaction)

[5] p-value for the change in estimate

An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

Table 4.21 Gene-environment interactions with the NQO1 genotype. Percent change[1] in intraday variability[2] of FEV_1 and peak flow (PF) associated with ambient levels[3] of SO_2 and PM_{10} from single pollutant linear regression models using generalized estimating equations (GEE)

Lung Function Outcome	Genotype	Lags	SO_2			PM_{10}		
			EST	P-Val	CI	EST	P-Val	CI
Intraday FEV1	NQO1 CC NQO1 CT/TT	Lag 1	0.33 0.44	0.10[5] 0.14[5] 0.75[4]	-0.06, 0.71 -0.15, 1.03 -0.57,0.79	-0.07 -0.11	0.71 0.68 0.91	-0.45, 0.30 -0.61, 0.39 -0.66,0.59
	NQO1 CC NQO1 CT/TT	Lag 2	-0.17 0.06	0.35 0.84 0.51	-0.54, 0.19 -0.56, 0.68 -0.47,0.94	0.05 0.01	0.75 0.95 0.88	-0.28, 0.39 -0.40, 0.43 -0.57,0.49
	NQO1 CC NQO1 CT/TT	Lag 3	-0.21 -0.04	0.28 0.89 0.60	-0.58, 0.17 -0.57, 0.50 -0.46,0.80	0.10 -0.08	0.57 0.73 0.53	-0.24, 0.43 -0.50, 0.35 -0.71,0.37
	NQO1 CC NQO1 CT/TT	Lag 4	0.04 -0.18	0.83 0.50 0.48	-0.31, 0.39 -0.69, 0.34 -0.81,0.38	0.17 0.11	0.35 0.65 0.82	-0.19, 0.54 -0.35, 0.56 -0.65,0.52
	NQO1 CC NQO1 CT/TT	Lag 5	0.22 0.28	0.20 0.30 0.86	-0.12, 0.57 -0.25, 0.80 -0.56,0.67	0.18 0.16	0.36 0.53 0.94	-0.20, 0.57 -0.33, 0.64 -0.64,0.59
	NQO1 CC NQO1 CT/TT	5 days average	-0.04 0.28	0.92 0.65 0.63	-0.79, 0.71 -0.90, 1.45 -0.95,1.58	0.12 0.01	0.54 0.96 0.74	-0.25,0.48 -0.47, 0.50 -0.70,0.50
Intraday PF	NQO1 CC NQO1 CT/TT	Lag 1	0.38 0.22	0.76 0.38 0.61	0.01, 0.73 -0.27, 0.72 -0.75,0.44	**0.37** -0.30	**0.04** 0.22 ***0.03***	**0.01, 0.73** -0.78, 0.17 -1.27,-0.08
	NQO1 CC NQO1 CT/TT	Lag 2	0.03 -0.18	0.88 0.50 0.50	-0.35, 0.41 -0.69, 0.34 -0.81,0.39	**0.43** -0.05	**0.01** 0.80 0.07	**0.10, 0.76** -0.47, 0.37 -1.02,0.04
	NQO1 CC NQO1 CT/TT	Lag 3	-0.07 -0.23	0.72 0.39 0.61	-0.47, 0.32 -0.76, 0.30 -0.78,0.46	0.48 -0.16	**<.005** 0.44 ***0.01***	**0.16, 0.81** -0.56, 0.24 -1.16,-0.13
	NQO1 CC NQO1 CT/TT	Lag 4	**0.45** -0.19	**0.03** 0.43 ***0.04***	**0.03, 0.86** -0.67, 0.28 -1.23,-0.04	**0.62** 0.01	**<.005** 0.97 ***0.04***	**0.26, 0.98** -0.47, 0.48 -1.20,-0.02
	NQO1 CC NQO1 CT/TT	Lag 5	0.25 -0.16	0.16 0.50 0.15	-0.10, 0.61 -0.64, 0.31 -0.99,0.16	**0.53** 0.05	**0.01** 0.83 0.11	**0.15, 0.92** -0.41, 0.51 -1.08,0.11
	NQO1 CC NQO1 CT/TT	5 days average	0.44 -0.11	0.27 0.83 0.34	-0.34, 1.23 -1.12, 0.90 -1.67,0.57	**0.56** -0.16	**<.005** 0.50 ***0.01***	**0.21, 0.92** -0.62, 0.30 -1.30,-0.15

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: PM_{10}: 29.4 ug m^{-3} and SO_2: 9.8 ppb.

[2] 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 (08h00, 09h45, 11h30, 13h20).
Intraday variability for PF is defined analogously to within-day variability for FEV_1.

[3] pollution levels used in regression models combine measured and imputed values
Covariates in each model: race, school, caregiver smokes, asthma severity, interactions between asthma severity and exposure, interaction between gene and pollutants

[4] Interaction p-value (gene-pollutant interaction)

[5] p-value for the change in estimate
An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

Table 4.22 Gene-environment interactions with the NQO1 genotype. Percent change[1] in nadir[2] FEV_1 and PF associated with ambient levels[3] of NO_2, and NO from single pollutant linear regression models using generalized estimating equations (GEE).

Lung Function Outcome	Genotype	Lags	NO_2			NO		
			EST	P-Val	CI	EST	P-Val	CI
Nadir FEV1	NQO1 CC	Lag 1	0.00	0.93[5]	-0.02, 0.02	-0.01	0.49	-0.02, 0.01
	NQO1 CT/TT		0.01	0.29[5]	-0.01, 0.03	0.01	0.46	-0.01, 0.03
				0.45[4]	-0.02,0.04		0.31	-0.01,0.04
	NQO1 CC	Lag 2	0.01	0.48	-0.01, 0.02	0.00	0.68	-0.01, 0.02
	NQO1 CT/TT		0.01	0.16	-0.01, 0.03	0.01	0.36	-0.01, 0.03
				0.52	-0.02,0.03		0.63	-0.02,0.03
	NQO1 CC	Lag 3	0.01	0.30	-0.01, 0.02	0.01	0.43	-0.01, 0.02
	NQO1 CT/TT		0.01	0.13	0.00, 0.03	0.01	0.25	-0.01, 0.03
				0.60	-0.02,0.03		0.67	-0.02,0.03
	NQO1 CC	Lag 4	0.00	0.53	-0.01, 0.02	0.00	0.60	-0.01, 0.02
	NQO1 CT/TT		0.01	0.22	-0.01, 0.03	0.01	0.25	-0.01, 0.03
				0.59	-0.02,0.03		0.56	-0.02,0.03
	NQO1 CC	Lag 5	0.00	0.97	-0.01, 0.02	0.00	0.82	-0.01, 0.02
	NQO1 CT/TT		0.01	0.24	-0.01, 0.03	0.01	0.41	-0.01, 0.03
				0.38	-0.01,0.03		0.62	-0.02,0.03
	NQO1 CC	5 days	0.01	0.51	-0.01, 0.02	0.00	0.72	-0.01, 0.02
	NQO1 CT/TT	average	0.01	0.18	-0.01, 0.03	0.01	0.27	-0.01, 0.03
				0.54	-0.02,0.03		0.52	-0.02,0.03
Nadir PF	NQO1 CC	Lag 1	**-3.72**	**0.01**	**-6.31, -1.14**	**-4.04**	**<.005**	**-6.50, -1.58**
	NQO1 CT/TT		0.68	0.63	-2.10, 3.46	0.65	0.67	-2.32, 3.62
				0.02	0.61,8.20		***0.02***	0.84,8.55
	NQO1 CC	Lag 2	**-3.28**	**0.01**	**-5.68, -0.87**	**-4.18**	**<.005**	**-6.65, -1.71**
	NQO1 CT/TT		0.99	0.48	-1.74, 3.72	0.10	0.95	-2.91, 3.10
				0.02	0.63,7.90		***0.03***	0.39,8.16
	NQO1 CC	Lag 3	**-2.43**	**0.03**	**-4.59, -0.28**	**-2.91**	**0.02**	**-5.31, -0.51**
	NQO1 CT/TT		0.56	0.65	-1.86, 2.99	-0.33	0.82	-3.12, 2.47
				0.70	-0.24,6.24		0.17	-1.10,6.27
	NQO1 CC	Lag 4	**-2.33**	**0.05**	**-4.63, -0.04**	-2.19	0.08	-4.66, 0.27
	NQO1 CT/TT		0.93	0.50	-1.79, 3.64	0.69	0.67	-2.46, 3.85
				0.07	-0.29,6.81		0.16	-1.11,6.89
	NQO1 CC	Lag 5	**-3.15**	**0.01**	**-5.61, -0.69**	-2.49	0.07	-5.18, 0.19
	NQO1 CT/TT		0.84	0.54	-1.87, 3.55	1.64	0.32	-1.61, 4.90
				0.03	0.33,7.64		***0.05***	-0.07,8.35
	NQO1 CC	5 days	**-3.31**	**0.02**	**-5.99, -0.63**	**-3.87**	**0.01**	**-6.75, -0.99**
	NQO1 CT/TT	average	0.86	0.56	-2.05, 3.77	0.52	0.76	-2.89, 3.94
				0.04	0.23,8.12		***0.05***	-0.06,8.85

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: NO_2: 8.19 ppb and NO: 29.7 ppb.

[2] Nadir FEV1 is defined as the minimum of the (up to 4) best FEV1s on a given day, Nadir PF is defined analogously

[3] pollution levels used in regression models combine measured and imputed values

Covariates in each model: race, school, caregiver smokes, asthma severity, interaction between gene and pollutants

[4] Interaction p-value (gene-pollutant interaction)

[5] p-value for the change in estimate

An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

Table 4.23 Gene-environment interactions with the NQO1 genotype. Percent change[1] in nadir[2] FEV_1 and PF associated with ambient levels[3] of SO_2, and PM_{10} from single pollutant linear regression models using generalized estimating equations (GEE).

Lung Function Outcome	Genotype	Lags	SO_2			PM_{10}		
			EST	P-Val	CI	EST	P-Val	CI
Nadir FEV1	NQO1 CC	Lag 1	0.00	0.42[5]	-0.01, 0.02	**0.02**	**0.03**	**0.00, 0.03**
	NQO1 CT/TT		0.00	0.72[5]	-0.02, 0.01	0.01	0.06	0.00, 0.03
				0.43[4]	-0.03,0.01		0.95	-0.02,0.02
	NQO1 CC	Lag 2	**0.01**	**0.03**	**0.00, 0.02**	**0.02**	**0.01**	**0.00, 0.03**
	NQO1 CT/TT		0.01	0.41	-0.01, 0.02	0.01	0.06	0.00, 0.03
				0.53	-0.03,0.01		0.77	-0.02,0.02
	NQO1 CC	Lag 3	**0.02**	**0.01**	**0.00, 0.03**	**0.02**	**0.03**	**0.00, 0.03**
	NQO1 CT/TT		0.01	0.12	0.00, 0.03	0.01	0.06	0.00, 0.03
				0.60	-0.02,0.01		0.93	-0.02,0.02
	NQO1 CC	Lag 4	0.01	0.09	0.00, 0.02	0.01	0.06	0.00, 0.03
	NQO1 CT/TT		0.01	0.10	0.00, 0.03	0.01	0.13	0.00, 0.03
				0.87	-0.02,0.02		0.91	-0.02,0.02
	NQO1 CC	Lag 5	0.00	0.79	-0.01, 0.01	0.01	0.13	0.00, 0.02
	NQO1 CT/TT		0.00	0.97	-0.01, 0.01	0.01	0.26	-0.01, 0.02
				0.90	-0.02,0.02		0.92	-0.02,0.02
	NQO1 CC	5 days	0.03	0.06	0.00, 0.06	**0.02**	**0.04**	**0.00, 0.03**
	NQO1 CT/TT	average	0.02	0.39	-0.02, 0.05	0.01	0.10	0.00, 0.03
				0.59	-0.06,0.03		0.94	-0.02,0.02
Nadir PF	NQO1 CC	Lag 1	-4.29	0.47	-15.87, 7.30	-1.05	0.31	-3.09, 0.99
	NQO1 CT/TT		0.38	0.82	-2.93, 3.69	1.56	0.23	-0.97, 4.10
				0.46	-2.35,5.16		0.11	-0.63,5.87
	NQO1 CC	Lag 2	0.21	0.82	-1.56, 1.98	-0.80	0.38	-2.58, 0.97
	NQO1 CT/TT		2.03	0.24	-1.38, 5.43	0.85	0.45	-1.38, 3.09
				0.35	-2.00,5.63		0.26	-1.19,4.51
	NQO1 CC	Lag 3	0.73	0.44	-1.14, 2.60	-1.13	0.26	-3.10, 0.83
	NQO1 CT/TT		1.99	0.26	-1.46, 5.44	1.16	0.30	-1.03, 3.35
				0.53	-2.63,5.15		0.13	-0.65,5.23
	NQO1 CC	Lag 4	-0.05	0.96	-1.98, 1.88	-1.26	0.24	-3.35, 0.82
	NQO1 CT/TT		1.77	0.37	-2.08, 5.61	0.95	0.43	-1.40, 3.30
				0.40	-2.45,6.08		0.17	-0.93,5.23
	NQO1 CC	Lag 5	-0.90	0.24	-2.42, 0.61	-1.90	0.07	-3.96, 0.17
	NQO1 CT/TT		0.51	0.75	-2.66, 3.68	0.20	0.86	-2.01, 2.40
				0.43	-2.07,4.90		0.17	0.92,5.11
	NQO1 CC	5 days	-0.22	0.92	-4.66, 4.21	-1.68	0.13	-3.87, 0.50
	NQO1 CT/TT	average	3.72	0.39	-4.66, 12.09	1.03	0.44	-1.57, 3.64
				0.41	-5.40,13.27		0.12	0.68,6.12

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: PM_{10}: 29.4 ug m^{-3} and SO_2: 9.8 ppb.

[2]Nadir FEV1 is defined as the minimum of the (up to 4) best FEV1s on a given day, Nadir PF is defined analogously

[3]pollution levels used in regression models combine measured and imputed values

Covariates in each model: race, school, caregiver smokes, asthma severity, interactions between asthma severity and exposure, interaction between gene and pollutants

[4]Interaction p-value (gene-pollutant interaction)

[5]p-value for the change in estimate

An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

Table 4.24 Gene-environment interactions with the GSTM1 and GSTP1 genotype combination. Percent change[1] in intraday variability[2] of FEV_1 and peak flow (PF) and nadir[3] FEV_1 and PF associated with ambient levels[4] of NO_2, and NO from single pollutant linear regression models using generalized estimating equations (GEE).

Lung Function Outcome	Genotype Combination	NO_2			NO		
		EST	**P-Val[5]**	**CI**	**EST**	**P-Val**	**CI**
Intraday FEV1	GSTM1null GSTP1AA	0.60	0.20	-0.31, 1.51	0.72	0.13	-0.20, 1.65
	GSTM1null GSTP1 AG/GG	0.28	0.48	-0.50, 1.06	0.25	0.60	-0.68, 1.17
	GSTM1pos GSTP1 AA	0.46	0.18	-0.22, 1.13	**0.76**	**0.04**	**0.05, 1.46**
	GSTM1 pos GSTP1AG/GG	0.26	0.34	-0.27, 0.79	0.44	0.17	-0.19, 1.08
Intraday PF	GSTM1null GSTP1AA	0.43	0.21	-0.25, 1.11	0.36	0.43	-0.53, 1.25
	GSTM1null GSTP1 AG/GG	**0.94**	**0.03**	**0.08, 1.79**	**1.16**	**0.02**	**0.19, 2.14**
	GSTM1pos GSTP1 AA	0.52	0.15	-0.18, 1.22	0.69	0.08	-0.08, 1.45
	GSTM1 pos GSTP1AG/GG	0.11	0.69	-0.42, 0.63	0.26	0.40	-0.35, 0.88
Nadir FEV1	GSTM1null GSTP1AA	-0.01	0.36	-0.04,0.02	-0.02	0.25	-0.05, 0.01
	GSTM1null GSTP1 AG/GG	0.02	0.06	0.00, 0.05	0.03	0.07	0.00, 0.06
	GSTM1pos GSTP1 AA	0.00	0.92	-0.03, 0.03	-0.01	0.70	-0.03, 0.02
	GSTM1 pos GSTP1AG/GG	0.01	0.44	-0.01, 0.03	0.01	0.49	-0.01, 0.03
Nadir PF	GSTM1null GSTP1AA	-3.31	0.35	-10.19, 3.56	-3.68	0.35	-11.44, 4.09
	GSTM1null GSTP1 AG/GG	-1.50	0.50	-5.84, 2.84	-1.91	0.48	-7.18, 3.37
	GSTM1pos GSTP1 AA	-2.48	0.23	-6.48, 1.53	-3.10	0.14	-7.17, 0.97
	GSTM1 pos GSTP1AG/GG	-0.11	0.95	-3.27, 3.06	-0.32	0.86	-3.77, 3.13

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: NO_2: 8.19 ppb and NO: 29.7 ppb.

[2] 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 (08h00, 09h45, 11h30, 13h20).
Intraday variability for PF is defined analogously to within-day variability for FEV_1.

[3] Nadir FEV1 is defined as the minimum of the (up to 4) best FEV1s on a given day, Nadir PF is defined analogously

[4] pollution levels used in regression models are the 5-day average
Covariates in each model: race, school, caregiver smokes, asthma severity, interactions between asthma severity and exposure, interaction between gene and pollutants

[5] p-value for the change in estimate
An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

Table 4.25 Gene-environment interactions with the GSTM1 and GSTP1 genotype combination. Percent change[1] in intraday variability[2] of FEV_1 and peak flow (PF) and nadir[3] FEV_1 and PF associated with ambient levels[4] of SO_2, and PM_{10} from single pollutant linear regression models using generalized estimating equations (GEE).

Lung Function Outcome	Genotype Combination	SO_2			PM_{10}		
		EST	P-Val[5]	CI	EST	P-Val	CI
Intraday FEV1	GSTM1null GSTP1AA	-0.68	0.39	-2.20, 0.85	0.66	0.16	-0.26, 1.57
	GSTM1null GSTP1 AG/GG	0.46	0.47	-0.78, 1.71	0.07	0.86	-0.72, 0.86
	GSTM1pos GSTP1 AA	0.13	0.80	-0.84, 1.10	0.14	0.63	-0.41, 0.69
	GSTM1 pos GSTP1AG/GG	0.28	0.60	-0.75, 1.31	-0.01	0.98	-0.44, 0.43
Intraday PF	GSTM1null GSTP1AA	-0.04	0.96	-1.66, 1.57	0.41	0.35	-0.46, 1.28
	GSTM1null GSTP1 AG/GG	1.06	0.22	-0.63, 2.74	0.76	0.09	-0.12, 1.64
	GSTM1pos GSTP1 AA	0.41	0.27	-0.32, 1.15	0.16	0.56	-0.37, 0.69
	GSTM1 pos GSTP1AG/GG	-0.37	0.48	-1.40, 0.66	0.02	0.92	-0.39, 0.43
Nadir FEV1	GSTM1null GSTP1AA	-0.02	0.57	-0.07, 0.04	-0.01	0.66	-0.04, 0.02
	GSTM1null GSTP1 AG/GG	0.03	0.17	-0.01, 0.07	**0.02**	**0.04**	**0.00, 0.04**
	GSTM1pos GSTP1 AA	-0.01	0.75	-0.04, 0.03	0.01	0.41	-0.02, 0.04
	GSTM1 pos GSTP1AG/GG	0.03	0.14	-0.01, 0.07,	0.01	0.12	0.00, 0.03
Nadir PF	GSTM1null GSTP1AA	-8.27	0.28	-23.30, 6.76	-2.45	0.43	-8.50, 3.61
	GSTM1null GSTP1 AG/GG	5.63	0.46	-9.14, 20.40	-0.38	0.86	-4.73, 3.96
	GSTM1pos GSTP1 AA	-3.15	0.26	-8.69, 2.38	-1.16	0.56	-5.00, 2.68
	GSTM1 pos GSTP1AG/GG	4.51	0.12	-1.16, 10.18	0.50	0.70	-2.03, 3.04

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: PM_{10}: 29.4 ug m^{-3} and SO_2: 9.8 ppb.

[2] 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 (08h00, 09h45, 11h30, 13h20).

Intraday variability for PF is defined analogously to within-day variability for FEV_1.

[3]Nadir FEV1 is defined as the minimum of the (up to 4) best FEV1s on a given day, Nadir PF is defined analogously

[4] pollution levels used in regression models are the 5-day average

Covariates in each model: race, school, caregiver smokes, asthma severity, interactions between asthma severity and exposure, interaction between gene and pollutants

[5]p-value for the change in estimate

An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

Table 4.26 Gene-environment interactions with the GSTM1 and NQO1 genotype combination. Percent change[1] in intraday variability[2] of FEV_1 and peak flow (PF) and nadir[3] FEV_1 and PF associated with ambient levels[4] of NO_2, and NO from single pollutant linear regression models using generalized estimating equations (GEE).

Lung Function Outcome	Genotype Combination	NO_2			NO		
		EST	P-Val[5]	CI	EST	P-Val	CI
Intraday FEV1	GSTM1null NQO1 CC	0.35	0.34	-0.37, 1.07	0.45	0.26	-0.33, 1.22
	GSTM1null NQO1 CC/TT	0.58	0.25	-0.41, 1.57	0.50	0.41	-0.69, 1.70
	GSTM1 pos NQO1 CC	0.45	0.11	-0.10, 1.01	**0.72**	**0.03**	**0.09, 1.35**
	GSTM1pos NQO1 CT/TT	0.13	0.67	-0.47, 0.74	0.35	0.35	-0.38, 1.08
Intraday PF	GSTM1null NQO1 CC	**0.90**	**0.03**	**0.09, 1.71**	**0.95**	**0.04**	**0.02, 1.87**
	GSTM1null NQO1 CC/TT	0.54	0.20	-0.29, 1.36	0.71	0.17	-0.29, 1.71
	GSTM1 pos NQO1 CC	**0.64**	**0.01**	**0.13, 1.14**	**0.87**	**<.005**	**0.31, 1.44**
	GSTM1pos NQO1 CT/TT	-0.07	0.83	-0.73, 0.59	0.08	0.84	-0.70, 0.86
Nadir FEV1	GSTM1null NQO1 CC	0.02	0.22	-0.01, 0.04	0.01	0.30	-0.01, 0.04
	GSTM1null NQO1 CC/TT	0.00	0.82	-0.03, 0.03	0.00	0.85	-0.03, 0.04
	GSTM1 pos NQO1 CC	0.00	0.90	-0.02, 0.02	0.00	0.89	-0.02, 0.02
	GSTM1pos NQO1 CT/TT	0.02	0.15	-0.01, 0.05	0.02	0.24	-0.01, 0.05
Nadir PF	GSTM1null NQO1 CC	-1.31	0.63	-6.57, 3.95	-1.21	0.68	-7.00, 4.59
	GSTM1null NQO1 CC/TT	-2.94	0.28	-8.22, 2.34	-4.21	0.21	-10.79, 2.37
	GSTM1 pos NQO1 CC	**-4.14**	**0.01**	**-7.20, -1.07**	**-4.97**	**<.005**	**-8.23, -1.71**
	GSTM1pos NQO1 CT/TT	2.85	0.10	-0.54, 6.24	2.88	0.14	-0.96, 6.72

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: NO_2: 8.19 ppb and NO: 29.7 ppb.

[2] 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 (08h00, 09h45, 11h30, 13h20).

Intraday variability for PF is defined analogously to within-day variability for FEV_1.

[3] Nadir FEV1 is defined as the minimum of the (up to 4) best FEV1s on a given day, Nadir PF is defined analogously

[4] pollution levels used in regression models are the 5-day average.

Covariates in each model: race, school, caregiver smokes, asthma severity, interactions between asthma severity and exposure, interaction between gene and pollutants

[5] p-value for the change in estimate

An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

Table 4.27 Gene-environment interactions with the GSTM1 and NQO1 genotype combination. Percent change[1] in intraday variability[2] of FEV_1 and peak flow (PF) and nadir[3] FEV_1 and PF associated with ambient levels[4] of SO_2, and PM_{10} from single pollutant linear regression models using generalized estimating equations (GEE).

Lung Function Outcome	Genotype Combination	SO_2			PM_{10}		
		EST	P-Val[5]	CI	EST	P-Val	CI
Intraday FEV1	GSTM1null NQO1 CC	0.55	0.32	-0.53, 1.64	0.23	0.51	-0.46, 0.93
	GSTM1null NQO1 CC/TT	-0.24	0.77	-1.82, 1.34,	0.08	0.89	-1.00, 1.15
	GSTM1 pos NQO1 CC	-0.19	0.66	-1.05, 0.66	0.08	0.72	-0.35, 0.51
	GSTM1pos NQO1 CT/TT	0.49	0.51	-0.97, 1.95	-0.02	0.95	-0.53, 0.50
Intraday PF	GSTM1null NQO1 CC	0.58	0.41	-0.81, 1.98	**0.87**	**0.04**	**0.05, 1.70**
	GSTM1null NQO1 CC/TT	0.83	0.42	-1.20, 2.87	0.16	0.74	-0.80, 1.12
	GSTM1 pos NQO1 CC	0.41	0.36	-0.46,1.29	**0.46**	**0.02**	**0.08, 0.84**
	GSTM1pos NQO1 CT/TT	-0.54	0.31	-1.57, 0.50	-0.29	0.26	-0.80, 0.22
Nadir FEV1	GSTM1null NQO1 CC	0.01	0.56	-0.03, 0.06	0.02	0.09	0.00, 0.04
	GSTM1null NQO1 CC/TT	0.01	0.59	-0.03, 0.06	0.01	0.42	-0.02, 0.04
	GSTM1 pos NQO1 CC	0.03	0.07	0.00, 0.07	0.01	0.13	0.00, 0.03
	GSTM1pos NQO1 CT/TT	0.02	0.48	-0.03, 0.06	0.02	0.15	-0.01, 0.04
Nadir PF	GSTM1null NQO1 CC	-2.34	0.65	-12.33, 7.65	-0.40	0.84	-4.28, 3.48
	GSTM1null NQO1 CC/TT	5.98	0.57	-14.73, 26.69	-1.08	0.73	-7.30, 5.14
	GSTM1 pos NQO1 CC	0.37	0.88	-4.45, 5.19	-2.13	0.11	-4.74, 0.48
	GSTM1pos NQO1 CT/TT	2.74	0.48	-4.80, 10.27	1.90	0.16	-0.76, 4.56

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: PM_{10}: 29.4 ug m^{-3} and SO_2: 9.8 ppb.

[2] 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 (08h00, 09h45, 11h30, 13h20).
Intraday variability for PF is defined analogously to within-day variability for FEV_1.

[3] Nadir FEV1 is defined as the minimum of the (up to 4) best FEV1s on a given day, Nadir PF is defined analogously

[4] pollution levels used in regression models are the 5-day average.
Covariates in each model: race, school, caregiver smokes, asthma severity, interactions between asthma severity and exposure, interaction between gene and pollutants

[5] p-value for the change in estimate
An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

Table 4. 28 Gene-environment interactions with the GSTP1 and NQO1 genotype combination. Percent change[1] in intraday variability[2] of FEV_1 and peak flow (PF) and nadir[3] FEV_1 and PF associated with ambient levels[4] of NO_2, and NO from single pollutant linear regression models using generalized estimating equations (GEE).

Lung Function Outcome	Genotype Combination	NO_2			NO		
		EST	P-Val[5]	CI	EST	P-Val	CI
Intraday FEV1	GSTP1 AA NQO1 CC	0.20	0.64	-0.63, 1.03	0.53	0.21	-0.29, 1.34
	GSTP1AA NQO1 CT/TT	**1.10**	**0.01**	**0.32, 1.89**	**1.30**	**<.005**	**0.47, 2.13**
	GSTP1AG/GG NQO1 CC	0.47	0.11	-0.10, 1.04	**0.65**	**0.05**	**0.00, 1.31**
	GSTP1AG/GG NQO1 CT/TT	0.00	1.00	-0.72, 0.72	-0.06	0.90	-0.99, 0.87
Intraday PF	GSTP1 AA NQO1 CC	0.43	0.29	-0.36, 1.22	0.51	0.25	-0.35, 1.37
	GSTP1AA NQO1 CT/TT	0.60	0.09	-0.10, 1.31	0.70	0.10	-0.14, 1.54
	GSTP1AG/GG NQO1 CC	**0.67**	**0.02**	**0.11, 1.22**	**0.85**	**0.01**	**0.21, 1.48**
	GSTP1AG/GG NQO1 CT/TT	-0.08	0.85	-0.85, 0.70	0.04	0.94	-0.91, 0.98
Nadir FEV1	GSTP1 AA NQO1 CC	0.01	0.63	-0.03, 0.04	0.00	0.87	-0.03, 0.03
	GSTP1AA NQO1 CT/TT	-0.01	0.30	-0.04, 0.01	-0.02	0.14	-0.05, 0.01
	GSTP1AG/GG NQO1 CC	0.00	0.66	-0.02, 0.03	0.00	0.72	-0.02, 0.03
	GSTP1AG/GG NQO1 CT/TT	0.03	0.07	0.00, 0.06	0.03	0.06	0.00, 0.06
Nadir PF	GSTP1 AA NQO1 CC	-3.95	0.24	-10.47, 2.57	-3.91	0.25	-10.51, 2.69
	GSTP1AA NQO1 CT/TT	-0.75	0.68	-4.34, 2.85	-1.65	0.39	-5.44, 2.14
	GSTP1AG/GG NQO1 CC	-2.47	0.13	-5.62, 0.69	-2.89	0.10	-6.30, 0.51
	GSTP1AG/GG NQO1 CT/TT	2.26	0.32	-2.22, 6.74	2.57	0.35	-2.79, 7.93

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: NO_2: 8.19 ppb and NO: 29.7 ppb.

[2] 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 (08h00, 09h45, 11h30, 13h20).

Intraday variability for PF is defined analogously to within-day variability for FEV_1.

[3] Nadir FEV1 is defined as the minimum of the (up to 4) best FEV1s on a given day, Nadir PF is defined analogously

[4] pollution levels used in regression models are the 5-day average

Covariates in each model: race, school, caregiver smokes, asthma severity, interactions between asthma severity and exposure interaction between gene and pollutants

[5] p-value for the change in estimate

An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

Table 4.29 Gene-environment interactions with the GSTP1 and NQO1 genotype combination. Percent change[1] in intraday variability[2] of FEV_1 and peak flow (PF) and nadir[3] FEV_1 and PF associated with ambient levels[4] of SO_2, and PM_{10} from single pollutant linear regression models using generalized estimating equations (GEE).

Lung Function Outcome	Genotype Combination	SO_2			PM_{10}		
		EST	P-Val[5]	CI	EST	P-Val	CI
Intraday FEV1	GSTP1 AA NQO1 CC	0.03	0.92	-0.46, 0.51	0.02	0.23	-0.01, 0.05
	GSTP1AA NQO1 CT/TT	0.54	0.18	-0.25, 1.32	-0.01	0.50	-0.04, 0.02
	GSTP1AG/GG NQO1 CC	0.42	0.10	-0.08, 0.92	0.01	0.15	0.00, 0.03
	GSTP1AG/GG NQO1 CT/TT	0.21	0.57	-0.52, 0.94	0.02	0.08	0.00, 0.05
Intraday PF	GSTP1 AA NQO1 CC	0.37	0.41	-0.50, 1.24	0.23	0.50	-0.43, 0.89
	GSTP1AA NQO1 CT/TT	0.31	0.55	-0.70, 1.31	0.24	0.47	-0.41, 0.89
	GSTP1AG/GG NQO1 CC	0.27	0.64	-0.87, 1.40	**0.53**	**0.03**	**0.06, 1.00**
	GSTP1AG/GG NQO1 CT/TT	-0.19	0.80	-1.62, 1.24	-0.23	0.50	-0.91, 0.44
Nadir FEV1	GSTP1 AA NQO1 CC	0.01	0.71	-0.04, 0.05	0.02	0.23	-0.01, 0.05
	GSTP1AA NQO1 CT/TT	-0.03	0.26	-0.07, 0.02	-0.01	0.50	-0.04, 0.02
	GSTP1AG/GG NQO1 CC	0.03	0.11	-0.01, 0.07	0.01	0.15	0.00, 0.03
	GSTP1AG/GG NQO1 CT/TT	0.03	0.18	-0.02, 0.08	0.02	0.08	0.00, 0.05
Nadir PF	GSTP1 AA NQO1 CC	-3.15	0.43	-10.98, 4.68	-1.83	0.50	-7.16, 3.50
	GSTP1AA NQO1 CT/TT	-2.91	0.34	-8.92, 3.11	-0.41	0.80	-3.65, 2.83
	GSTP1AG/GG NQO1 CC	2.10	0.45	-3.29, 7.49	-0.89	0.51	-3.54, 1.76
	GSTP1AG/GG NQO1 CT/TT	8.22	0.19	-4.01, 20.46	1.94	0.34	-2.04, 5.91

[1] the percent change value shown is for an increase of one interquartile range in each respective pollutant: PM_{10}: 29.4 ug m^{-3} and SO_2: 9.8 ppb.

[2] 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 (08h00, 09h45, 11h30, 13h20).

Intraday variability for PF is defined analogously to within-day variability for FEV_1.

[3] Nadir FEV1 is defined as the minimum of the (up to 4) best FEV1s on a given day, Nadir PF is defined analogously

[4] pollution levels used in regression models are the 5-day average.

Covariates in each model: race, school, caregiver smokes, asthma severity, interactions between asthma severity and exposure, interaction between gene and pollutants

[5] p-value for the change in estimate

An increase in the estimate for intraday FEV_1 and PF and a decrease in the estimate for nadir FEV_1 and PF would indicate a negative impact or decline on lung function.

In order to highlight general trends in the change of estimates from lag 1 to lag 5 after exposure to pollutants, graphs for each genotype were compared (16 graphs per genotype and 48 graphs in total). Due to the large number of lag-trend graphs, a representative selection of graphs (Figure 4.5 to 4.20) were chosen for each of the pollutants in order to show patterns of change in estimate versus lagged exposures. Adverse lung function was indicated by the higher estimates for intraday variability in FEV_1 and PF and by lower estimates for nadir FEV_1 and PF. While there was generally a lack of consistency between outcome and exposure among these trend graphs, there is an indication that the adverse outcome is somewhat worse on lag 1, followed by an improvement, then subsequent deterioration. Exposure to NO_2 showed a decrease in effect at lag 3 and a considerable increased adverse effect at lags 4 and 5. Change in estimate trends across the 5 lags were less consistent with NO exposure for the 3 genotypes tested. The general trend in estimate change from one lag to another for PM_{10} exposure was significantly different from the three gaseous pollutants. There was a lower adverse effect at lag 1 with a trend of increasing adverse effect from lags 2-5.

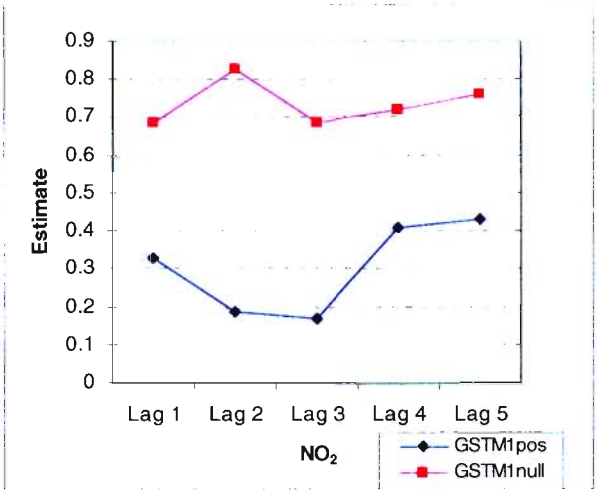


Figure 4.5: Trend in percent change (estimate) in intraday variability in PF for an increase in one interquartile range of NO_2 (8.19 ppb) across lags 1-5 using GSTM1 as an effect modifier.

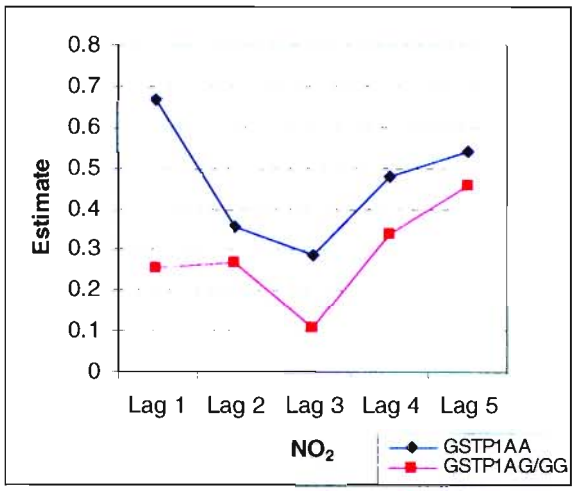


Figure 4.6: Trend in percent change (estimate) in Intraday variability of FEV1 for an increase in one interquartile range of NO_2 (8.19 ppb) across lags 1-5 using GSTP1 as an effect modifier.

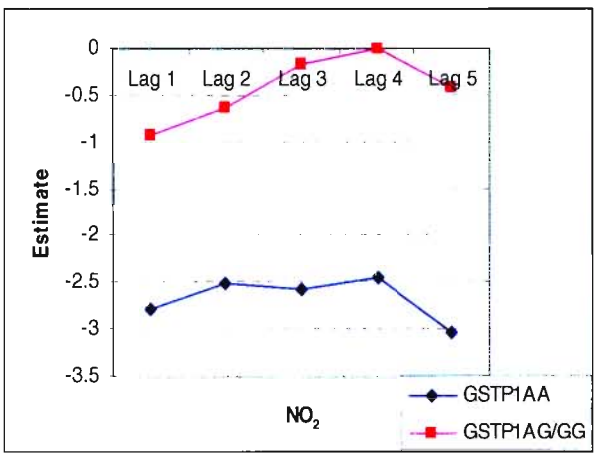


Figure 4.7: Trend in percent change (estimate) in Nadir PF for an increase in one interquartile range of NO_2 (8.19 ppb) across lags 1-5 using GSTP1 as an effect modifier.

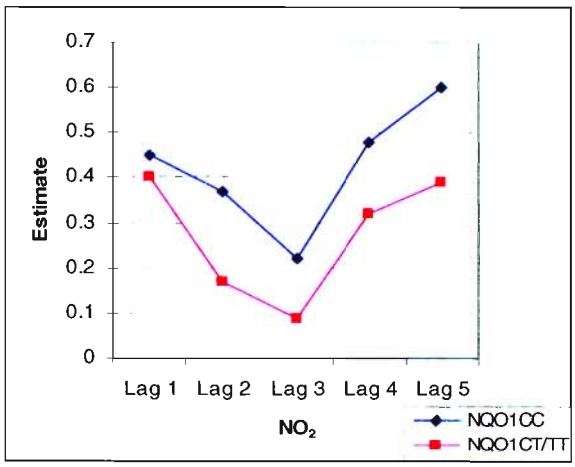


Figure 4.8: Trend in percent change (estimate) in intraday FEV1for an increase in one interquartile range of NO_2 (8.19 pb) across lags 1-5 using NQO1 as an effect modifier

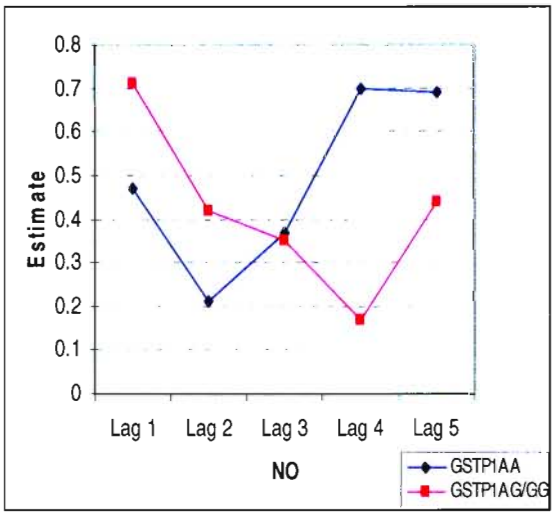


Figure 4.9: Trend in percent change (estimate) in intraday variability in PF for an increasein one interquartile range of NO (29.7 ppb) across lags 1-5 using GSTP1 as an effect modifier.

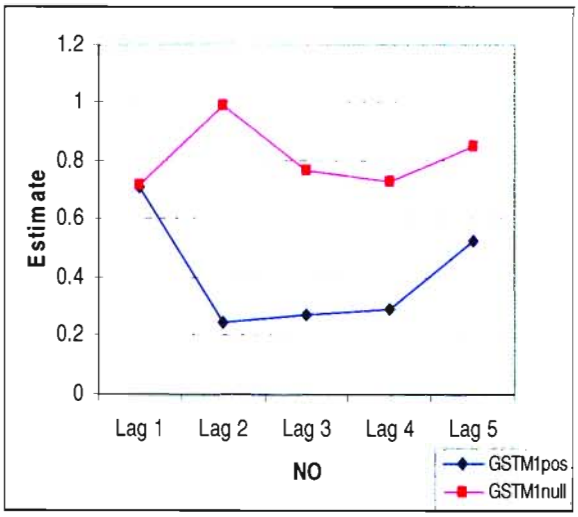


Figure 4.10: Trend in percent change (estimate) in intraday variability in PF for an increase in one interquartile range of NO (29.7 ppb) across lags 1-5 using GSTM1 as an effect modifier.

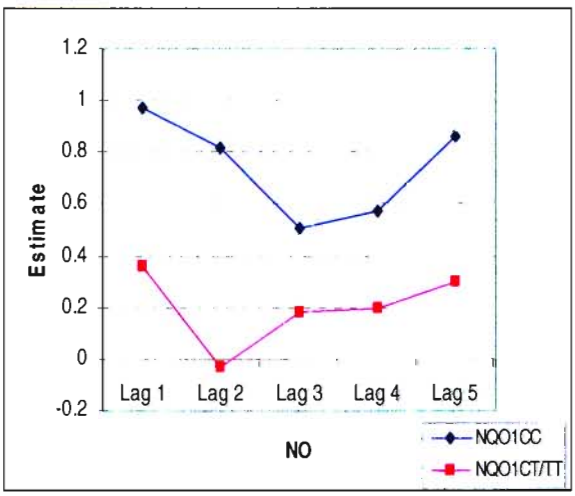


Figure 4.11: Trend in percent change (estimate) in Intraday variability in PFfor an increase in one interquartile range of NO (29.7 ppb) across lags 1-5 using NQO1 as an effect modifier.

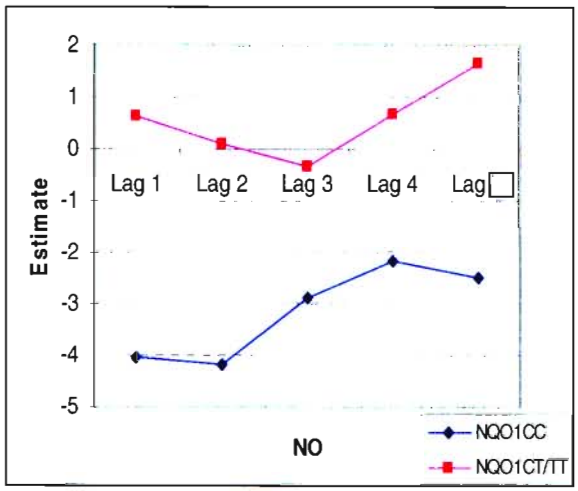


Fig 4.12: Trend in percent change (estimate) in nadir PF for an increase in one interquartile range of NO (29.7 ppb) across lags 1-5 using NQO1 as an effect modifier

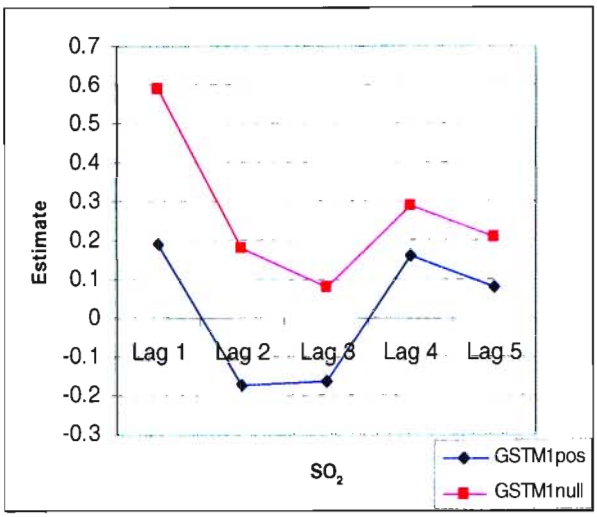


Figure 4.13: Trend in percent change (estimate) in Intraday variability in PF for an increase in one interquartile range of SO_2 (9.8 ppb) across lags 1-5 using GSTM1 as an effect modifier.

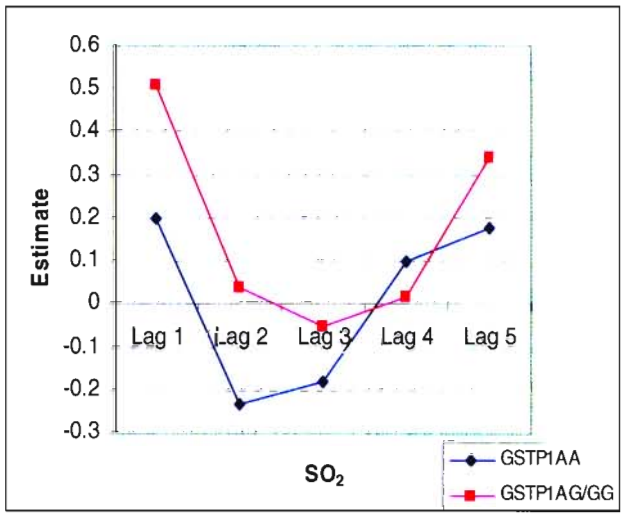


Fig 4.14: Trend in percent change (estimate) in Intraday variability in FEV1 for an increase in one interquartile range of SO_2 (9.8 ppb) across lags 1-5 using GSTP1 as an effect modifier.

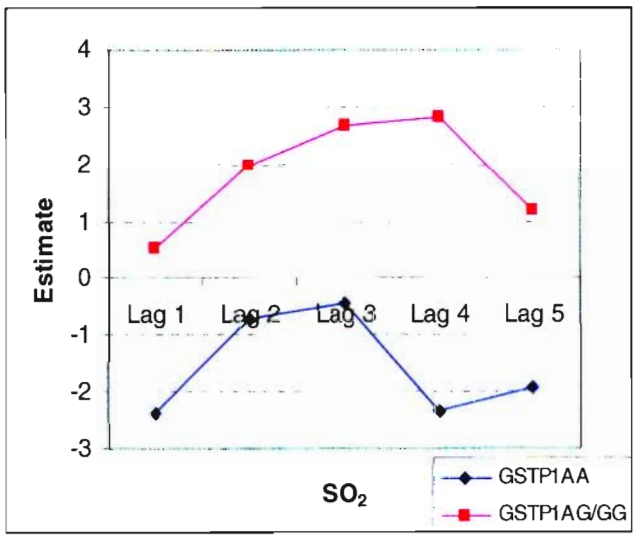


Figure 4.15: Trend in percent change (estimate) in nadir PF for an increase in one interquartile range of SO_2 (9.8 ppb) across lags 1-5 using NQO1 as an effect modifier.

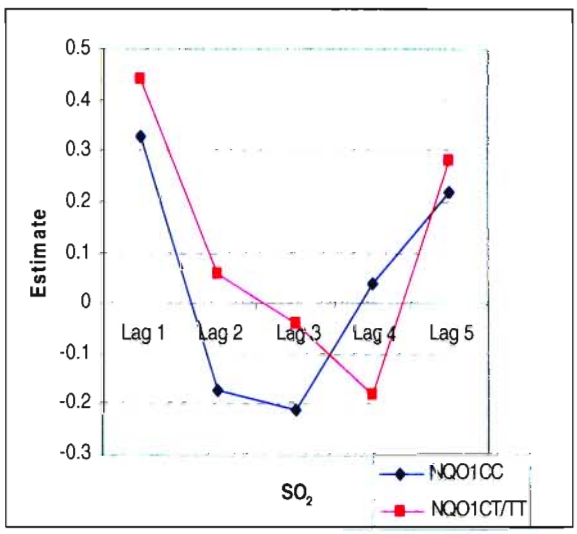


Figure 4.16: Trend in percent change (estimate) in intraday variability in FEV1for an increase in one interquartile range of SO_2 (9.8 ppb) across lags 1-5 using GSTP1 as an effect modifier.

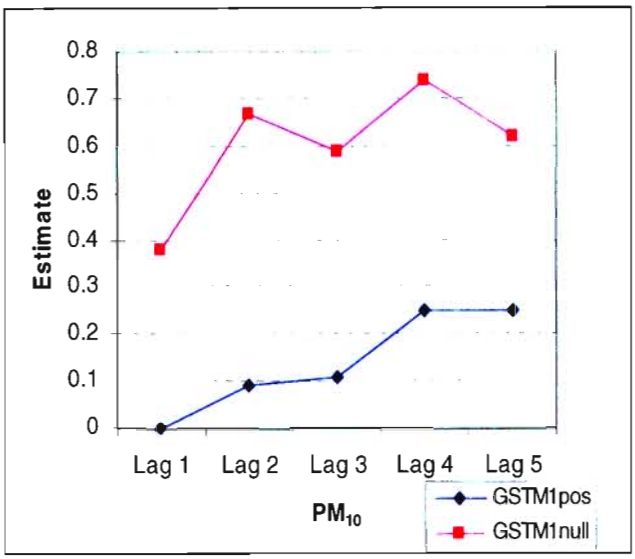


Figure 4.17: Trend in percent change (estimate) in Intraday PF for an increase in one interquartile range of PM_{10} (29.4 ug/m^{-3} ppb) across lags 1-5 using GSTM1 as an effect modifier.

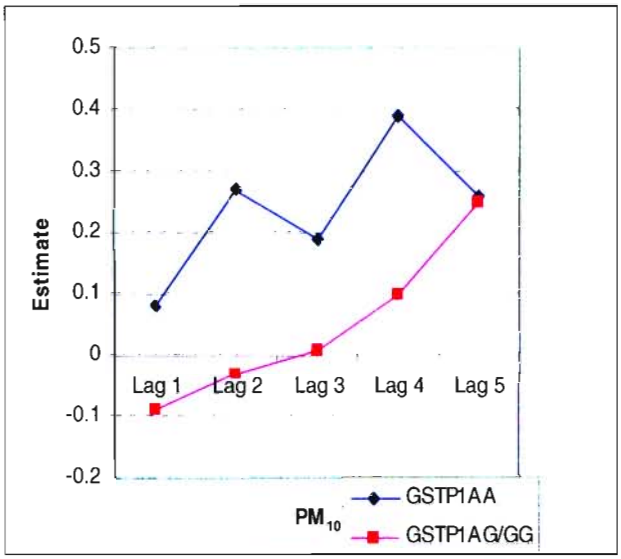


Figure 4.18: Trend in percent change (estimate) in Intraday FEV1 for an increase in one interquartile range of PM_{10} (29.4 ug/m^{-3} ppb) across lags 1-5 using GSTP as an effect modifier.

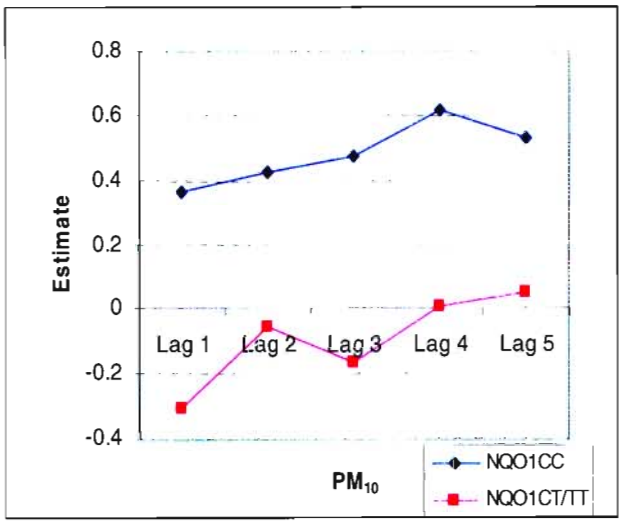


Fig 4.19: Trend in percent change (estimate) in nadir PF for an increase in one interquartile range of PM_{10} (29.4 ug/m^{-3} ppb) across lags 1-5 using NQO1 as an effect modifier.

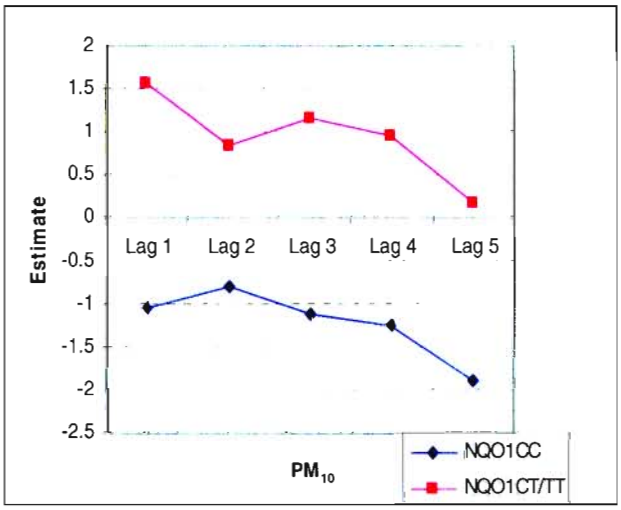


Figure 4.20: Trend in percent change (estimate) in Intraday PF for an increase in one interquartile range of PM_{10} (29.4 ug/m^{-3} ppb) across lags 1-5 using NQO1 as an effect modifier.

CHAPTER 5:

DISCUSSION

Asthma is a complex disease associated with many genes and susceptibility may involve multiple genes, gene-gene and gene-environment interactions. In this sample of South African schoolchildren we investigated three genes with functional polymorphisms (GSTM1, GSTP1 and NQO1) associated with oxidative stress and found that increased risk to certain respiratory outcomes, especially persistent asthma, may be in part determined by genetic polymorphisms and to some extent, gene-ambient pollution interaction. The most striking finding in our study was that pollutant exposure, especially oxides of nitrogen and PM_{10}, even at levels below the recommended limits of South African guidelines, is associated with poorer lung function and that this association is significantly modified by an individual's genotype, particularly the GSTM1null, GSTP1AA and NQO1CC genotypes. Significant gene*pollutant or gene*environment interactions ($p_{int}<0.05$) were observed for the GSTP1 and NQO1 variants SO2 with nadir PF; and NO, NO_2 and PM_{10} with both intraday variability in PF and nadir PF respectively. Furthermore, polymorphisms associated with oxidative stress, GSTP1 AG/GG and GSTP1 GG variants, influence health outcomes among those with persistent asthma, irrespective of pollution. Interestingly, our findings suggested that the GSTM1 and NQO1 polymorphisms both individually and in combination were not associated with the development of asthma and related phenotypes when air pollution exposure was ignored.

We chose a particular set of genes for the purposes of this study, recognising the limitations of this narrow choice. This choice was based on the study of the literature, and driven by sample size and resource constraints. The literature identified more than 100 reports of genetic variants associated with asthma and related phenotypes. No more than 8-10 such genes have been replicated in three or more studies, and none of these genes have been consistently associated with same asthma phenotype in studies to date (Yeatts *et al.,* 2006). Polymorphisms of each gene may impart only a small relative risk of disease and we can speculate that several polymorphic variants coexist to manifest in the disease phenotype. Our strategy has been to select genes that have been well documented in literature for having a role in cellular protection against oxidative stress and where there are common highly functional polymorphisms. In this respect GSTs and NQO1 are critical in the protection of cells from toxic products of ROS mediated reactions (Spiteri *et al.*, 2000).

Because the frequency of homozygosity at the GSTP1 GG and the NQO1 TT loci in our multiethnic population was low, we used the dominant gene model with the heterozygote and homozygote genotypes combined. According to the literature on metabolic gene frequencies in control populations, a GSTM1 null frequency of 40-60% is common in both Caucasians and Asians, while a lower frequency is usually found in the African populations (16-36%) (Garte *et al.,* 2001; Adams *et al.*, 2003). Our results with GSTM1 confer with other African populations in that a relatively low genotypic frequency of the null genotype was found (29%) (Table 4.2). This was similar to the 24% GSTM1null genotype found in Zimbabweans and 23% and 21% GSTM1 null found in South African

Vendas and Xhosas respectively (Adams *et al.*, 2003). Fewer studies have been done with GSTP1. One study found a 75.6% frequency of GSTP1AA and 24.4% GSTP1 AG/GG in a Asian population (Lee *et al.*, 2005), while the frequency of this variant in a South African Xhosa population was 22% for GSTP1AA and 78 % of GSTP1 AG/GG (Adams *et al.*, 2003). In our study, the GSTP1 AG/GG (65%) and GSTP1AA (35%) frequencies were similar to the Adams *et al* study. Other studies have shown that the frequency of the NQO1 TT homozygous genotype varies across ethnic groups, 4% in Caucasians, 5% in African Americans and 22% in Asians (Ross *et al.*, 2000). In this study, we found the frequency of the NQO1 TT polymorphism was 4.3% and the combined heterozygote and homozygote (NQO1CC+CT) was 36%.

The African and Coloured populations had the lowest GSTM1null frequencies (20.6% and 33.3% respectively) which are much lower than that of other populations reported in literature (Garte *et al.*, 2001) (Table 4.3). Similarly, Africans and Coloureds also had the highest GSTP1AG/GG frequencies (78.5 and 69.0%) compared to Indians and Caucasians. Similar frequencies in African and Coloured populations may be due to their closely linked ancestries. More Indians had the NQO1 CT/TT genotype (56.3%). However, whether these frequencies are reflective of the general population is debatable; our relatively small sample size has limited statistical power.

Multivariate regression showed that children with the GSTP1GG genotype were at increased odds of presenting with persistent asthma (OR= 2.8, CI: 1.2-5.9, p<.005) (Table 4.9). GSTP1 is expected to be the major enzyme to be involved in detoxification

of xenobiotics in the lung (Cheng *et al.*, 2004) and this increased risk may be attributed to the possibility that children with the GSTP1GG genotype may be less able to defend their airways from the adverse effects of excess oxidative stress associated with asthma and may therefore be more susceptible.

Results from other studies of associations between asthma and GST polymorphisms often contradict each other. However these studies include different populations and varied study designs, which may account for these differences. Studies in a large cohort study in Southern California demonstrated a modest but significant association between decreased lung function (FEV_1) and the GSTM1null and GSTP1GG variants (Gilliland *et al.*, 2002a). These authors suggested that children with these genotypes, especially those with asthma, may have lower attained lung function at maturity and be more susceptible to adverse respiratory outcomes associated with oxidative stress. Tamer *et al.*, (2004) reported a higher prevalence of the GSTM1 null genotype (63.4%) in asthma patients than the control group (40.8%) OR=2.3 (CI 1.3, 4.2). In addition, they found that GSTP1 GG genotype had a 3.5 fold higher risk of atopic asthma and the combined GSTM1null and GSTP1GG genotypes were also more frequent among asthma patients (22.8%) than in the control group (7.8%).

Conversely, Fryer *et al.*, (2000) found that GSTP1 GG was considerably lower in asthmatics than in the control population with a 6-fold lower risk of asthma than GSTP1 AA. In their study, GSTP1 GG correlated with a decreasing severity of airway obstruction/BHR and 10 times lower risk of atopy defined by skin test positivity. These

authors reported no association with GSTM1 and BHR. This finding was corroborated in other studies. Gilliland *et al.*, (2002b) documented school absences in relation to respiratory illnesses and found that GSTP1GG was both protective against acute respiratory illnesses and was associated with a lower risk compared to the GSTP1AA genotype. The GSTM1 null genotype was associated with a slightly higher rate of respiratory illness than those with the GSTM1 pos genotype. Similarly, Lee *et al* (2005) found that homozygous GSTP1 AA was significantly associated with physician diagnosed asthma (adj OR =1.9). Since gene frequencies are different among various racial groups, this may account for the different associations between gene polymorphisms and asthma in the different studies. Additionally, each locus may be in linkage disequilibrium with an unknown causal gene(s), which is a fundamental limitation of the candidate gene approach. Different patterns of linkage disequilibrium could explain ethnic differences (Gilliland *et al.*, 2002a).

We considered the effects of pair-wise interactions between genes on each outcome parameter (Table 4.9 and 4.10). Evaluating the effect of 2 genotypes in combination may not necessarily translate to a simple additive effect, in fact some authors have suggested that the effects of the two genes may be competitive, thus decreasing the expected joint effect of the combination genotype (Lee *et al.,* 2005). Since most of the joint effects were reduced (most of the odds ratios for the combination genotypes in our population were to some extent protective, although not statistically significant), there may be competitive effects from the two genotypes studied. Subjects with the GSTM1 positive and the GSTP1 AG/GG genotype combination showed a significant association to persistent

asthma (OR=2.4, CI: 1.2, 4.9, p = 0.01). Similarly, in the GEE models this gene-gene combination showed significant effect modification with NO, NO_2 and PM_{10} in terms of decreased lung function.

The first report of NQO1 genotype in relation to asthma risk was published by David and coworkers in 2003, so research on NQO1 and asthma is relatively new. Although no significant associations were found in our study with the NQO1 genotype and any of the respiratory linked symptoms, we were interested in the interaction between GSTM1null and NQO1 CC genotypes. Bergamaschi and colleagues (2001) reported that subjects with the combined NQO1 CC and GSTM1 null genotypes are more susceptible to adverse effects of ambient ozone. Their work and that of David *et al.* (2003) showed decreased susceptibility to ozone among subjects carrying at least one Ser allele (NQO1 CT or NQO1 TT) and who are GSTM1null. Similarly in our population, we found a protective effect for those carrying the GSTM1null and at least one Serine allele (NQO1 CT/TT) for persistent asthma and marked BHR (OR=0.7, CI: 0.3-1.5 and OR=0.3, CI: 0.0-1.9 respectively). This protective effect in children with the variant NQO1 Ser allele, which is expected to have reduced activity, is consistent with the role of NQO1 in metabolic activation. Although often detoxifying, the wild type NQO1 CC can catalyze the reduction of some quinones to hydroquinones, which are more reactive and autooxidise to generate ROS (Bergamaschi *et al*, 2001).

The lead finding in our study was that the pollution-outcome relationship is modified by genotype. Asthma prevalence is likely a consequence of environmental factors increasing

the risk in genetically susceptible individuals. This view implies that a particular genotype will only manifest in phenotype, depending on environmental exposure, so it is important to study the gene-environment interaction in order to understand etiology of asthma.

Our GEE models provide evidence that participants experienced adverse effects on pulmonary function related to prior exposure to NO, NO_2, SO_2 and PM_{10}. These pollutants are related to oxidative stress and its associated role in respiratory illnesses. NO, NO_2 and SO_2 may produce free oxidative radicals while the mechanism for PM_{10} is that certain metal components in the particles may contribute to damage to the respiratory system via the generation of free radicals (Seaton *et al.*, 1995, Hong *et al.*, 2007). Genes involved in antioxidant and detoxifying reactions such as the GSTs and NQO genes are thus important in the response to oxidative stress.

Our null hypothesis was that the change in lung function measure when exposed to a unit increase in pollutant does not differ between wild type and polymorphic genotypes (i.e. the interaction between genotype and pollutant is zero). Children with the GSTM1null, GSTP1AA and the wild type NQO1CC genotypes showed adverse effects on lung function which were generally statistically significant for NO_2 and NO and to a lesser extent PM10. There were very few significant gene-environment interactions with SO2 and the 3 SNPs tested (Tables 4.11-4.22). Statistically significant lagged decrements in pulmonary function were more frequent among the children with the genetic polymorphisms GSTM1null and GSTP1AA perhaps as a consequence of their decreased

capacity to mount an effective cytoprotective response to pollutants. Direct support for this idea comes from human nasal provocation studies examining variation in responses to diesel exhaust particles (DEPs) (Gilliland *et al.*, 2004) where individuals with the genotypes GSTM1null and GSTP1AA showed enhanced susceptibility to DEPs. Researchers estimate that 15-20% of the population has both genetic variations so this represents a large group of people that are potentially susceptible to the adverse effects of air pollution. Additional proof of involvement of these genes comes from a randomized trial of children who live in high ozone areas in Mexico. The beneficial effect of antioxidant supplementation was seen primarily in the GSTM1null individuals and more pronounced among GSTM1null children with moderate to severe asthma (4.4%, p=0.04). Supplementation with antioxidant vitamins C and E above the daily minimum requirement might compensate for this genetic susceptibility (Romieu *et al.*, 2005).

Children with the NQO1CC wild type genotype also showed adverse lung function outcomes when exposed to NO, NO_2 and PM_{10}. Significant gene*environment effects with NO_2, NO and PM_{10} were observed for this genotype. Most of the literature on NQO1 to date focuses on its role as a detoxifying and antioxidant enzyme (Ross *et al.*, 2000). However, some of the hydroquinones produced by NQO1 reduction are less stable and prone to auto-oxidation with resulting ROS production (Zheng *et al.*, 2007). Given the epidemiologic data linking wild type NQO1 CC genotype with asthma and pulmonary susceptibility to ozone (Bergamaschi *et al.*, 2001, David *et al.*, 2003), it is speculated in this study that NQO1 may be activated in response to air pollutants leading to airway obstruction and decreased pulmonary function.

General trends in the change of estimates from lag 1 to lag 5 after exposure to pollutants were compared for each genotype. Adverse lung function was indicated by the higher estimates for intraday variability in FEV_1 and PF and by lower estimates for nadir FEV_1 and PF. While there was generally a lack of consistency between outcome and exposure among these trend graphs, there is an indication that the adverse outcome is somewhat worse on lag 1, followed by an improvement in lag 2 and 3 then subsequent deterioration at lags 4 and 5. The general trend in estimate change from one lag to another for PM_{10} exposure was significantly different from the three gaseous pollutants. There was a lower adverse effect at lag 1 with a trend of increasing adverse effect from lags 2-5.

We also evaluated different gene-gene combinations as effect modifiers of pulmonary response using the interquartile ranges for a 5 day average pollutant exposure (Table 4.23-28). Although power in the statistical models may have been reduced by stratifying by 12 different genotype combinations, 3 of these genotype combinations; GSTM1nullGSTP1AG/GG, GSTP1AG/GG NQO1CC and GSTM1pos NQO1CC consistently showed a significant interaction with NO, NO_2 and PM_{10} with decrements in lung function measures. Our findings with the GSTM1nullGSTP1AG/GG genotype is corroborated by Romieu *et al.,* (2006) who found that Mexican children with both the GSTM1null and the GSTP1 GG genotypes showed increased breathing difficulty in association with increase in ozone exposure.

These findings in single pollutant regression models suggest that the response to the level of air pollutants, as indicated by variability in pulmonary function measures, is modified by genotype and therefore demonstrates a gene-environment interaction. We studied the effects of 4 pollutants independently and accept that there are limitations to this approach since we 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. There have been few studies to date that have considered the gene-environment effect in relation to asthma and associated phenotypes. Lee *et al.* (2004) examined the relationship between the GSTP1 polymorphism, outdoor air pollution (designated high and moderate areas) and childhood asthma using 61 asthmatic schoolchildren and 95 controls in Taiwan. Similar to our study, they found that GSTP1AA conferred an increased risk of asthma in the moderate air pollution district (OR=1.5, 95% CI 0.7-3.1) and high pollution district (OR=2.9, CI 1.4-6.0). Research conducted by David *et al.*, (2003) suggested that the NQO1TT allele conferred a protective effect to risk of asthma among GSTM1-null children subjected to increased ozone exposure. In a Mexico City population of 159 asthmatics, Romieu *et al.* (2006) found that increases in breathing difficulty were associated with O_3 exposure in children with the GSTM1null (8%) per 20 ppb increase in daily 1h maximum daily average and GSTP1 GG (14%) which is contrary to our findings with GSTP1.

This study involved a large number of analyses. Three genes with six variants (also combined into twelve different gene-gene combinations) were evaluated with four markers of ambient air quality, six time lags of exposure and two functional outcomes, adjusted by six covariates, with attempts at determining associations among these. This resulted in several complex models, with a lack of consistency among the associations.

Multiple comparisons will always be an issue in any study that involves multiple outcomes. There is much controversy about correcting for multiple comparisons. It is argued that multiple comparisons can increase the overall error in significance testing. Errors in inference, including confidence intervals that fail to include their corresponding population parameters, or hypothesis tests that incorrectly reject the null hypothesis, are more likely. The type 1 error (α), under the hypothesis of no association between two factors, indicates the probability of the observed association from the data at hand being attributable to chance. The likelihood of false positives due to random error is thus increased with multiple comparisons (Savitz and Olshan, 1995; Schulz and Grimes, 2005).

While there are a variety of statistical methods available to adjust for multiple comparisons (e.g. Bonferroni adjustments), these are in general quite conservative. Bonferroni's correction for multiple comparison works reasonably well for moderately correlated variables. The conservatism of Bonferroni increases when the correlation between endpoints increases as in this study, where all four outcomes are different ways of measuring pulmonary function. In reality, multiple endpoints are not usually equi-correlated and normally distributed, even more, for discrete outcomes (such as symptoms) (Pocock et al., 1987). In epidemiological terms, invoking the concept of "multiple comparisons" does not provide an explanation of why a particular association was or was not found; analyzing other aspects of the data does not influence the data bearing on the hypothesis of interest. In fact, epidemiologists have expressed little enthusiasm for formal correction methods since they diminish statistical power (Savitz

and Olshan, 1995). However, in this study, it is prudent that we consider certain point estimates with caution, given that we did not correct for multiple comparisons, but rather look at patterns of significance in our data. We have attempted to address this multiple comparisons issue by disaggregating the data (Figure 4.5-4.20), and found little consistency between genotypes, pollutants, lag exposures and lung function outcomes.

A major impetus for this study was to determine whether the risk for adverse respiratory health outcomes is greater in south Durban compared to similar communities in the north of Durban. Both are well established communities with minimal migration (Naidoo *et al.*, 2006), the children that were sampled were very likely to have lived in these areas all their lives, so although there is no historical ambient pollution data available for North Durban, we can assume that the children in north Durban have had a lower lifetime exposure to pollutants than the children in south Durban. Students in the south schools are at almost twice the risk for persistent asthma (OR=1.9; CI: 1.2-3.2; p<.005) and 3 times the risk of BHR (OR=3.5, CI: 1.4-8.4, p<.005) than those in the north. The prevalence of doctor diagnosed asthma was identical in both regions (11%) which is in the similar range of findings of other studies conducted in South Africa (Erlich *et al.*, 1995; Nriagu *et al,* 1999).

The prevalence of reported symptom based asthma of any severity was very high in both areas (43.7% in the north and 48.8% in the south schools). In this study, a broad definition of "asthma of any severity" included the following: moderate to severe, mild persistent and mild intermittent asthma and included questions on cough, shortness of breath, chest tightness, wheeze and other symptoms. This may account for the high

prevalence of asthma of any severity in both regions. This prevalence of 48.8% of any asthma in the south correlates with the results from the Settlers school study conducted in south Durban in 2001 which found a 52% prevalence of asthma of any severity among learners, higher than any other South African report of asthma prevalence (Robins *et al.*, 2002). An earlier study by Nriagu and coworkers found a 37% self reported wheeze in south central Durban (1999). These rates are higher than that reported in other parts of South Africa and worldwide. Poyser and others found a 10% wheeze and a 12% severe asthma among children in Cape Town (Poser *et al.*, 2002), while the ISAAC study found that the prevalence of asthma among 13-14 year olds was 17% in North America, 13% in Western Europe, 10% in Africa and 15% in Cape Town (South Africa) (ISAAC, 1998).

Based on symptoms, 20.4% of children from the Type A classrooms had some grade of persistent asthma, compared to the methacholine challenge testing, which indicated that 10.3% had marked BHR ($PC_{20} \leq 2$ mg/ml) (Table 4.4). The differences in respiratory health between north and south schools were highlighted by tests of airway responsiveness: a greater number of students in the south schools had marked BHR (13.3%) compared to those from the northern schools (3.8%). When we included probable, possible and marked BHR, classified according to ATS guidelines, in a category of positive evidence of airway hyperreactivity, we found a prevalence of 28% positive evidence of airway hyperreactivity with 30.9% of children in the south presenting with any evidence of airway reactivity compared to 20.6% of children in the north. As an objective marker of airway disease, the overall rate of any grade of BHR is considerably higher than that reported in other populations, e.g. 12.5% reported in

African Americans and 14% BHR found among 10 year old children in the UK (Joseph *et al.*, 2002; Kurukulaaratchy *et al.*, 2002). We found a high prevalence of atopy of 40.4% in the type A classrooms with 38% atopic in the north and 43.8% in the south. This correlates with the high prevalence of any grade of asthma found in these areas.

Only 185 people provided information for caregiver smoking, therefore 50% of the data for this variable was missing. Similarly for the ETS variable, 21% was missing, and 48% of those with missing ETS information had persistent asthma. Parents may have deliberately withheld smoking information, since they did not want to be held accountable for exacerbating their child's asthma. Had the missing data been available, we would have been able to test for an interaction between smoking and genotype

There have been many studies documenting the effect of various air pollutants on respiratory symptoms independent of genetic risk, including SO_2, particulates and oxides of nitrogen (Schwartz *et al.*, 1993; Delfino *et al*, 1998; ISAAC, 1998; Roemer, 1998; Vedal *et al.*, 1998, Brauer *et al.*, 2002; McConnell *et al*, 2002). Generally, the findings in most of these studies provide adequate support for the air pollutant-health outcome associations seen in our study. This was most pronounced for the oxides of nitrogen, and to a lesser extent for PM_{10} and SO_2. The lack of a consistent finding with particulates and SO_2, the most important points of departure in comparison with previous studies, is explained in part to our poor exposure datasets for these pollutants – only the first intensive phase SO_2 data was used in the analysis – and also because of the extremely low (almost undetectable levels) of SO_2 seen in the northern areas.

Various hypotheses can be proposed to explain our gene-environment-health outcome findings. In the detoxification pathway, both Phase I and Phase II enzymes work in tandem to metabolize and excrete toxic substances. It is possible that those with defective GST and NQO1 enzymes cannot effectively detoxify and excrete intermediate metabolites produced in the Phase I detoxification step. These metabolites may provoke oxidative stress thus exacerbating respiratory symptoms. Therefore, individuals with lowered antioxidant capacity are at an increased risk for asthma. Oxidants in ambient air pollution produce oxidative stress in respiratory epithelial cells and individuals carrying specific genotypes may be better equipped to defend against the adverse affects of excessive oxidative stress.

When a pollutant first enters the lung, the first interface it encounters is the lung lining fluid. Antioxidant enzymes present in the lung lining fluid protect the lung against oxidative challenge arising from the air pollutants. It is the oxidized species arising from a reaction between the pollutant and the lung lining fluid compartment that is responsible for initiating the signaling cascade which brings the inflammatory cells into the lung (Kelly *et al.*, 2003). High concentrations of oxidants and pro-oxidants contained in ambient air pollution such as PM, NO, NO_2 and SO_2 promote oxidative stress and respiratory inflammatory responses (Kunzli and Tager, 2005). Spiteri *et al.* (2000) propose that apart from the detoxification of ROS, GSTP1 may also influence the synthesis of eicosanoids which are crucial mediators in the atopic asthmatic response. The conflicting GSTP result in the logistic models with respiratory symptoms compared

to the GEE models may be related to the smaller sample size used in the logistic models (n =369) compared to the repeated measures used in the GEE analysis.

Our study provided some notable findings, however, there were several limitations to our study, not least of which was the restriction on the number of genes that we were able to study. Twelve separate genotype combinations were analyzed. Although GSTM1, GSTP1 and NQO1 are situated on different chromosomes and linkage disequilibrium is not likely, these genes could be in linkage disequilibrium with other genes on the same chromosomes. Secondly, when conducting analysis of genotype combinations, the number of subjects in each grouping dropped drastically because of our small sample size, which may have introduced bias. Thirdly, our sample consisted of different race groups and effects of population stratification are always of concern in genetic epidemiology studies. This study did not have sufficient power to stratify by race group. The original sample was chosen for the South Durban Health Study which required a representative sample of the study area.

There are a limited number of studies that have investigated the gene-environment-disease triad and to our knowledge; this is the first to do so in the context of asthma in sub-Saharan Africa. The clearest examples of genetic interactions for inhaled pollutants exist for endotoxin, environmental tobacco smoke and ozone (London, 2007) but only a few studies have investigated NO, NO_2, SO_2 and PM_{10} in this context. Although this study was modest in sample size, repeated measurements of pulmonary function and daily and hourly pollutant measurements increased the power to detect interactions with

these genotypes. Effect modification of lung function reponse to pollutants was evident for children with the GSTM1null, GSTP1AA and the NQO1CC genotypes. Our results suggest that these genes are strong determinants of lung function decline in this population of South African schoolchildren. The suggested mechanism is that children with compromised oxidative defense ability are at increased risk of adverse pulmonary outcomes. This study supports the importance of further investigations on these and other genotypic variants involved in oxidative stress responses and respiratory linked phenotypes in larger cohorts.

CHAPTER 6:

CONCLUSIONS AND FUTURE DIRECTIONS

Our study provides additional support for the findings in numerous studies that genetic polymorphisms modify the risk for asthma and other respiratory outcomes in the presence of ambient pollution. Our cross sectional analysis has indicated that GSTP1 AG/GG and GSTP1 GG variants, which are involved in an individual's capacity to address oxidative stress, may influence the risk of persistent asthma. Additionally, GSTP1 is strongly expressed in the respiratory epithelium and the dominant GST in the lung could account for why our data indicated that a variation in GSTP1 function had larger effects on asthma than the other two genes tested. However, genetic polymorphisms of the GSTM1 and NQO1 genes both individually and in combination were not associated with the development of asthma and related phenotypes in our multiple logistic regression models. The increased risk conferred by the GSTP1 genotype may have clinical and public health importance since the variant is common in many populations and acute respiratory illnesses are frequent causes of morbidity.

It is likely that the risk of developing asthma is greatest when both genetic and environmental risk factors are present simultaneously. GSTM1 showed no association with any asthma related phenotype in our bivariate testing and logistic regression, but when air pollution was factored into the GEE models, GSTM1 was significantly associated with decrements in lung function. It is possible that GSTM1 is associated with

acute changes in respiratory health given an adverse environmental exposure, but the genotype does not influence the prevalence of asthma.

We have also demonstrated that pollutant exposure, especially with NO and NO_2, even at levels below the recommended South African guidelines, is associated with poorer lung function and that this association is significantly modified by an individual's genotype. Children with the GSTM1null, GSTP1AA and NQO1CC genotypes appear to be more susceptible to developing adverse respiratory symptoms related to air pollutant exposure especially to NO and NO_2. Since these genes are found in high frequencies in the general population, this has important implications for public health. This suggests a significant gene-environment interaction between the GSTM1, GSTP1 and NQO1 genotypes and air pollution.

In our study, it was disturbing to note that relatively modest increases in the concentrations of ambient pollutants affect respiratory health. In light of the substantial and consistent associations between ambient concentrations of the 4 pollutants assessed and adverse effects on lung function among children who are genetically predisposed, strategies for reducing ambient environmental pollution should be urgently considered. Current standards and guidelines should be reviewed. By presenting the distribution of risks across populations risk assessment can be far more effective in shaping public policy that is both preventative and fair. However, there is controversy around the ethical, economic, and legal ramifications of the use of genetic information.

The data on genetic susceptibility do not support a policy of large-scale individual screening, because there are too many polymorphisms involved that contribute to asthma risk and the costs would be prohibitive. Although genetic testing is not feasible or desirable, disease prediction might become feasible in the future. Although predictive testing for single gene disorders (e.g. cancers) is useful, predicted health gains for multifactorial diseases such as asthma are greater from those strategies directed at the whole population rather than targeted at a high risk group. Also, resource allocation to support genomics technologies is a problem in developing countries, where funding to treat disease epidemics such as HIV and TB is more important than allocating money to research in genetics. Therefore the gap is growing between those countries that can use this technology and those that cannot.

Public health associated benefits linked to genetic epidemiological information include tailored treatment regimens, prevention and management of disease. Genetic information may be used pre-symptomatically for targeted interventions including diet, medication or lifestyle modifications. Increased risk may advocate certain behavioral changes, but this is not always a successful strategy. Educating and advising smokers to quit smoking because it is known that smoking is a significant risk factor for lung diseases has not really provided the impetus to quit.

The increasing prevalence of asthma and other related respiratory diseases is an important public health concern. Studies such as ours, although preliminary, that evaluate the functional significance of particular polymorphisms according to whether their molecular

actions are influenced by environmental exposures, are important. With increasing industrialization, one can reason that genetically vulnerable high risk populations are being increasingly exposed to environmental influences that have altered in recent decades. In the near future, an understanding of the biology of candidate genes and gene-environment interaction may lead to development of more effective strategies to prevent or treat complex respiratory diseases.

CHAPTER 7:

REFERENCES

Adams, C.H., Werely, C.J., Victor, T.C., Hoal, E.G., Rossouw, G. and van Helden, P.D. 2003. Allele frequencies for glutathione S-transferase and N-acetyltransferase 2 differ in African population groups and may be associated with oesphageal cancer and tuberculosis incidence. *Clini chem lab med* 41(4):600-605.

Abbey, D.E., Petersen, F., Milis, P.K. and Beeson WL.1995. Long-term ambient concentrations of total suspended particulates, ozone, and sulfur dioxide and respiratory symptoms in a nonsmoking population. *Arch Environ Health* 8:33-46.

Aligne, C.A., Auinger, P., Byrd, R.S. and Weitzman, M. 2000. Risk factors for pediatric asthma: Contributions of poverty, race and urban residences. *Am J Respir Crit Care Med* 162:873-877.

American Thoracic Society. 2000. What Constitutes an Adverse Health Effect of Air Pollution? *Am J Respir Crit Care Med* 161:665-673.

Arif, A.A., Delclos, G.L., Lee, E.S., Tortorlero, S.R. and Whitehead, L.W. 2003. Prevalence and risk factors of asthma and wheezing among US adults: an analysis of the NHANES III data. *Eur Respir Journal* 21:827-833.

Arruda, L.K., Sole, D., Beana-Cagnani, C.E. and Naspitz ,C.K. 2005. Risk factors for asthma and atopy. *Curr Opinion Allergy Clin Immunol* 5(2):153-159.

Aynacioglu, A.S., Nacak, M., Filiz, A., Ekinci, E. and Roots, I. 2003. Protective role of glutathione S-transferase P1 (GSTP) Val105Val genotype in patients with asthma. *British J clin pharm* 57(2):213-217.

Bailey, R., Weingarten, S., Lewis, M.and Mohsenifar, Z. 1998. Impact of clinical pathways and practice guidelines on the management of acute exacerbations of bronchial asthma. *Chest* 113(1): 28-33.

Barnes, K.C. 2000. Atopy and asthma genes-where do we stand? 2000. *Allergy* 55:803-817.

Bell, D.A., Taylor, J.A., Paulson, D.F., Robertson, C.N., Mohler, J.L. and Lucier, G.W. 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 of the Natl Cancer Ins* 85(14):1159-1164.

Bennett, D.A. and Waters, M.D. 2000. Applying Biomarker Research. *Environ Health Perspect* 108(9):907-910.

Bergamaschi, E., De Palma, G., Mozzoni, P., Vanni, S., Vettori, M.V., Broeckaert, F., Bernard, A. and Mutti, A. 2001. Polymorphism of quinone-metabolizing enzymes and susceptibility to ozone-induced acute effects. *Am J Respir Crit Care Med* 163:1426-1431.

Bernstein, J.A., Alexis, N., Barnes, C., Bernstein, I.L., Bernstein, J.A., Nel., A. *et al.* 2004. Health effects of air pollution. *J Allergy Clin Immunol* 114:1116-23.

Bleecker, E.R., Postma, D.S., and Meyers, D.A. 1997. Evidence for multiple genetic susceptibility loci for asthma. *Am J Respir Crit Care med* 156:5113-5116.

Borish, L. 1999. Genetics of allergy and asthma. *Ann Allergy Asthma Immunol* 82:413-426.

Brasch-Anderson, C., Christiansen, L., Tan, Q., Haagerup, A., Vestbo, J. and Kruse, T.A. 2004. Possible gene dosage effect of Glutahione-S-Transferases on atopic asthma: using real time PCR for the quantification of GSTM1 and GSTT1 gene copy numbers. *Hum Mutation* 24:208-214.

Brauer, M., Hoek, G. and Van Vliet, P. 2002. Air pollution from traffic and the development of respiratory infections and asthmatic and allergic symptoms in children. *Am J Respir Crit Care Med* 166(8):1092-1098.

Buch, S.C., Notani, P.N. and Bhisey, R.A. 2002. Polymorphism at GSTM1, GSTM3 and GSTT1 gene loci and susceptibility to oral cancer in an Indian population. *Carcinogenesis* 23(5):803-807.

Burney, P. 1999. Air pollution and asthma: The dog that doesn't always bark. *The Lancet* 353, 9156 Health Module pg 859.

Carlisle, A.J. and Sharp, N.C.C. 2001. Exercise and outdoor ambient air pollution. British *J Sports Med* 35: 214-222.

Carroll, W.D., Lenney, W., Jones, P.W., Strange, R.C., Child, F., Whyte, M.K., Primhak, R.A. and Fryer, A.A. 2005. Effects of glutathione S-transferase M1, T1 and P1 on lung function in asthmatic families. *Clin Exp Allergy* 35:1155-1161.

Castro-Giner, F., Kauffmann, F., de Cid, R and Kogevinas, M. 2004. Gene-Environment interactions in asthma. *Occup. Environ. Med.* 63:776-786.

Chen, C.L., Lui, Q. and Relling, M.V. 1996. Simultaneous characterization of glutathione S-transfer Mi and T1 polymorphism by polymerase chain reaction in American whites and blacks. *Pharmacogenetics* 6:187-191.

Cheng, S-L., Yu C-J., Chen C-J. and Yang, P-C. 2004. Genetic Polymorphism of epoxide hydrolase and glutathione S-transfer in COPD. *Eur Respir J* 23:818-824

Child, F., Lenney, W., Clayton, S., Davies, S., Jones, P.W., Strange, R.C. and Fryer, A.A. 2003. Correction of bronchial challenge data for age and size may affect the results of genetic association studies in children. *Ped Allergy Immunol* 14:193-200.

Chipps, B.E. 2004. Determinants of asthma and its clinical course. *Annals Allergy, asthma and immunol* 93(4):309.

Cookson, W.O.C.M., Sharp, P.A., Faux, J.A. and Hopkin, J.M. 1989. Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. *Lancet* 1:1292-1295

CSIR. 2002. Air Pollution and Health effects in South Durban-A review of existing data, Final Draft. CSIR report ENV-D-C 2002-011. Project: JX 337(020). P.O. Box 17001, Congella, 4013.

D'Amato, G., Liccardi, G., D'Amato, M. and Holgate, S. 2005. Environmental risk factors and allergic bronchial asthma. *Clin Exp Allergy* 35:1113-1124.

Daniels, S.E., Bhattacharrya, S., James, A. *et al.*1996. A genome-wide search for quantitative trait loci underlying asthma. *Nature* 383:247-250.

David, G.L., Romieu, I. *et al.* 2003. Nicotinamide adenine dinucleotide (phosphate) reduced: quinone oxidoreductase and glutathione-S-tranfersae M1 polymorphisms and childhood asthma. *Am J Respir Crit Care Med* 168:1199-1204.

Delfino, R.H., Zieger, R.S., Seltzer, J.M., and Street, D.H. 1998. Symptoms in pediatric asthmatics and air pollution: differences in effects by symptom severity, anti-inflammatory medication use and particulate averaging time. *Environ Health Perspect* 106: 751-761.

Dworski, R. 2000. Oxidant stress in asthma. *Thorax* 55(Suppl 2):S51-S53.

ECOSERVE.1998. Air quality monitoring annual report, South Durban Environmental Monitoring Management Committee, Durban.

Erlich, R.I., Du Toit, D. *et al.* 1995. Prevalence and reliability of asthma symptoms in primary school children in Cape Town. *International J epidemiol* 24:1138-1145.

Erlich, R. 2002. The prevalence of asthma in South Africa. *Curr allergy and clin immunol* 15(1):4-8.

European Community Respiratory Health Survey (ECRHS).1997. Variations in the prevalence of respiratory symptoms, self-reported asthma attacks, and use of asthma medication in the European Community Respiratory Health Survey (ECRHS). *Eur Respir J* 10:2495-2501.

Fryer, A.A., Bianco, A., Hepple, M., Jones, P.W., Strange, R.C. and Spiteri, M.A. 2000. Polymorphism at the Glutathione S-tranferase GSTP1 locus: A new marker for bronchial hyperresponsiveness and asthma. *Am J Respir Crit Care med* 161:1437-1442.

Garte, S., Gaspari, L., Alexandrie, A.K. *et al.* 2001. Metabolic gene polymorphism frequencies in control populations. *Cancer epidemiol, Biomarkers and Prevention* 10: 1239-1248.

Gauderman, W.J., Avol, E., Gilliland, F. *et al.* 2004. The effect of air pollution on lung development from 10-18 years of age. *N. Engl J Medicine* 351(11):1057-106

Gauderman, W.J. 2006. Air pollution and Children: a unhealthy mix. *New England J Med.* 355(1):78-79.

Gehring, U., Cyrys, J., Sedlmeir, G. *et al.* 2002. Traffic-related air pollution and respiratory health during the first 2 yrs of life. *Eur Respir Journal* 19(4):690-698.

Gilliland, F.D., McConnell, R., Peters, J. and Gong, H. 1999. A theoretical basis for investigating ambient air pollution and childrens respiratory health. *Environ Health Perspect* 107(Suppl 3):403-407.

Gilliland, F.D., Gauderman, W.J., Vora, H., Rappaport, E. and Dubeau, L. 2002a. Effects of glutathione-S-transferase M1, T1 and P1 on childhood lung function growth. *American Journal of respiratory and critical care medicine* 166:710-716.

Gilliland, F.D., Rappaport, E.B., Berhane, K., Islam, T., Dubeau, L., Gauderman, W.J. and McConnell, R. 2002b. Effects of Glutathione S-Tranferase P1, M1 and T1 on acute respiratory illness in school children. *Am J Respir Crit Care med* 166:346-351.

Gilliland, F.D., Li Y, Dubeau L *et al.* (2002c). Effects of glutathione-S-transferase M1, maternal smoking during pregnancy, and environmental tobacco smoke on asthma and wheezing in children. *Am J Respir Crit Care Med.* 166:457-463.

Gilliland, F.D., Li, Y., Saxon, A. and Diaz Sanchez, D. 2004. Effect of glutathione-S-tranferase M1 and P1 genotypes on xenobiotic enhancement of allergic responses: randomized, placebo-controlled crossover study. *The Lancet* 10(363) 9403 p 119.

Gilmour, M.I., Jaakkola, M.S., London, S.J., Nel, A.E. and Rogers, C.A. 2006. How exposure to environmental tobacco smoke, outdoor air pollutants, and increased pollen burdens influences the incidence of asthma. *Environ Health Perspect* 114(4):627-633.

Gold, D.R. and Wright, R. 2005. Population disparities in asthma. *Annual Reviews in Public Health* 26:89-113.

Green, S.L., Gaillard, C., Song, E., Dewar, J.B. and Halkas, A. (1998). Polymorphisms of the beta chain of the high-affinity immunoglobulin E receptor (FcαRIβ) in South African

black and white asthmatic and nonasthmatic individuals. *Am J Respir Crit Care Med* 158:1487-1492.

Halliday, J.L., Collins, V.R., Aitken, M.A., Richards, M.P.M. and Olsson, C.A. 2004. Genetics and public health-evolution or revolution? *J epidemiol community health* 58:894-899.

Hayes, J.D. and Strange, R.C. 1995. Potential contribution of the glutathione S-transferase supergene family to resistance to oxidative stress. *Free Radic Res* 22(3):195-207.

He, J., Ruan, J., Connett, J.E., Anthonisen, N.R., Pare, P.D. and Sanford, A.J. 2002. Antioxidant gene polymorphisms and susceptibility to a rapid decline in lung function in smokers. *Am J Respir Crit Care Med* 166:323-328.

Hemmingsen, A., Fryer, A.A., Hepple, M., Strange, R.C. and Spiteri, M.A. 2001. Simultaneous identification of GSTP1 Ile105-Val 105 and Ala114-Val 114 substitutins using an amplification refractory mutation system polymerase chain reaction assay: studies in patients with asthma. *Respir Res* 2:255-260.

Hill, M.R. and Cookson, W.O.C.M. 1996. A new variant of the β subunit of the high-affinity receptor for Immunoglobulin E (FcαRIβ E327G): associations with measures of atopy and bronchial hyper-responsiveness. *Hum Molec Genetics* 5(7):959-962.

Howard, T.D., Whittaker, P.A., Zaiman, A.L., Koppelman, G.H., Zu, J., Hanley, M.T., Meyers, D.A., Postma, D.S. and Bleecker, E.R. 2001. Identification and association of polymorphisms in the interleukin-13 gene with asthma and atopy in a Dutch Population. *Am J Respir Cell Mol Biol.* 25:377-384.

Holla, L.I., Stejskalova, I. and Vasku, A. 2006. Polymorphisms of the GSTM1 and GSTT1 genes in patients with allergic diseases in the Czech population. *Allergy* 61:265-267.

Hong, Y.C., Hwang, J.H.K., Kim, J.K *et al.* 2007. Metals in Particulate Pollutants affect peak expiratory flow of schoolchildren. *Environ Health Perspect* 115 (3): 430-434.

Iannuzzi, M.C., Maliarik, M. and Rybicki, B. 2002. Genetic polymorphisms in lung disease: bandwagon or breakthrough? *Respir Res* 3:15.

Illig, T and Wjst, M. 2002. Genetics of asthma and related phenotypes. *Pediatr Respir Rev* 3:47-51.

International Study of Asthma and Allergies in Childhood (ISAAC) steering committee. 1998. Worldwide variations in the prevalence of asthma symptoms: ISAAC. *Eur Respir J* 12:315-335.

Ishii, T., Matsuse, T., Teramoto, S., Matsui, H., Miyao, M., Hosoi, T., Fukuchi, Y. and Ouchi, Y. 1999. Glutathione-S- transferase P1 (GSTP1) polymorphism in patients with chronic obstructive pulmonary disease. *Thorax* 54:693-696.

Ivaschenko, T.E., Sideleva, O.G. and Baranov, V.S. 2002. Glutathione-S-transferase u and theta gene polymorphisms as new risk factors of atopic bronchial asthma. *J Mol Med* 80:39-43.

Joseph, C.L., Ownby, D.R. et al. 2002. Does Low birth weight help to explain the increased prevalance of asthma among African Americans*?* *Annals allergy Asthma Immunol* 88:507-512.

Kabesch, M., Hoefler, C., Carr, D., Leupold, W., Weiland, S.K. and Von Mutius, E. 2004. Glutathione S-Transferase deficiency and passive smoking increase childhood asthma. *Thorax* 59:569-573.

Kabesch, M. 2006. Gene by environment interactions and the development of asthma and alergy. *Toxicology Letters* 162:43-48.

Kaley, D.J., Neill, P. and Gallwin, S. 1991.Comparison of the prevalence of reversible airway obstruction in Zimbabwean children. *Thorax* 46:549-553.

Kelly, F.J., Buhl, R. and Sandstrom, T. 1999. Measurement of antioxidants, oxidants and oxidant products in bronchoalveolar lavage fluid. *Eur Respir Rev*. 9:93-8.

Kelly, F.J. 2003. Oxidative stress: its role in air pollution and adverse health effects. *Occ Environ Med* 60:612-616.

Kelly, F.J. and Sandstrom, T. 2004. Air pollution, oxidative stress, and allergic responses. *The Lancet*. 363:95.

Kleeberger S.R. 2003. Genetic aspects of susceptibility to air pollution. *Eur Respir J.* 21:52S-56S.

Koren, H.S. 1995. Associations between criteria air pollutants and asthma. *Environ Health Perspect* 103(Suppl 6):235-242.

Kunzli N., Ackermann-Liebrich U., Brandli O., Tschopp J.M., Schindler C. and Leuenberger P. 2000. Clinically small effects of air pollution on FVC have a large public health impact. *Eur Respir J.* 15:131-136.

Kunzli, N. and Tager, I.B. 2005. Air pollution: from lung to heart. *Swiss Med Weekly* 135:697-702.

Kurukulaaratchy, R.J., Fenn, M., Twiselton, R., Matthews, S. and Arshad, S.H. 2002. The prevalence of asthma and wheezing illnesses amongst 10 year-old –school children. *Respir Med* 96(3):163-9.

Lagorio, S., Forastiere, F., Pistelli, R. *et al.* 2006. Air pollution and lung function among susceptible adult subjects: a panel study. *Environmental Health: A Global Access Science Source* 5:11.

Lee, Y.L., Lin, Y.C., Lee, Y.C., Wang, J.Y., Hsiue, T.R. and Guo, Y.L. 2004. Glutathione-S-transferase P1 gene polymorphism and air pollution as interactive risk factors for childhood asthma *Clin Exp Allergy* 34:1707-1713.

Lee,Y.L., Hsiue, T.R., Lee, Y.C., Lin, Y.C. and Guo, Y.L. 2005. The association between glutathione-S-transferase P1, M1 polymorphisms and asthma in Taiwanese schoolchildren. *Chest* 128:1156-1162.

LeSouef, P. 1997. Genetics of Asthma: What we need to know? *Pediatric Pulmonol* 15:3-8.

Li, X.Y., Gilmour, P.S., Donaldson, K. *et al.* 1996. Free radical activity and pro-inflammatory effects of particulate air pollution (PM_{10}) in vivo and in vitro. *Thorax* 51:1216-22.

Li, N, Hao, M., Phalen, R.F., Hinds, W.C. and Nel, A.E. 2003. Particulate air pollutants and asthma: a paradigm for the role of oxidative stress in PM-induced adverse health effects. *Clin Immunol* 3:250-265.

London, S.J. 2007. Gene-Air Pollution Interactions in Asthma. *Proceedings American Thoracic Soc.* 4:217-220.

Los, H., Koppelman, G.H. and Postma, D.S. 1999. The importance of genetic influences in asthma. *Eur Respir Journal* 14:1210-1227.

Matooane, M. 2002. Air pollution and health effects in South Durban - a review of existing data. CSIR Report. ENV-D-C 2002-011. June 2002.

Matooane, M. and Diab, R. 2002. Initial Survey of sulphur dioxide (SO_2) pollution health risk in the South Durban Industrial Basin. Paper read at the National Association for Clean Air Proceedings, Durban, 2002.

McConnell, R., Berhane, K., Gilliland, F. *et al.* 2002. Asthma in exercising children exposed to ozone: a cohort study. *Lancet* 359(9304):386-391.

McConnell, R., Berhane, K., Yoa L. *et al.* 2006. Traffic, Susceptibility, and childhood asthma. *Environ Health Perspect* 114 (5):766-772.

McDonnell, W.F., Abbey, D.E., Nishino, N. and Lebowitz, M.D. 1999. Long-term ambient ozone concentration and the incidence of asthma in nonsmoking adults: the AHSMOG study. *Environ Res* 80:110-121.

Meyers, D.A., Postma, D.S., Panhuysen, C.I.M. *et al.* 1994. Evidence for a locus regulating total serum levels mapping to chromosome 5. *Genomics* 23:464-470.

Miller, R.L. 1999. Breathing freely: the need for asthma research on gene-environment interactions. *Am J of Public Health* 89(6):819-822.

Moffatt, M.F. and Cookson, W.O.1997. Linkage and candidate gene studies in asthma. *Am J Respir Crit Care Med.*156(4):S110-2.

Nadel, J.A. and Busse, W.M. 1998. Asthma. *Am J Respir Crit Care Med* 157: s130-s138.

Nagel, F.O. 1993. The prevalence of childhood asthma in white primary schoolchildren in the Southern suburbs of Cape Town. M. Med Degree (Part Three), Department of Pediatrics, University of Cape Town.

Naidoo, R., Gqaleni, N., Batterman, S. and Robins, T. 2006. Final Project Report: South Durban Health Study.

Nelson, D.A., Johnson, C.C., Divine, G.W., Strauchman, C., Joseph, C.L. and Ownby, D.R.1997. Ethnic differences in the prevalence of asthma in middle class children. *Ann Allergy Asthma Immunol* 78:21-26.

NHLBI: National asthma Education and Prevention program: Guidelines for the Diagnosis and Management of Asthma.1997. Bethesda: National Institutes of Health

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

Ober, C. and Moffatt, M.F. 2000. The pathobiology of asthma: implications for treatment. *Clinics in Chest Med* 21(2).

Ober, C. 2005. Perspectives on the past decade of asthma genetics. *J Allergy clin Immunol* 116:274-278.

Ohtsuka, Y., Clarke, R.W., Mitzner,W., Brunson, K., Jakab, G.J. and Kleeberger, S.R. 2000. Interstrain variation in murine susceptibility to inhaled acid coated particles. *Am J Physiol Lung Cell Mol Physiol* 278:L469-L476.

Palmer, L.J., Daniels, S.E., Rye, P.J., Gibson, N.A., Tay, G.K., Cookson, W.O.C.M., Goldblatt, J., Burton, P.R. and LeSouef, PN. 1998. Linkage of chromosome 5q and 11q

gene markers to asthma-associated quantitative traits in Australian children. *Am J Respir Crit Care Med* 158:1825-1830.

Pearce, N., Sunyer, J., Cheng, J. *et al.*. 2000. Comparison of asthma prevalence in the ISAAC and the ECRHS. *Eur Respir J* 16:420-426.

Peden, D.B. 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. 515-28.

Peden, D.B. 2005. The epidemiology and genetics of asthma risk associated with air pollution. *J allergy Clin Immunol* 115:213-219.

Pediatrics: Policy Statement. 2004. Ambient air pollution: Health hazards to children. *Pediatrics* 114:1699-1707.

Peters, J.M., Avol, E., Gauderman, W., *et al.* 1999. A study of twelve Southern California Communities with differing levels and types of air pollution. *Am J Respir Ctrit Care Med* 159:768-775.

Peters, A., von Klot, S., Heier, M. *et al.*, 2004. Exposure to traffic and the onset of of mycardial infarction. *N Engl J Med* 351:1721-30.

Piirila, P., Wikman, H., Ritva, L., Katja, K., Rosenberg, C., Nordman, H., Hannu, N., Harri, V. and Hirvonen, A. 2001. Glutathione-S-transferase genotypes and allergic responses to diisocyanate. *Pharmacogenetics* 11:437-445.

Pocock, S.J., Geller, N.L., Tsiatis, A.A. 1987. The analysis of multiple endpoints in clinical trials. *Biometrics* 43:487-498.

Pope III, C.A. 1989. Respiratory disease associated with community air pollution and a steel mill, Utah Valley. *Am J Public Health* 79:623-8.

Pope, C.A., Burnett, R.T., Thurston, G.D. *et al.,* 1995. Particulate air pollution as a predictor of mortality in a prospective study of US adults. *Am J Respir Crit Care Med* 151:669-74.

Postma, D.S, Koppelman, G.H. and Meyers, D.A. 2000. The genetics of atopy and airway responsiveness. *Am J Respir Crit Care Med.* 162:S118-S123.

Poyser, M.A., Nelson, H., Erlich, R.T., Bateman, E.D., Puterman, A. and Wanberg, E. 2002. Socioeconomic deprivation and asthma prevalence and severity in young adolescents. *Eur. Respir J* 19 (5):892-8.

Robins, T., Batterman, S., Lalloo, U., Irusen, E., Naidoo, R., Kistnasamy, B., Kistnasamy, J., Baijnath, N. and Mentz, G. 2002. Air contaminant exposures, acute

symptoms and disease aggravation among students and teachers at the Settlers' School in South Durban. Interim Report, University of Natal Faculty of Health Sciences/University of Michigan School of Public Health.

Romieu, I., Meneses, F., Ruiz, S., Sienra, J.J., *et al.* 1996. Effects of air pollution on the respiratory health of asthmatic children living in Mexico City. *Am J Respir Crit Care Med* 154:300-307.

Roemer, W., Hoek, G., Brunekreef, B., et al., 1998. Daily variations in air pollution and respiratory health in a multicenter study, the PEACE project. Pollution effects on Asthmatic children in Europe. *Eur Respir* J 12(6):1354-61.

Romieu, I. *et al.,* 2002. Antioxidant supplementation and lung functions among children with asthma exposed to high levels of air pollutants. *Am J Respir Crit Care Med* 166:703-709.

Romieu, I., Sienra-Monge, J., Ramirez, M., Moreno-Macias, H., Reyes-Ruiz, N., Rio-Navarro, B.E., Avila, M.H. and London, S.J. 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, J.J. *et al.* 2006. GSTM1 and GSTP1 and respiratory health in asthmatic children exposed to ozone. *Eur Respir Journal* 28:953-959.

Ross, D., Kepa, J.K., Winski, S.L., Beall, H.D., Anwar, A. and Siegel, D. 2000. NAD(P)H: quinine oxidoreductase 1 (NQO1) : chemoprotection, bioactivation, gene regulation and genetic polymorphisms. *Chemico-Biological Interactions* 129:77-97.

Roth, M., Johnson, P.R., Borger, P. *et al.* 2004. Dysfunctional interaction of C/EBP alpha and the glucocorticoid receptor in asthmatic bronchial smooth-muscle cells. *N Engl J Med.* 351(6):560-74.

Salvi, S. and Holgate, S. 1999. Mechanisms of particulate matter toxicity. *Clin Exp Allergy* 29:1187-1194.

Samet, J.M. and White, R.H. 2004. Urban air pollution, health and equity. *J Epidemiol Community Health* 58(1):3-5.

Sandford, A., Weir, T. and Pare, P. 1996. The genetics of asthma. *Am J Respir Crit Care Med* 153:1749-1765.

Sandford, A.J. and Pare, P.D. 2000. The Genetics of asthma. *Am J Respir Crit Care Med* 161(3):S202-S206.

Savitz, D.A. and Olshan A.F. 1995.Multiple comparisons and related issues in the interpretation of epidemiologic data. *Am J Epidemiology* 142:904-908.

Saxon, A. and Diaz-Sanchez, D. 2005. Air pollution and allergy: you are what you breathe. *Nature Immunol* 6(3) 223-226.

Schulz, K.F and Grimes, D.A. 2005. Multiplicity in randomized trials 1: endpoints and treatments. *Lancet* 365:1591-95.

Schwartz, J., Slater, D., Larson, T.V., Pierson, W.E. and Koenig, J.Q. 1993. Particulate air pollution and hospital emergency room visits for asthma in Seattle. *Am Rev Respir Dis* 147:826-31.

Scirica, C.V. and Celedon, J.C. 2007. Genetics of Asthma. *Chest.* 132: 770S-781.

Sears, M.R., Burrows, B., Flannery, E.M., Herbison, G.P., Hewitt, C.J. and Holdaway, M.D. 1991. Relation between airway responsiveness and serum IgE in children with asthma and apparently normal children. *N Eng J Med* 325:1067-1071.

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

Seidegard, J., Vorachek, W.R., Pero, R.W. and Pearson, W.R. 1988. Hereditary differences in the expression of the human glutathione transferase active on trans-stilbene oxide are due to a gene deletion. *Proc Natl Sci USA* 85(19):7293-7297.

Shannon, M.W., Best, D. and Binns, H.J. *et al.* 2004. American Academy of Pediatrics: Policy Statement. Ambient Air Pollution: Health Hazards to children. 114(6):1699.

Sorenson, M., Skov, H., Autrup, H., Hertel, O. and Loft, S. 2003. Urban benzene exposure and oxidative DNA damage: Influence of genetic polymorphisms in metabolism genes. The Science of the Total Environment 309: 69-80.

Spiteri, M.A., Bianco, A., Strange, R.C., and Fryer, A.A. 2000. Polymorphisms at the glutathione S-transferase, GSTP1 locus: a novel mechanism for susceptibility and development of atopic airway inflammation. *Allergy* 55 Suppl 61:15-20.

Strachan, D.P. 2000. Family size, infection and atopy: the first decade of the "hygiene hypothesis" *Thorax* 55(Suppl.1):S2-10.

Suk, W.A. and Collman, G.W. 1998. Genes and the environment : their impact on childrens health. *Environ Health Perspect* 106:S3:817-820.

Suk, W.A., Murray, K. and Avakian, M.D. 2003. Environmental hazards to children's health in the modern world. *Mutation Res* 544(2-3):35-242.

Tamer, L., Calikoglu, M., Ates, N.A., Yidirim, H., Ercan, B., Saritas, E., Unlo, A. and Atik, U. 2004. Glutathione-S-Transferase gene polymorphisms (GSTT1 and GSTM1 and GSTP1 as increased risk factors for asthma. *Respirology* 9:493-498.

Trasande, L. and Thurston, G.D. 2005. The role of air pollution in asthma and other pediatric morbidities. *J Allergy Clin Immunol* 115:689-99.

Traver, R.D., Horiksohi, T., Danenberg, P.V.et al. 1992. NAD(P)H: quinone oxidoreductase gene expression in human colon carcinoma : characterisation of a mutation which modulates DT-diaphorase activity and mitomycin sensitivity. *Cancer Res* 52:797-802.

United Nations Environment Program and WHO report. 1994. Air pollution in the world's megacities. A report from the UN environment program and WHO. *Environment.* 5-37.

Van Strien, R.T., Koopman, L.P., Kerkhof, M. et al. 2002. Mite and pet allergen levels in homes of children born to allergic and non allergic parents: the PIAMA study. *Environ Health Perspect* 110:A693-A698.

Vedal, S., Petkau, J., White, R. and Blair, J. 1998. Acute effects of ambient inhalable particles in asthmatic and nonasthmatic children. *Am J Respir Crit Care Med* 157:1034-1043.

Von Mutius, E., Martinez, F.D., Fritzsch, C., Nicolai, T., Roell, G., Thiemann, H.E. 1994 Prevalence of asthma and atopy in two areas of west and East Germany. *Am J Respir Crit Care Med* 149:358-364.

Wang, Z., Chen, C., Niu, T., Wu, D., Yang, J., Wang, B., Fang, Z., Yandava, CN., Dreazen, J.M., Weiss, S.T. and Xu, X. 2001. Association of Asthma with the b2-adrenergic receptor gene polymorphism and cigarette smoking. *Am J Respir Crit Care Med* 163:1404-1409.

Weiss, S.T. Issues in phenotype assessment. In Liggett S.B, Meyers, D.A, editors. The Genetics of Asthma. New York: Marcel Dekker; 1996, pp 401-419.

Wiesch, D.G. and Meyers, D.A. 2000. Strategies for analyzing genotype-phenotype relationships in asthma. *J Allergy Clin Immunol* 105:S482-6.

Witte, J.S., Palmer, L.J., O' Connor, R.D. Hopkins, P.J. and Hall, J.M. 2002. Relation between tumour necrosis factor polymorphism TNF-308 and risk of asthma. *Eur J Hum Genet* 10(1):82-85.

Yeatts, K., Sly, P., Shore, S., Weiss, S., Martinez, F., Geller, A., Bromberg, P., Enright, P., Koren, H., Weissman, D. and Selgrade, M. 2006. A brief targeted review of

susceptibility factors, environmental exposures, asthma incidence, and recommendations for future asthma incidence research. *Environ Health Perspect* 114(4):634-640.

Zheng, S., Byrd, A.S., Fischer, B.M. *et al.* 2007. Regulation of MUC5AC expression by NAD(P)H: quinone oxidoreductase 1. *Free Radic Biol Med.* 42(9):1398-1408.

Zhong, S., Howie, A.F., Ketterer, B., Taylor, J., Hayes, J.D., Beckett, G.J., Wathen, C.G., Wolf, C.R. and Spurr, N.K. 1991. Glutathione S-transferase mu locus:use of genotyping and phenotyping assays to assess association with lung cancer susceptibility. *Carcinogenesis* Sep 12(9):1533-7.

Websites:

1. NHLBI/WHO. GINA workshop report, global strategy for asthma management and prevention, updated April 2002. Scientific information and recommendations for asthma programs. Washington, DC: US Department of Health and Human Services. NIH Publication no. 02-3659;2002. Available at: htpp://www.ginasthma.com.

2. *Asthma: How is asthma treated?* National Heart, Lung and Blood Institute [online]. USA. Available from: http://nhlbi.nih.gov/health/dci/Diseases/Asthma/Asthma_Treatments. Html [Accessed on 23rd July 2004].

3. GINA (Global Initiative for Asthma) Reports: Global Burden of asthma. Available :http://ginasthma.com/Reportitem.asp. [Accessed on 12 March 2006].

4. South African Air Quality guidelines (DEAT). Available from: http://ceroi.net/reports/durban/issues/air/guidelin.html [Accessed on 5 August 2006].

5. EPA guidelines. USA [online]. Available from: http://epa.gov/air/criteria.html [Accessed on 19 January 2005].

6. US Environmental Protection Agency: Latest findings on national air quality: 2000 status and trends. Research Triangle Park, NC: Environmental Protection Agency; 2001. Publication No EPA 454/K-01-002. Available at www.epa.gov/airtrends/reports.html Accessed April 18, 2004.

ANNEXURES

APPENDIX 3.1: SDHS methodology for the collection of epidemiological data including lung function measures, symptoms and allergy status

APPENDIX 3.2: Environmental monitoring of ambient pollutants

APPENDIX 3.3: Child Screening Questionnaire

APPENDIX 3.4: Child caregiver baseline survey

APPENDIX 3.5: Parent Informed Consent

APPENDIX 3.6: Child Informed Assent

APPENDIX 3.7: Information Sheet

APPENDIX 3.1

SDHS methodology for the collection of epidemiological data including lung function measures, symptoms and allergy status

3.1.1 Collection of symptom logs and bihourly lung function data

A central aspect of the health data collection was bihourly symptom logs and the measures of lung function collected five days per week over three week period in each of four seasons. This was the data used to determine whether there was an association between daily fluctuations in ambient air pollution levels and fluctuations in health status.

3.1.2 Bihourly measures of pulmonary function during the schoolday

The AirWatch® (iMetrikus, Carlsbad, California, USA) brand airway monitor was used to monitor fluctuations in peak expiratory flow (PEF) and forced expiratory volume at one second (FEV_1) of each participant. This portable, hand-held device has a number of distinct advantages over methods used previously to obtain repeated measures of a forced expiratory manoeuvre in field studies. First, unlike the case with traditional peak flow meters, the FEV_1 is also obtainable. FEV_1 has inherently greater reproducibility than PEF and is a more clinically relevant measure (Thiadens *et al.*, 1999). Second, results of up to 500 expiratory manoeuvres was digitally stored in each Air Watch. A unique patient identifier and the time and date of each expiratory manoeuvre was manually downloaded into a data base. Each participant received his/her own peak flow device, which were kept at the school, and was clearly labeled with the participant's full name to avoid inadvertent exchange of devices.

The quality of such peak flow and FEV1 measures collected in the field tend to be quite variable, but is responsive to focused training of participants in good technique with frequent reinforcement. An intensive training session was conducted at the school with the participants in the proper performance of peak flow maneuvers. As part of this training each participant was individually coached and observed by field supervisors to ensure his or her ability to perform valid and reproducible expiratory maneuvers. In addition, during the actual intensive phases of data collection when participants used the peak flow meters, supervisors observed expiratory maneuvers to ensure proper technique. Participants were retrained at the beginning of each of the four-week intensive data collection periods.

On each of the five schooldays during the week, participants were be asked to perform a session of three consecutive maneuvers every one and a half to two hours (four times per 5.5 hour schoolday: approximately 08h00, 09h45, 11h30 and 13h20), and immediately

prior to completion of the bihourly logs described above. The highest PEF and highest FEV_1 from each session, even if from different maneuvers, was used in data analyses. All schools were studied simultaneously.

3.1.3 Collection of baseline pulmonary data :Baseline spirometric assessments and methacholine challenge tests

Baseline spirometric assessments and methacholine challenge tests was conducted on all schoolchildren participating in the three-week intensive data collection sessions. All American Thoracic Society (ATS) guidelines for conducting spirometry were followed (ATS, 1995). Spirometers were calibrated at least twice a day with a three-liter syringe. Technologists who had undergone training in standard technique conducted spirometry, which was performed in a sitting position without nose clips. The lung function indices of primary interest included forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1). Special instructions were given to participants to ensure that tested individuals did not take any anti-asthmatic inhalers (12 hours before) or oral asthma medications (48 hours before) prior to the test. Participants with an obstructive pattern at baseline ($FEV_1/FVC < 0.75$) were administered an inhaled bronchodilator and had testing repeated. Those without a baseline obstructive pattern underwent methacholine or histamine nonspecific challenge testing according to an abbreviated protocol used in epidemiological surveys (Yan *et al.,* 1983). Special precautionary measures included having readily available oxygen and B_2-adrenergic agents for nebulization. Additionally, emergency medical personnel were either physically on site or within quick access at all times during nonspecific challenge testing. All students were assessed during school hours (Naidoo *et al.,* 2006)

3.1.4 Collection of questionnaire data

Carefully selected interviewers drawn from the communities or from students at the involved universities and Technikons were trained and supervised to conduct baseline interviews with participants and their caregivers. Training included techniques and practice in conducting interviews in a consistent and neutral fashion. Components of this questionnaire included demographic information; assessment of presence and severity of respiratory and other relevant symptoms using standardized validated questions from sources such as the British Medical Research Council and American Thoracic Society; validated questions to specifically address the presence and severity of asthma among participants including 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; potential confounding factors such as exercise, viral respiratory infections, exposure to cigarette smoke, pre-existing medical conditions. (Annexure1: Child screening questionnaire). All questionnaires were available in English, isiZulu and Afrikaans, and was conducted in the

language of choice of the interviewee by an interviewer fluent in that language. Child participants were interviewed at school and their caregivers at the homes of the participants.

3.1.5 Assessment of allergic status

All pupils who participated in the three-week intensive data collection study were requested to participate in skin prick testing. Antigens tested included mixed cockroach, mixed dust mite, mould mix (*Aspergillus*, *Cladosporium* and *Penicillium*), cat, dog, mouse, rat, ragweed, mixed grasses, plus histamine as a positive control and saline as a negative control. These tests were conducted at school on a different day than the methacholine challenge testing. Participants were informed to stop any antihistamines and any other reactive medication (H_2 antagonists, tricyclic antidepressants, corticosteroids etc) at least 24 – 72 hours pre-test. The test was applied to the volar surface of the forearm, and read approximately 15 – 20 minutes later. The wheal and erythema were read and measured according to a standardised method, and an outline of the wheal and erythema was recorded on see through tape for a permanent record. A greater than 2 mm difference in mean diameter between allergen and control wheal was considered as positive. Emergency health personnel were on site to clear each participant receiving skin testing and were equipped with proper medications and resuscitation equipment in the unlikely event that any individual had a severe reaction to a skin test. Collection of this data allowed for the assessment of whether skin test positivity is associated with genotype.

APPENDIX 3.2

ENVIRONMENTAL MONITORING OF AMBIENT POLLUTANTS

Conventional Pollutants

3.2.Nitrogen Dioxide (NO_2)

Monitoring. This pollutant was sampled continuously at 8 monitoring sites that can be grouped into 3 categories:

- Lower DSIB with 3 monitoring sites (Southern Works, Jacobs, Wentworth) that capture industrial sources.
- Central DSIB/traffic sites with 4 monitoring sites (Warwick, City Hall, King Edward, Ganges) that reflect primarily vehicular sources.
- Northern area with 1 monitoring (Ferndale) which is some distance from major roads and industry.

Monitoring of NO_2 (and NO) used conventional continuous gas-phase chemiluminescence's detection (Monitor Europe, model ML 9841 B, set to operate in a 0 to 1000 ppb range). These monitors are designated under US EPA regulations as an equivalent method.

Data processing/quality/status. At each site, data were collected as 5-min averages, which were processed to 1 hr averages if at least half of the data for that hour were available. The 1-hr averages were processed to 24 hr averages, from noon to noon, if at least half of the hourly data in the period were available. Data capture rates were good, e.g., for the period 2.1.04 through 1.10.05, the overall capture rate for valid 24-hour observations was 83.4% (range from 76% at King Edward to 94% at Ganges). The distribution plots do not show strikingly high statistical outliers, though a number of higher observations are seen at Ferndale, City Hall, Warwick, King Edward, etc.

Spatial variation. Concentrations across the 8 monitoring sites show that the lowest levels are in the north (11 ppb), highest concentrations in the center city and industrial areas (19 - 24 ppb), and somewhat lower levels at Southern Works and Wentworth in the south (12 - 14 ppb). As expected, concentrations were generally highest at traffic-impacted sites (City Hall, Warwick, and King Edward) and some of the industrial sites (especially Jacobs).

Temporal variation. At all sites, concentrations show very strong seasonality with the highest levels in the winter period (March - August, roughly 20–25 ppb), and the lowest levels in summer (October – February, 16-17 ppb).
Across much of the region, daily levels were moderately to highly correlated, e.g., correlation coefficients range from 0.51 to 0.84 among the lower basin monitors. Concentrations at the northern site are lower and have only low-to-moderate correlations

with the other sites (0.13 to 0.53). Some of the highest concentrations at all sites (except the northern site) were seen on July 21-23, 2004, a period that bears more investigation. Autocorrelation was high, about 0.7 for 1 day lags. All sites show small day-of-week effects, with levels about 10% lower on the weekends. Distributions at each site show several (1 to 6) 24-hour observations that might be considered "modest" statistical outliers. Traffic impacted sites (City Hall, Ganges, Warwick) had more statistical outliers.

Exposure estimates. This pollutant was not monitored at the school sites. To reflect a mixture of industrial and vehicular sources and derive population-oriented exposure estimates, several options were considered for the southern Durban area:

a) Averaging concentrations at the central and lower basin sites (7 monitoring locations).

b) Using the more representative lower basin sites (Wentworth, Jacobs, Southern Works and Ganges), excluding downtown and highly traffic impacted sites (Warwick, City Hall, King Edward). Although Ganges was originally considered to be traffic-impacted site, traffic influence was gauged to be only moderate and thus was included in the southern average.

Given the similar levels, high correlation, and the advantage of additional observations that can increase the representativeness of the data, we opted to use option b. This is supported by trends that show that the industrial sites appear to be occasionally influenced by local sources. Averaging across the 5 monitoring sites will diminish such effects.
For the northern Durban area, the northern site (Ferndale) was used to estimate exposures.

3.2.2 Nitrogen Oxide (NO)

Monitoring. This pollutant was sampled continuously at 8 monitoring sites as described for NO_2. (The same equipment is used to monitor NO and NO_2.) Many of the same results and conclusions apply for these closely related pollutants. This section discusses only significant differences.

Data processing/quality/status. At each site, data were collected as 5 min averages, which were processed to 1 hr averages if at least half of the data for that hour were available. The 1 hr averages were processed to 24 hr averages, from noon to noon, if at least half of the hourly data in the period were available. Data capture rates were good. For the period 2.1.04 through 1.1005, the overall capture rate for valid 24-hour observations was 83.4% (range from 76% at King Edward to 94% at Ganges).

Spatial variation. Based on averages, concentrations at Ganges and Warwick, the most traffic-impacted sites, were considerably higher than levels elsewhere, while levels at Wentworth and Southern Works, away from traffic but near industrial sources, were by far the lowest. High peak concentrations (> 200 ppb, 24-hr average maximum) were

occasionally observed at City Hall and King Edward, in addition to Warwick and Ganges (where levels reached or exceed 300 ppb).
Larger spatial differences were seen for NO compared to NO_2, reflecting the influences of local sources and the short lifetime of NO. As seen for NO_2, Levels at the northern site (Ferndale) were considerably lower than levels measured at most of the southern sites.

Temporal variation. At all sites, concentrations show very strong seasonality with the highest levels in winter (March - August, but peaking in July at up to 140 ppb), and lowest levels in summer (October – February, generally below 20 ppb). Given that NO (and NO_2) emissions are likely relatively uniform over the year, this variation is likely to result from the poorer dispersion conditions occurring the winter (as seen for other pollutants), and from the shorter lifetime of NO in the summer (due to faster reaction including scavenging by O_3).
Across the region, levels were highly correlated at the 24-hr level, e.g., correlation coefficients ranged from 0.5 to 0.9 among the 8 monitoring sites. Unlike NO_2, NO concentrations at the northern site remained highly correlated with NO levels at the other sites, though concentrations were lower. As for NO_2, some of the highest concentrations at all sites (except the northern site) were seen on July 21 - 23, 2004.
Autocorrelation was moderate, about 0.5 for 1 day lags. All sites showed moderately strong day-of-week effects, and concentrations fell by ~30% on the weekends, except at Ferndale where changes were smaller. Distributions at each site show a few 24-hour values that might be considered "modest" statistical outliers; however, the data generally performed consistently. Overall, NO patterns are consistent with vehicular and industrial emission sources.

Exposure estimates. This pollutant was not monitored at the school sites. The same options as discussed for NO_2 are appropriate. In this case, however, distance to major roads will likely be even more important.

3.2.3 Sulfur Dioxide (SO_2)

Monitoring. SO_2 was monitored continuously at 16 monitoring sites by ultraviolet fluorescence spectrometry using US EPA reference methods. Monitors were located at 7 schools:

1. Assegai Primary School initially used an API 100A but this was swapped out to an ML 2015 in May 2004 due to problems;
2. Dirkie Uys had a API 100A which failed and was replaced with a Dasibi 4108 in January 2005 (also problematic);
3. Nizam Primary School used an API 100A, which was replaced in January 2005 with a similar instrument (taken from Lamontville).
4. Lamontville (Entuthukweni) School used an API 100A, which failed, was repaired, and then moved to Nizam.
5. Briardale used a TECO 43A instrument.

6. Ferndale used a Monitor Labs ML 9850B instrument (serial no. M1873–M702)
7. Ngazana Primary School used a Monitor Labs ML 9850B instrument.

In addition, eThekwini Municipality monitored SO_2 at 9 stations in 2004, with the Settlers' monitor doubling as an H_2S analyzer (providing measurements every 10 min as compared to every 5 min at the other sites). Due to the need for H_2S data to resolve complaints in Merebank, the City Hall SO_2 analyser was moved to Southern Works in July 2004 to measure H_2S (eThekwini, 2004). Of the Municipality's 9 SO_2 analysers, 8 were relatively new (2003, Monitor Lab 9850B); the 9th instrument was from the Settlers School caravan (API 110A).

Data processing/quality/status. At each site (except one as noted above), data were collected as 5 min averages, which were processed to 1 hr averages if at least half of the data for that hour were available. The 1 hr averages were processed to 24 hr averages, from noon to noon, if at least half of the hourly data in the period were available.
Several of the monitors at the school sites experienced drift problems, probably a result of inadequate temperature control in the instrument or in the enclosure. Drift resulted in slowly varying negative or positive biases that was easily detected. This bias was corrected on a monitor-specific basis by subtracting the long term baseline, computed as a running average (typically considering a 400 h window) of low (1st) percentile hourly concentrations in a time window typically 68 hours before and 4 hours after the current value. Minima were allowed to vary only slowly (< 5 ppb) otherwise a new window was utilised. This approach is reasonable since background SO_2 values at all sites approached zero almost every day due to strong variation in source emissions and meteorology, and since background levels were negligible. Statistical and visual checks ensured that this approach yielded reasonable and robust values. Some small (< -1 ppb) negative values remain after this correction, a normal result for this measurement, even at monitors that do not experience excessive drift.
Overall, 6 324 valid 24-hr observations were collected across the 16 monitors for the period 1.1.04 through 6.10.05. The data capture rates varied across the monitoring sites. Due to equipment moves and instrument failures, SO_2 records are incomplete for certain periods at certain sites. For example, as few as 97 days of data were available for Lamontville, and 129 days at Briardale.

Spatial variation. Average concentrations across the 16 sites varied widely. SO_2 monitoring results are grouped into three categories:

- Low concentrations (1-3 ppb) at Briardale, Ferndale, Ngazana
- Medium concentrations (6-10 ppb) at Dirkie Uys, Nizam, Lamontville, City Hall, Grosvenor, and Prospecton
- High concentrations (12-20 ppb) at Assegai, Warwick, Jacobs, Settlers, Ganges, and Southern Works.

Southern Works had by far the highest concentrations, averaging 20 ppb with 24-hr peaks reaching 127 ppb, which shows the influence of nearby SO_2 sources (e.g., Mondi, Sapref and Engen). Overall, the spatial distribution reflects the distribution of industry in the South Basin.

Temporal variation. At all sites except Southern Works, concentrations show moderately strong seasonality with the highest levels in winter (March - August, but peaking in June/July) and the lowest levels in summer (December to February). Levels within an area showed low to moderate correlation. The highest intersite correlation was observed at the northern school sites (correlation coefficients of 0.5 – 0.7). In the Southern Basin, correlation coefficients were lower (typically 0.2 to 0.5). Correlations between Southern Works and the other monitoring sites were smaller (-0.1 to 0.5). Correlations were also low for the Settlers School and Prospecton sites with other sites.

Autocorrelation was low to moderate, ranging from 0.2 to 0.7. Day-of-week effects varied by site. Jacobs, Ganges and Southern Works showed the greatest variation, with weekend levels decreasing by 20 to 50% from weekday levels. However, weekend SO_2 concentrations increased by 25 to 35% at Dirkie Uys and Briardale. Insufficient data were available at Lamontville to quantify day-of-week patterns.
Time-of-day patterns often help to confirm the influence of local sources. Mornings have the highest concentrations at Dirkie Uys, Nizam, Wentworth, and Settlers.

- Afternoons have the highest concentrations at Assegai, Lamontville, Briardale, Ferndale, and Ngazana.

In part, this reflects the influence of the sea/shore breeze, e.g., the monitoring sites closest to the coast tend to be flushed with cleaner air in the afternoon, lowering concentrations. However, actual patterns are complex, and likely depend on season, the direction of local sources, the rotation of the wind field, and dispersion characteristics.
The distributions show that apparent statistical outliers occur at most of the monitoring sites, with the exception of Prospecton, which is some distance from large SO_2 sources.

Exposure estimates. While SO_2 was monitored at all schools, this pollutant shows considerable spatial variation. Moreover, large amounts of data were missing at several sites. We believe that the best approach to reflect spatial and temporal variation is to utilise school-based monitoring, after imputing missing data using SO2 concentrations (and other variables) collected across the entire network. With respect to averaging time, we considered using exposure measures based on hourly peaks and possibly 15-min averages, however, this was not done for several reasons: (1) Symptom data was not always collected at the same time; (2) some of the symptom data represented the previous 24-hr period; (3) our previous experience indicated 1-hr and 24-hr data are highly correlated; (4) typically 24-hr data provide more reliable results in the health models; and (5) imputing very short-term data was both difficult and unreliable.

3.2.4 Particulate Matter under 10 μm Diameter (PM_{10})

Monitoring. PM_{10} was monitored using several types of monitoring systems.

- Five TEOM (tapered element oscillating microbalance, Rupprecht & Patashnick, 1400a) samplers, a US EPA-approved method, were used by the eThekwini Municipality at five stations (Warwick, Ferndale, City Hall, King Edward and Ganges). TEOMs provide continuous measurements.

samplers were tested simultaneously at this site. The Partisol 2025 sampler was used as a reference sampler and was operated continuously throughout this period. Concentrations obtained by the reference sample (average = 37.4 ± 11.6 μg m^{-3}) closely matched simultaneous 24-hr PM_{10} TEOM measurements obtained at three nearby sites operated by eThekwini Municipality (City Hall, King Edward, Ganges; average = 37.0 ± 11.5 μg m^{-3}). This suggests that the results of the reference monitor were accurate, assuming that PM_{10} gradients are small (as shown below) and that losses of volatile and semi-volatile PM components on the heated TEOM are small relative to gravimetric measurements. Previous studies have indicated that TEOM losses are generally below 10% for $PM_{2.5}$, and a considerably lower loses are expected for PM_{10}.

The collocation comparisons included 19 days in which 126 PM_{10} measurements were obtained (average of 10.5 measurements per sampler, 18 – 38 measurements per sampler type). Concentrations during this period spanned a large range (19 to 102 μg m^{-3} based on the reference sampler), thus providing an excellent test. Study results indicated that 97% of the samples fell within 20% of the reference sampler. (One sampler type, a Topaz, failed to operate properly and is excluded from this analysis.) Some small biases were identified by sampler type, as indicated by the regression results below:

C(Partisol 2000) = 1.01 C (Partisol 2005) R^2 = 0.91 n = 18

C(DHA 80 HiVol) = 0.919 C(Partisol 2005) R^2 = 0.84 n = 32

C(FG 95 MedVol) = 0.921 C(Partisol 2005) + INTERCEPT R^2 = 0.89 n = 38

Mean and absolute mean biases were 0.6 and 6 μg m^{-3}, respectively. Overall, the agreement, high correlation, and small biases, indicate that PM_{10} measurements obtained by the five types of samplers (Partisol 2000, Partisol 2005, DHA Hivol, FG MedVol, TEOM) were comparable.

Collocation study 2. A second "collocation" study was obtained by maintaining a TEOM monitor at Ferndale throughout the study, in addition to the filter-based method (low volume Thermo sampler) also used at the same site. Both TEOM and filter-based PM_{10} measurements were available for 129 days. Several statistical outliers, e.g., much higher PM_{10} concentrations obtained from the filter-based measurement, were noted on several days (e.g., 26.9.04 and 9.7.05). Comparing all available PM_{10} data (including these outliers), the correlation coefficient was 0.71, 84% of observations were within a factor of 2, the mean bias was 9 μg/m^3, and the relative bias was 11% (the filter-based method yielded higher concentrations). Overall, this performance does not match the performance obtained in the first collocation study. The poorer performance may have resulted from many possible reasons: (1) This distant site (Ferndale) involved the most sample transport which may have resulted in particle loss, and thus may represent a worst-case situation, though it is recognised that all of the school-based sites involved sample transport. (2) Losses of volatile and semi-volatile components from the TEOM measurements might cause differences from the gravimetric measurements, as mentioned above. Differences might be larger at this site since ambient temperatures are slightly cooler at the higher elevation of Ferndale (thus increasing the temperature difference

between the TEOM and ambient air). Still, this seems unlikely to cause large differences. (3) The presence of strong localised sources that produce a range of particle sizes that are sampled differentially by the TEOM and the Thermo Low Volume samplers, a result of different sampling efficiencies or "cut-points." However, mean concentrations did not vary much between the TEOM and gravimetric measurements, suggesting this was not a major factor. (4) Experimental error, including sampler, operator and laboratory errors. We cannot definitively identify the error source, and all of these (and perhaps other) errors might be responsible. As discussed in the next section, we identified 5 outliers at this site, the removal of which improve results significantly, e.g., the correlation between TEOM and filter-based measurements at Ferndale increased to 0.79 (from 0.70).

Data processing/quality/status. Data capture rates varied across the monitoring sites and by monitoring type. The TEOMs had capture rates exceeding 80%. Filter-based samples at the schools were collected daily during intensives, and less frequently, on an every 6th day schedule. The number of 24-hr filter samples at the sites ranged from 131 at Briardale to 168 at Nizam. The filter-based methods did not achieve the precisions of the TEOM measurements, a result of many factors, e.g., sampler errors, particle/fibre shedding during filter handling and transport, and errors in pre- and post-exposure weighing (that in turn are partly attributable to fluctuations in the weighing room temperature and humidity). Because the TEOM monitoring was continuous and filter-based methods focused on the intensive periods, these monitoring types are analysed separately. TEOM measurements are not adjusted for possible losses of volatile and semi-volatile compounds. We also noted that while the TEOM PM_{10} levels were correlated across the region (though at times certain areas were elevated due to local influences as discussed below), a small number of the filter-based PM_{10} measurements were low, particularly at Ferndale where a few observations fell below the collocated TEOM PM_{10} measurements. The reasons for such discrepancies are unknown (these samples passed the normal QA checks) as discussed above. To identify such low values, we identified the 2nd lowest daily TEOM PM_{10} measurement in the network as a background concentration, which was decreased by 25% to account for possible experimental errors. Then, filter-based TEOM measurements falling below this value were considered to be erroneous and thus deleted. Over 21 months of daily data, this procedure removed only 16 observations (2 to 5 observations at each site). We also considered high-end outliers by visually examining the trend plots using both TEOM and filter data, and looked for filter-based measurements that were a factor of 2 or more above TEOM measurements. Five such observations were located, from 133 to 267 $\mu g\ m^{-3}$. These outliers quite clearly were erroneous and their removal greatly enhanced the performance of the imputation procedures, which provides additional support for the validity of this procedure.

Spatial variation. Based on averages, TEOM-based PM_{10} concentrations were nearly identical, 38 – 39 $\mu m\ m^{-3}$, at four sites, and slightly elevated, 46 $\mu g\ m^{-3}$, at Ganges. Concentrations at other percentiles were also closely matched. For the filter-based measurements, average concentrations at the 7 school sites were in the same range, 41 – 58 $\mu g\ m^{-3}$. Thus, only modest spatial differences are observed for average PM_{10} concentrations. Maximum 24-hr average concentrations approached or exceeded 150 μg

m^{-3} at most sites. The highest concentrations were observed at Assegai (south) and Ngazana (north), two widely separated monitors. Assegai may be affected by a combination of industrial, vehicular and open burning emissions; Ngazana may be largely affected by open burning emissions.

Temporal variation. All sites showed very strong seasonality with the highest levels in winter (March – August), typically peaking in July (when 24-hr concentrations exceeded 150 $\mu g\ m^{-3}$ at 11 sites), and the lowest levels in summer (December – April).
The five TEOM-based measurements tracked extremely closely ($r > 0.92$). This also applied to the TEOM PM_{10} concentrations at the northern site (Ferndale) when compared to levels at the four DSIB TEOM monitors, though Ferndale concentrations were slightly lower. The TEOM measurements were moderately to highly correlated with the filter-based measurements, with correlation coefficients from 0.53 to 0.82. Thus, PM_{10} levels were moderately-to-highly correlated across all sites.
PM_{10} levels showed moderate to high autocorrelation, about 0.6 to 0.9 for 1 day lags. No day-of-week effects were indicated. As mentioned, distributions most sites include several 24-hour values that might be considered modest statistical outliers.

Exposure estimates. This pollutant shows significant temporal variation, but relatively little spatial variation. There are several approaches:

a) Filter-based measurements are available at the 7 schools and could be used.

b) A Durban-wide PM_{10} measurement based on TEOMs could be derived, e.g., as a 5-site average.

c) A DSIB-wide average based on TEOMS could be derived, e.g., as a 4-site average, and the TEOM measurements at Ferndale would be used for the three northern schools.

d) Combined approach, e.g., using filter based measurements at the 7 school sites but imputed and bounded using TEOM data.

The TEOM-based approaches would provide similar results, given the high correlation, but would offer the advantage that TEOMs provide more reliable and continuous coverage. On the other hand, the filter-based methods may reflect local spatial gradients at the schools. Also, the filter-based methods collected samples from noon-to-noon. The TEOM data are hourly, and thus can be manipulated to obtain 24-hr average values for various start/stop times. We selected approach d, which relied upon school-based filter-measurements with imputation and validation checks that used, among other, information provided by the TEOM measurements.

Results for Air Pollutant Monitoring

Compliance with guidelines and standards
Below are the monitoring results and exposure assessment approach for pollutants monitored in this study. The WHO guidelines are used with several "lower" target levels. Analysis included measurements collected at permanent sites in the eThekwini

monitoring network and monitoring conducted in the SDHS conducted in schools and elsewhere.

- Average SO2 values in 2005 achieved the limit value of 19 ppb at all sites. The Southern Works site was the network's hotspot with a value of 22 ppb in 2004 and 16 ppb in 2005. The same site exceeded the daily limit value (48 ppb) 34 times in 2004 and 1 times in 2005, and the 10 min value (191 ppb) 796 times in 2004 and 240 times n 2005. The 2005 reductions are attributed to the installations of SO2 scrubbers at Mondi in May 2005. There were additional exceedances of the 10-min and 24-hr limit values at Settlers, Ganges (not in 2005), Grosvenor, Wentworth and Jacobs, but not at outlying sites (Prospection and Ferndale). In the school based monitoring, no sites exceeded the annual limit value, and two exceedances of the 24 hr limit value were noted at Assegai and Lamontville, 1 each).
- Average PM10 concentrations at at Ganges (46 and 43 ug/m3 in 2004 and 2005, respectively) exceeded the annual limit (40 ug/m3) in 2005. The annual PM10 target value (30ug/m3) was exceeded at all 5 PM10 monitoring sites. The 24-hr limit values (75 ug/m3) was exceeded 18-36 times at each of the five sites monitoring PM10. The number of exceedances of the 24-hr PM10 target value (50ug/m3) was not specified, however it was calculated that all sites had multiple exceedances ranging from 34 (King Edward) to 64 (Ganges) times for about 570 study days at each site. In the school based monitoring, all 7 sites also exceeded the annual limit value, and all sites also exceeded the 24-hr limit multiple times, from 2 (Nizam) to 36 (Assegai) times over the studt period (based on 130 daily samples at each site).
- Average NO2 at Warwick and Ganges (21 ppb) exceeded or attained the annual limit value (21 ppb) in 2005. The 1-hr limit (106 ppb) was exceeded from 0-5 times at the 8 sites measuring this pollutant in 2005, down from 0-9 times per site in 2004.

APPENDIX 3.3

Child Screening Questionnaire

South/North Durban Health Study

S1. Date:____/____/____ S2. Study Identification No. | | | | - | | | | - | | | |

Day Month

Year

Screening Questionnaire

Screening Questionnaire about

_____ ***[full name of child must be printed here by study staff or teacher before the questionnaire is brought home]***

(This questionnaire is available in isiZulu. Please request this version if this is your preference)

This questionnaire should be completed by the person who most often takes care of the child.

Please put a tick ☑ in the correct box for each question.

BACKGROUND INFORMATION

S3. Child's full name **[should be filled in before child brings this home]**	_____ First _____ Middle _____

	Surname
S4. Child's current grade in school	____
S5. Child's date of birth	_____/_____/_____ day month year
S6. Is the child:	☐1 Male ☐2 Female
S7. Usual language spoken at home:	☐1 English ☐2 Zulu ☐3 Xhosa ☐9 Other (Specify:_____)
S8. Your name:	_____ _____ First Middle _____ Surname
S9. Your telephone numbers:	home: _____ work: _____ cell: _____ or ☐1 I do not have a telephone

We may need to contact you again to obtain additional information. Please give me the name, address and telephone number of two relatives or friends who would know where you could be reached in case we have difficulty in contacting you.

S10. Name of first contact person:

_____

S11. Telephone number of first contact person: _____

S12. Address of first contact person:

_____ _____

House No. Road/Street

_____

_____

Suburb/Township

Postal Code

S13. Relationship of contact person to you: _____

S14. Name of second contact person:

_____

S15. Telephone number of second contact person: _____

S16. Address of second contact person:

_____

House No. Road/Street

_____ _____

Suburb/Township **Postal Code**

S17. Relationship of contact person to you: _____

S18. What is the complete address of the household where the child sleeps most often ?	_____ House No. _____ Road/Street _____ Suburb/Township _____ Postal Code
S19. How does the child usually get to school?	☐1 walks ☐2 driven in a private vehicle ☐3 driven in a taxi ☐4 takes a bus ☐9 Other (Specify:_____)
S20. Are you the main person who takes care of this child?	☐1 Yes ☐2 No
S21. How are you related to **this child**?	☐1 Mother ☐2 Father ☐3 Grandmother ☐4 Grandfather

	☐5 Aunt ☐6 Uncle ☐9 Other (**specify:** _____)

A. CHILD'S SYMPTOMS INFORMATION

S22. In the past 12 months, how often has your child had a **cough that won't go away**?	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never
S23. In the past 12 months, how often has your child had **wheezing** (a whistling sound from the chest) **with a cold**?	☐1 more than 1 time per month ☐2 3 to 12 times in the whole year ☐3 1 or 2 times in the whole year ☐4 never
S24. In the past 12 months, how often has your child had **wheezing** (a whistling sound from the chest) ***without* a cold**?	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never
S25. In the past 12 months, how often has your child had an attack of **wheezing** that made it **hard to breathe or catch his or her breath**?	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never
S26. In the past 12 months, how often has your child **wheezed while exercising, running or playing?**	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never
S27. In the past 12 months, how often has your child **coughed while exercising, running or playing**?	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never
S28. In the past 12 months, how often has your child complained that his or her **chest felt tight or heavy**?	☐1 every day ☐2 more than 2 times per week ☐3 more than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 never

S29. In the past 12 months, how often has your child's **sleep been disturbed due to wheezing, coughing, chest tightness or shortness of breath**?	☐1 most nights ☐2 more than 1 time per week ☐3 more than 2 times per month ☐4 more than 1 time per month ☐5 3 to 12 times in the whole year ☐6 1 or 2 times in the whole year ☐7 never
S30. Has a doctor or nurse **EVER** said that your child had: (check ALL that apply)	☐1 Asthma ☐2 Bronchitis or Bronchiolitis ☐3 Reactive Airway Disease (RAD) ☐4 Pneumonia ☐5 Asthmatic Bronchitis
S31. In the past 12 months has your child **taken any medications, nebulisers, or inhalers (pumps) prescribed by a doctor** for any of the conditions listed above?	☐1 Yes ☐2 No
S32. Does your child take **any of these doctor-prescribed medications every day**, even when he/she is **not** having trouble breathing?	☐1 Yes ☐2 No ☐8 Does not apply
S33. In the past 12 months how many times has your child **had to make an unplanned visit to a doctor's office for breathing problems?**	☐1 0 times ☐2 1 time ☐3 2 times ☐4 3 or 4 times ☐5 5 or 6 times ☐6 7 times or more
S34. In the past 12 months how many times has your child **been to the emergency room** (but not stayed overnight in the hospital) **for breathing problems?**	☐1 0 times ☐2 1 time ☐3 2 times ☐4 3 or 4 times ☐5 5 or 6 times ☐6 7 times or more
S35. In the past 12 months how many times has your child **had to stay in the hospital for one night or more because of breathing problems?**	☐1 0 times ☐2 1 time ☐3 2 times ☐4 3 or 4 times ☐5 5 or 6 times ☐6 7 times or more

B. MEMBERS OF YOUR HOUSEHOLD

S36. How many people live in your household? (Include the child, yourself, and all other adults and children whether related to you or not)	_____ (write in number)
S37. Write in the name of the person who you consider to be the head of the household -- this will usually be the person who owns or rents this home	**Head of Household** _____ _____ _____ **first name** **middle name** **surname** **OR** ☐1 I am the Head of Household
S38. Does this person (or you, if you are the head of the household) have a husband or wife who also lives in this household?	☐1 Yes ☐2 No
S39. If "yes", write in the name of this person	**Husband or Wife of Head of Household** _____ _____ _____ **first name** **middle name** **surname** **OR** ☐1 I am the husband or wife of the Head of Household
S40. Write in the name of the person who most often takes care of the child.	**Person who most often takes care of the child** _____ _____ _____ **first name** **middle name** **surname** **OR** ☐1 I am the person who most often takes care of the child

TABLE 1: TABLE OF PERSONS LIVING IN YOUR HOUSEHOLD – LIST EVERYONE WHO USUALLY SLEEPS AT YOUR HOUSEHOLD – BOTH ADULTS AND CHILDREN. INCLUDE PERSONS WHO ARE NOT RELATED TO THE HEAD OF THE HOUSEHOLD, AS WELL AS FAMILY MEMBERS. START WITH THE THE CHILD, FOLLOWED BY YOURSELF.

[PLEASE PRINT CLEARLY!]

S41. FIRST NAME	**S42.** MIDDLE NAME	**S43.** SURNAME	**S44.** RELATIONSHIP TO HEAD OF HOUSEHOLD	**S45.** AGE OF PERSON IN YEARS	**S46.** SEX OF PERSON	
a.	**a.**	**a.**	**a.**	**a.**	☐1 Male	☐2 Female
b.	**b.**	**b.**	**b.**	**b.**	☐1 Male	☐2 Female
c.	**c.**	**c.**	**c.**	**c.**	☐1 Male	☐2 Female
d.	**d.**	**d.**	**d.**	**d.**	☐1 Male	☐2 Female
e.	**e.**	**e.**	**e.**	**e.**	☐1 Male	☐2 Female
f.	**f.**	**f.**	**f.**	**f.**	☐1 Male	☐2 Female
g.	**g.**	**g.**	**g.**	**g.**	☐1 Male	☐2 Female
h.	**h.**	**h.**	**h.**	**h.**	☐1 Male	☐2 Female
i.	**i.**	**i.**	**i.**	**i.**	☐1 Male	☐2 Female
j.	**j.**	**j.**	**j.**	**j.**	☐1 Male	☐2 Female
k.	**k.**	**k.**	**k.**	**k.**	☐1 Male	☐2 Female
l.	**l.**	**l.**	**l.**	**l.**	☐1 Male	☐2 Female
m.	**m.**	**m.**	**m.**	**m.**	☐1 Male	☐2 Female
n.	**n.**	**n.**	**n.**	**n.**	☐1 Male	☐2 Female

S47. Do you have any comments about the project or the questionnaire?

_____
_____
_____
_____
_____

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE

Please have your child return it by _____, _____ to his or her teacher.

APPENDIX 3.4

Child caregiver baseline survey

South/North Durban Health Study

G1. Date:____/____/____ G2. Study Identification No. | | | | - | | | | - | | | |

Day Month

Year

Child Caregiver Baseline Survey

Cover Sheet

G3. Name of respondent:	_____ First _____ Middle _____ Surname
G4. Relationship to Head of Household:	_____
G5. Phone numbers:	**home:** _____ **work:** _____ **cell:** _____
G6. Name of child participating in the study:	_____ First _____ Middle _____ Surname
G7. Child's birth date:	____/____/____

	Day Month Year
G8. Child's sex:	☐1 Male ☐2 Female
G9. Child's school:	_____
G10. What is the complete address of this household?	_____ House No. _____ Road/Street _____ City _____ Postal Code
G11. Interviewer's Name:	_____
G12. Interview time started:	Time: _ _:_ _ am/pm
G13. [INTERVIEWER: Enter gender of respondent]	☐1 Male ☐2 Female
G14. Who is the person most responsible for care of **[child]** or most familiar with any health problems (s)he has?	_____
[If answer is not "me" then assess informally whether the person knows enough to complete the questionnaire.]	
G15. How are you related to **[child]**?	☐1 Mother ☐2 Father ☐3 Grandmother ☐4 Grandfather ☐5 Aunt ☐6 Uncle ☐7 Other (**SPECIFY:** _____)

[INTRODUCTION: INTERVIEWER READS TO RESPONDENT]

The purpose of this questionnaire is to collect information about your child's health status.

Your answers will help us figure out how to assist you and your child in protecting your child's health. If there is a question you do not want to answer, please let me know and we can skip it. All of your responses are confidential and will not shown to anyone outside the study team without your written consent.

A. BIRTH HISTORY

G16. In what country was **[CHILD]** born?	_____ country name
G17. What is the highest grade or year of regular school has **[CHILD]** completed?	Grade_____
G18. How old was the biological mother of **[CHILD]** when **[CHILD]** was born?	_____ years age
G19. Did **[CHILD]'s** biological mother smoke at anytime while she was pregnant with **[CHILD]?**	☐1 Yes ☐2 No ☐8 Don't know
G20. At any time during the pregnancy did **[CHILD]'s** biological mother quit or refrain from smoking for the rest of the pregnancy?	☐1 Yes ☐2 No ☐8 Don't know
G21. Did **[CHILD]** receive any newborn care in an intensive care unit, premature nursery or any other type of special care facility?	☐1 Yes **[if yes: How many days: _____ days]** ☐2 No
G22. How much did **[CHILD]** weigh at birth?	_____grams number ☐8 Don't know
G23. Did **[CHILD]** weigh more than 2.5 kg or less?	☐1 more than 2.5 kg ☐2 2.5 kg or less ☐8 Don't know
G24. Did **[CHILD]** weigh more than 4.0 kg or less?	☐1 more than 4.0 kg ☐2 4.0 kg or less ☐8 don't know

B. DIET

G25. How often does **[CHILD]** eat breakfast: everyday, on some days, rarely, never, or on weekends only?	☐1 everyday ☐2 some days ☐3 rarely ☐4 never ☐5 weekends only
G26. During the past 12 months, has **[CHILD]** changed eating habits to try to lose weight?	☐1 Yes ☐2 No
G27. During the past 12 months, has **[CHILD]** changed what you eat for any medical reason or health condition?	☐1 Yes ☐2 No
G28. What was the medical reason or health condition that caused **[CHILD]** to change	☐1 High Blood Pressure/Hypertension ☐2 High Blood Cholesterol

what he/she eats? **[Mark all that apply – USE HAND CARD CCG-1]**	☐3 Allergy ☐4 Diabetes ☐5 Formula-related reason ☐6 Due to infections ☐7 Gastro-intestinal Problems ☐8 Overweight/Obesity ☐9 Nutrition/Anemia ☐10 Other, specify: _____
G29. Do you consider **[CHILD]** to be overweight, underweight, or about the right weight?	☐1 overweight ☐2 underweight ☐3 about the right weight

C. HEALTH SERVICES AND HEALTH IMPAIRMENT

G30. Would you say that **[CHILD]** s health in general is excellent, very good, good, fair, or poor?	☐1 excellent ☐2 very good ☐3 good ☐4 fair ☐5 poor
G31. Is there a particular clinic, health center, doctor's office, or other place that you usually go to if **[CHILD]** is sick, or needs advice about health or for routine care?	☐1 Yes ☐2 No
G32. Is there one particular doctor or health professional that **[CHILD]** usually sees?	☐1 Yes ☐2 No
G33. About how long has it been since you or anyone last saw or talked to a medical doctor or other health professional about **[CHILD]** ? Include doctors seen while a patient in a hospital.	☐1 less than 1 year ☐2 more than 1 year, but less than 2 years ☐3 more than 2 years, but less than 5 years ☐4 5 years or more ☐5 never ☐8 don't know
G34. Since **[CHILD]** was born, how many different times has **[CHILD]** stayed in the hospital overnight or longer? **Do not include the hospitalization when [child] was born.**	☐0 none _____ times number
G35. Is **[CHILD]** able to take part at all in any of the usual kinds of activities done by most children of his/her age?	☐1 Yes ☐2 No
G36. Is **[CHILD]** limited in the kind or amount of activities can do because of an impairment or health problem?	☐1 Yes ☐2 No
G37. Does any impairment or health problem now keep **[CHILD]** from attending school?	☐1 Yes ☐2 No
G38. Does **[CHILD]** need to attend a special	☐1 Yes

school or special classes because of any impairment or health problem?	☐2 No
G39. How long ago was the impairment or health problem first noticed?	_____ number ☐8 don't know } ☐1 months ☐2 years
G40. Did a doctor ever say that **[CHILD]** had rheumatic fever/rheumatic heart disease?	☐1 Yes ☐2 No **[GO TO G44]**
If yes:	
G41. How old was **[CHILD]** when he/she first had this illness?	_____ number } ☐1 months ☐2 years
G42. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 Don't know
G43. Has **[CHILD]** ever been treated by a Doctor for this illness?	☐1 Yes ☐2 No ☐8 Don't know
G44. Did a doctor ever say that **[CHILD]** had epilepsy/fit/convulsion?	☐1 Yes ☐2 No **[GO TO G48]**
If yes:	
G45. How old was **[CHILD]** when he/she first had this illness?	_____ number } ☐1 months ☐2 years
G46. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 Don't know
G47. Has **[CHILD]** ever been treated by a Doctor for this illness?	☐1 Yes ☐2 No ☐8 Don't know
G48. Did a doctor ever say that **[CHILD]** had Cerebral palsy?	☐1 Yes ☐2 No **[GO TO G52]**
If yes:	
G49. How old was **[CHILD]** when he/she first had this illness?	_____ number } ☐1 months ☐2 years
G50. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 Don't know

G51. Has **[CHILD]** ever been treated by a Doctor for this illness?	☐1 Yes ☐2 No ☐8 Don't know
G52. Did a doctor ever say that **[CHILD]** had mental retardation?	☐1 Yes ☐2 No **[GO TO G56]**
If yes:	
G53. How old was **[CHILD]** when he/she first had this illness?	_____ number } ☐1 months ☐2 years
G54. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 don't know
G55. Has **[CHILD]** ever been treated by a Doctor for this illness?	☐1 Yes ☐2 No ☐8 don't know
G56. Did a doctor ever say that **[CHILD]** had muscle weakness or paralysis of the arms?	☐1 Yes ☐2 No **[GO TO G60]**
If yes:	
G57. How old was **[CHILD]** when he/she first had this illness?	_____ number } ☐1 months ☐2 years
G58. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 don't know
G59. Has **[CHILD]** ever been treated by a Doctor for this illness?	☐1 Yes ☐2 No ☐8 don't know
G60. Did a doctor ever say that **[CHILD]** had muscle weakness or paralysis of the legs?	☐1 Yes ☐2 No **[GO TO G64]**
If yes:	
G61. How old was **[CHILD]** when he/she first had this illness?	_____ number } ☐1 months ☐2 years
G62. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 don't know
G63. Has **[CHILD]** ever been treated by a Doctor for this illness?	☐1 Yes ☐2 No ☐8 don't know
G64. Did a doctor ever say that **[CHILD]** had	☐1 Yes

asthma?	☐2 No **[GO TO G68]**
If yes:	
G65. How old was **[CHILD]** when he/she first had this illness?	_____ number ☐1 months ☐2 years
G66. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 don't know
G67. Has **[CHILD]** ever been treated by a Doctor for this illness?	☐1 Yes ☐2 No ☐8 don't know
G68. Did a doctor ever say that **[CHILD]** had chronic bronchitis?	☐1 Yes ☐2 No **[GO TO G72]**
If yes:	
G69. How old was **[CHILD]** when he/she first had this illness?	_____ number ☐1 months ☐2 years
G70. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 don't know
G71. Has **[CHILD]** ever been treated by a doctor for this illness?	☐1 Yes ☐2 No ☐8 don't know
G72. Did a doctor ever say that **[CHILD]** had hay fever?	☐1 Yes ☐2 No **[GO TO G76]**
If yes:	
G73. How old was **[CHILD]** when he/she first had this illness?	_____ number ☐1 months ☐2 years
G74. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 don't know
G75. Has **[CHILD]** ever been treated by a doctor for this illness?	☐1 Yes ☐2 No ☐8 don't know
G76. Did a doctor ever say that **[CHILD]** had hypertension or high blood pressure?	☐1 Yes ☐2 No **[GO TO G80]**
If yes:	
G77. How old was **[CHILD]** when he/she	

first had this illness?	_____ number ☐1 months ☐2 years
G78. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 don't know
G79. Has **[CHILD]** ever been treated by a doctor for this illness?	☐1 Yes ☐2 No ☐8 don't know
G80. Did a doctor ever say that **[CHILD]** had high blood cholesterol?	☐1 Yes ☐2 No **[GO TO G84]**
If yes:	
G81. How old was **[CHILD]** when he/she first had this illness?	_____ number ☐1 months ☐2 years
G82. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 don't know
G83. Has **[CHILD]** ever been treated by a doctor for this illness?	☐1 Yes ☐2 No ☐8 don't know
G84. Has **[CHILD]** ever seen a psychiatrist, psychologist, or psychoanalyst about any emotional, mental, or behavioral problems?	☐1 Yes ☐2 No
G85. During the past 12 months, has **[CHILD]** taken any prescribed medicines or drugs to help control activity or behavior?	☐1 Yes ☐2 No
G86. During the past 12 months, how often did **[CHILD]** complain of headaches? Would you say never, rarely, sometimes, frequently, or always?	☐1 never ☐2 rarely ☐3 sometimes ☐4 frequently ☐5 always
G87. During the past 12 months, how often did **[CHILD]** complain of stomach aches? Would you say never, rarely, sometimes, frequently, or always? [**Do not include menstrual cramps**]	☐1 never ☐2 rarely ☐3 sometimes ☐4 frequently ☐5 always
G88. Does **[CHILD]** have any speech defect, such as stuttering, stammering, or lisping?	☐1 Yes ☐2 No

G89. Has **[CHILD]** ever had anemia, sometimes called "tired blood" or "low blood?"	☐1 Yes ☐2 No ☐8 don't know
G90. Now I will ask about some immunizations that may have received. It may be easier to recall this information if you have a record of **[CHILD]**'s shots. Do you have a vaccinationrecord for **[CHILD]** that I can see?	☐1 Vaccination record available ☐2 Vaccination record NOT available
G91. Has **[CHILD]** ever received a DPT or tetanus shot? A DPT shot is to prevent diphtheria, tetanus, and pertussis or whooping cough. **[Verify with vaccination record if available]**	☐1 Yes ☐2 No **[GO TO G93]** ☐8 don't know **[GO TO G93]**

G92. How long ago was **[CHILD]** 's last DPT or tetanus shot?	_____ number □1 months □2 years
G93. During the past 12 months, how many times did **[CHILD]** have an accident, injury or poisoning, excluding lead poisoning, that required medical attention?	□0 None _____ number times □8 don't know

D. RESPIRATORY CONDITIONS AND ALLERGY

Cough	
G94. Does **[CHILD]** usually cough on most days for 3 consecutive months or more during the year?	□1 Yes □2 No
G95. Does **[CHILD]** usually cough first thing in the morning in the winter?	□1 Yes □2 No
if no to the above, SKIP to PHLEGM:	
G96. Does **[CHILD]** usually cough at all during the rest of the day?	□1 Yes □2 No
Ignore an occasional cough	
G97. For how many years has **[CHILD]** had this cough?	_____ Number years
Phlegm	
Count phlegm on first going outdoors. Exclude phlegm from the nose. Count swallowed phlegm.	
G98. Does **[CHILD]** usually bring up any phlegm/sputum/mucus from your chest first thing in the morning in the winter?	□1 Yes □2 No
G99. Does **[CHILD]** usually bring up any phlegm/sputum/mucus from his/her chest during the day in the winter?	□1 Yes □2 No
if no to above, SKIP to EPISODES OF COUGH and PHLEGM	
G100. Does **[CHILD]** bring up phlegm like this on most days for as much as three months each year?	□1 Yes □2 No
G101. Does **[CHILD]** usually bring up phlegm at all on getting up or first thing in the morning?	□1 Yes □2 No

G102. For how many years has **[CHILD]** had trouble with phlegm?	_____ years
G103. Has **[CHILD]** ever coughed up blood?	☐1 Yes ☐2 No
G104. Was this in the past year?	☐1 Yes ☐2 No
Episodes Of Cough And Phlegm	
G105. Has **[CHILD]** had periods or episodes of (increased) cough and phelgm lasting for 3 weeks or more each year?	☐1 Yes ☐2 No
Breathlessness	
G106. Is **[CHILD]** troubled by shortness of breath when hurrying on level ground?	☐1 Yes ☐2 No
G107. Does **[CHILD]** get short of breath walking with other children of his/her own age on level ground?	☐1 Yes ☐2 No
G108. Does **[CHILD]** have to stop for breath when walking at his/her own pace on level ground?	☐1 Yes ☐2 No
G109. Is **[CHILD]** too breathless to leave the house or breathless on dressing or undressing?	☐1 Yes ☐2 No
Wheezing	
G110. Does **[CHILD]** chest ever sound wheezy or whistling?	☐1 Yes ☐2 No **[GO TO G125]**
[If no, GO to WEATHER] if yes to above, is it:	
G111. When **[CHILD]** has a cold?	☐1 Yes ☐2 No
G112. Occasionally apart from colds?	☐1 Yes ☐2 No
G113. Most days or nights?	☐1 Yes ☐2 No
G114. For how many years has this been Present?	_____ years
G115. How many episodes of wheezing or Whistling has **[CHILD]** had in the past 12 Months?	_____ number
G116. How many times in the past 12 Months was **[CHILD]** hospitalised overnightfor these episodes of	_____ number

wheezing or whistling?	
G117. Can you estimate the total cost of all these hospitalizations for the past year? **[HELP RESPONDENT FIGURE OUT BY SUMMING ACROSS COST OF EACH HOSPITALIZATION]**	**R**_____,____ ☐8 Don't know
G118. How many times in the past 12 Months has **[CHILD]** gone to a doctor's Surgery or hospital emergency room for one of these episodes of wheezing or whistling?	_____ number
G119. Can you estimate the total cost of all these visits for the past year? **[HELP RESPONDENT FIGURE OUT BY SUMMING ACROSS COST OF EACH HOSPITALIZATION]**	**R**_____,____ ☐8 Don't know
G120. Has **[CHILD]** ever had an ATTACK of wheezing that has made him/her feel short of breath?	☐1 Yes ☐2 No
if yes to above	
G121. How old was **[CHILD]** when he/she had your first such attack?	_____ Age in years
G122. Has **[CHILD]** had 2 or more such Episodes?	☐1 Yes ☐2 No
G123. Has **[CHILD]** ever required medicine or treatment for the(se) attack(s)?	☐1 Yes ☐2 No
G124. Is/Was **[CHILD]'s** breathing Absolutely normal between attacks?	☐1 Yes ☐2 No
Weather	
G125. Does the weather affect **[CHILD]'s** chest?	☐1 Yes ☐2 No
Only record "YES" if adverse weather definitely and regularly causes chest symptoms if yes to above	
G126. Does the weather make **[CHILD]** short of breath?	☐1 Yes ☐2 No
G127. What kind of weather?	_____
Other Symptoms And Allergies	
During the past 12 months, has **[CHILD]** had	

any episodes of:	
G128. Stuffy, itchy, running nose?	☐1 Yes ☐2 No
G129. Watery, itchy eyes?	☐1 Yes ☐2 No
G130. During the past 12 months, how many episodes of stuffy, itchy, running nose or watery, itchy eyes has **[CHILD]** had?	☐1 none ☐2 constantly/continuously _____ episodes
Are ANY of the above symptoms (wheezing, whistling, runny nose, watery eyes etc), brought on by:	
G131. Exercise or cold air?	☐1 Yes ☐2 No

G132. Animals?	☐1 Yes ☐2 No
G133. Housedust?	☐1 Yes ☐2 No
G134. pollen?	☐1 Yes ☐2 No
G135. Wool clothing	☐1 Yes ☐2 No
G136. Cigarette smoke	☐1 Yes ☐2 No
G137. Soaps, sprays or detergents	☐1 Yes ☐2 No
G138. Colds or 'flu	☐1 Yes ☐2 No
G139. Air pollution	☐1 Yes ☐2 No
G140. Strong odours/smells	☐1 Yes ☐2 No
G141. Other things	☐1 Yes ☐2 No ☐9 please specify _____
G142. During which months of the year does pollen make **[CHILD]'s** symptoms worse? **[circle months that apply]**	☐1 ALL months J F M A M J J A S O N D

ALLERGY

G143. Within an hour after eating something, has [CHILD] ever had a severe reaction, such as itching all over, trouble breathing, flushing, or swelling of the hands and feet?	☐1 Yes ☐2 No
G144. Within an hour after receiving allergy shots or allergy tests, has [CHILD] ever had a severe reaction, such as itching all over, trouble	☐1 Yes ☐2 No ☐3 Never had allergy shots or tests ☐8 Don't know

breathing, flushing, or swelling of the hands and feet?	
G145. **Has [CHILD] ever given up or had to avoid a pet because of allergies?**	☐1 Yes ☐2 No

EAR INFECTION

G146. Did **[CHILD]** ever have an ear infection or an earache?	☐1 Yes ☐2 No ☐8 don't know
G147. How many times has **[CHILD]** had an ear infection or an earache?	☐0 never ☐1 once ☐2 twice ☐3 3 – 5 times ☐4 6 or more times ☐8 don't know
G148. How old was **[CHILD]** when had the first ear infection or earache?	☐1 less than 1 year old ⇨ ___months age ☐2 1 year old or older ⇨ _____ years age
G149. Was **[CHILD]** ever treated by a doctor for ear infection(s) or earache(s)?	☐1 Yes ☐2 No ☐8 don't know
G150. Has **[CHILD]** ever had trouble hearing with one or both ears? Do not include any problems which lasted just a short period of time such as during a cold.	☐1 Yes ☐2 No ☐8 don't know
G151. Does **[CHILD]** still have trouble hearing with one or both ears?	☐1 Yes ☐2 No ☐8 don't know
G152. Does **[CHILD]** use a hearing aid?	☐1 Yes ☐2 No ☐8 don't know
G153. How long ago did **[CHILD]** last have hearing tested?	☐1 never ☐2 6 months or less ☐3 more than 6 months, but less than 12 months ☐4 more than 12 months, less than 2 years ☐5 more than 2 years, less than 5 years ☐6 more than 5 years ☐8 don't know

SCHOOL ATTENDANCE

G154. During the past 12 months, about how many whole days was **[CHILD]** absent from school because of illness, playing truant, or for other reasons?	☐0 none _____ days number ☐8 don't know
G155. Has **[CHILD]** ever skipped any grades for any reason?	☐1 Yes ☐2 No
G156. Has **[CHILD]** repeated any grades for any reason?	☐1 Yes ☐2 No
G157. What grade did **[CHILD]** repeat?	1 2 3 4 5 6 7 8
Why did **[CHILD]** repeat the grade(s)? **[USE HAND CARD CCG-2]**	☐1 Academic failure ☐2 Immature/acted too young ☐3 Frequently absent ☐4 Moved into a more difficult school ☐5 Language problem ☐6 Learning/behavior problem ☐7 Hearing/vision problem ☐8 Health problem ☐9 Relocation problem ☐10 Language problem ☐11 Other, specify: _____
G158. Has **[CHILD]** ever been suspended, excluded or expelled from school?	☐1 Yes ☐2 No
G159. How many times has **[CHILD]** been suspended, excluded or expelled from school?	_____ times number
G160. On the average during the school year, how many hours per week does **[CHILD]** work in a paid or unpaid job?	☐1 none ☐2 5 or fewer hours ☐3 6-9 hours ☐4 10-14 hours ☐5 15-19 hours ☐6 20-24 hours ☐7 25 or more hours

ASTHMA SEVERITY

G161. Has a *doctor or nurse* ever told you that **[child]** has asthma?	☐1 Yes **[GO TO G162]** ☐2 No **[READ PASSAGE BELOW]** ☐8 Don't know **[READ PASSAGE BELOW]**
If NO or DON'T KNOW say: From now on, when I say asthma, I will be talking about breathing problems such as episodes of wheezing, coughing, tightness of the chest, heaviness in the chest or shortness of breath that **[child]** may sometimes experiences. I understand that [he or she] may or may not be having any problems like this. Okay? **[GO TO G164]**	

G162. How old was **[child]** when a doctor or nurse told you that he/she had asthma?	____ years old
G163. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 don't know
G164. In the past 12 months, how often has your Child had a cough that won't go away? Would you say… **[USE HAND CARD CCG-3]**	☐1 Every day ☐2 More than 2 times per week ☐3 More than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 Never
G165. In the past 12 months, how often has your child had wheezing (a whistling sound from the chest) with a cold? **[USE HAND CARD CCG-3]**	☐1 Every day ☐2 More than 2 times per week ☐3 More than 1 time per month ☐4 3 to 12 times in whole year ☐5 1 or 2 times in the whole year ☐6 Never
G166. In the past 12 months, how often has your child had wheezing (a whistling sound from the chest) *without* a cold? **[USE HAND CARD CCG-3]**	☐1 Every day ☐2 More than 2 times per week ☐3 More than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 Never
G167. In the past 12 months, how often has your child had an attack of wheezing that made it hard for him or her to breathe or catch his or her breath? **[USE HAND CARD CCG-3]**	☐1 Every day ☐2 More than 2 times per week ☐3 More than 1 time per month ☐4 3 to12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 Never
G168. In the past 12 months, how often has your child wheezed with exercise or running or playing hard? **[USE HAND CARD CCG-3]**	☐1 Every day ☐2 More than 2 times per week ☐3 More than 1 time per month ☐4 3 to12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 Never
G169. In the past 12 months, how often has your child coughed with exercise or running or playing hard? **[USE HAND CARD CCG-3]**	☐1 Every day ☐2 More than 2 times per week ☐3 More than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 Never
G170. In the past 12 months, how often has your child complained that his or her chest felt tight or heavy? **[USE HAND CARD CCG-3]**	☐1 Every day ☐2 More than 2 times per week ☐3 More than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year

	☐6 Never
G171. In the past 12 months, how often has your child's sleep been disturbed due to wheezing, coughing, chest tightness or shortness of breath?	☐1 Most nights ☐2 More than 2 times per week ☐3 More than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 Never
G172. Are there any particular seasons or months when **[child's]** symptoms are worse?	☐1 YES **[GO TO G173]** ☐2 NO **[GO TO G174]**
G173. During which season (or months) does **[child]** have the most breathing problems? **[CHECK ALL THAT APPLY]**	☐1 Spring (September, October, November) ☐2 Summer (December, January, February) ☐3 Autumn (March, April, May) ☐4 Winter (June, July, August) ☐5 Never has breathing problems
G174. I am going to read a list of things that might bring on wheezing, tightness in the chest, cough, or shortness of breath in some children. I would like to know whether each of these things brings on these symptoms for **[child]**. **[CHECK ALL THE RESPONSES THAT R. MENTIONS, REMEMBER TO REPEAT QUESTION FROM TIME TO TIME]** **[USE HAND CARD CCG-4]**	☐1 Being active (running, playing, swimming, or exercising) ☐2 Sprays or strong smells (such as colognes, perfumes, or cleaning supplies) ☐3 Colds or flu ☐4 Cold air ☐5 Change in weather ☐6 Laughing or crying hard ☐7 Dust ☐8 Pets ☐9 Truck or car exhaust ☐10 Hot summer days ☐11 Pollen, trees, fresh cut grass ☐12 Mold and mildew ☐13 Smoke ☐14 Cockroaches ☐15 Certain foods ☐16 Nothing causes breathing problems ☐99 Other (**SPECIFY:** _____)
Has a doctor *ever* told you that **[child]** has.... **[READ ALL CHOICES]**	
G175. Allergies	☐1 Yes ☐2 No
G176. Eczema	☐1 Yes ☐2 No
G177. Reactive airway disease	☐1 Yes ☐2 No
G178. Asthmatic bronchitis	☐1 Yes ☐2 No

G179. Any other lung/breathing condition	☐$_1$ Yes (**SPECIFY:** _____) ☐$_2$ No

B. HEALTH SERVICES UTILIZATION

G180. Not including the emergency room, does **[child]** have a *regular* family doctor or health care provider that you usually go to for his/her health care?	☐$_1$ Yes What is Doctor or Clinic's name _____ ☐$_2$ No **[GO TO G182]**
G181. When is the last time you visited this doctor or clinic?	_____ month/year

C. ASTHMA MEDICATION

Can you bring all the medications in the home that **[child]** is has ever taken for asthma, wheezing, tightness in the chest, shortness of breath, or cough. This includes those medications that a doctor or clinic has prescribed and those that a doctor did not prescribe, for example, over-the-counter drugs or home remedies. [Ask respondent to bring you all containers of medication in that house that the child has used. Fill in names from containers] **[IF CHILD HAS NEVER TAKEN ANY MEDICATION FOR ASTHMA, SKIP TO ASTHMA HEALTH SERVICES UTILIZATION SECTION, Q 18]**

Medication Name	Code **[LEAVE BLANK]**	Is the container present and have you seen it...	Is this a. . .	Was this medicine prescribed by a doctor?	How often did the doctor say to take it or use it?		Can you tell me when [child] last used this medicine?	How much does a one month supply of the medication [if taken as prescribed] cost you?	Does **[child]** use this medication less often than needed or prescribed because of the cost?
G182.	**G183**	**G184.** ☐1 container seen ☐2 container not seen	**G185.** ☐1 pill ☐2 liquid (to swallow) ☐3 inhaler/pump ☐4 added to a breathing machine or nebulizer	**G186.** ☐1 Yes ☐2 No	**G187.** PRN (as needed) ☐1	____ times/day or ____ puffs/day	**G188.** ☐1 today ☐2 yesterday ☐3 last week ☐4 last month ☐5 more than 1 month ago	**G189.** **R**_____,_____	**G190.** ☐1 Yes ☐2 No
G191.	**G192.**	**G193.** ☐1	**G194.**	**G195.** ☐1 Yes	**G196.** PRN	____	**G197.** ☐1 today	**G198.**	**G199.**

		container seen ☐2 container not seen	☐1 pill ☐2 liquid ☐3 inhaler/puffer ☐4 breathing machine or nebulizer	☐2 No	(as needed) ☐1	times/day or ____ puffs/day	☐2 yesterday ☐3 last week ☐4 last month ☐5 more than 1 month ago	**R**_____,_____	☐1 Yes ☐2 No
G200.	**G201.**	**G202.** ☐1 container seen ☐2 container not seen	**G203.** ☐1 pill ☐2 liquid ☐3 inhaler/puffer ☐4 breathing machine or nebulizer	**G204.** ☐1 Yes ☐2 No	**G205.** PRN (as needed) ☐1	____ times/day or ____ puffs/day	**G206.** ☐1 today ☐2 yesterday ☐3 last week ☐4 last month ☐5 more than 1 month ago	**G207.** **R**_____,_____	**G208.** ☐1 Yes ☐2 No
G209.	**G210.**	**G211.** ☐1 container seen ☐2 container not seen	**G212.** ☐1 pill ☐2 liquid ☐3 inhaler/puffer ☐4 breathing machine or	**G213.** ☐1 Yes ☐2 No	**G214.** PRN (as needed) ☐1	____ times/day or ____ puffs/day	**G215.** ☐1 today ☐2 yesterday ☐3 last week ☐4 last month ☐5 more than 1 month ago	**G216.** **R**_____,_____	**G217.** ☐1 Yes ☐2 No

			nebulizer						
G218.	**G219.**	**G220.** ☐1 container seen ☐2 container not seen	**G221.** ☐1 pill ☐2 liquid ☐3 inhaler/puffer ☐4 breathing machine or nebulizer	**G222.** ☐1 Yes ☐2 No	**G223.** PRN (as needed) ☐1	____ times/day or ____ puffs/day	**G224.** ☐1 today ☐2 yesterday ☐3 last week ☐4 last month ☐5 more than 1 month ago	**G225.** **R**_____,_____	**G226.** ☐1 Yes ☐2 No

G227. Are there any other medications that **[child]** has taken in the last month for asthma that aren't here?	☐1 Yes **(GO TO G228)** ☐2 No
Complete chart below	

Medication Name	Code **[LEAVE BLANK]**	Is the container present and have you seen it...	Is this a. . .	Was this medicine prescribed by a doctor?	How often did the doctor say to take it or use it?		Can you tell me when [child] last used this medicine?	How much does a one month supply of the medication [if taken as prescribed] cost you?	Does **[child]** use this medication less often than needed or prescribed because of the cost?
G228.	**G229.**	**G230.** ☐1 container seen ☐2	**G231.** ☐1 pill ☐2 liquid	**G232.** ☐1 Yes ☐2 No	**G233.** PRN (as needed)	____ times/day	**G234.** ☐1 today ☐2 yesterday	**G235.** **R**_____,_____	**G236.** ☐1 Yes ☐2 No

		container not seen	(to swallow) ☐3 inhaler/pump ☐4 added to a breathing machine or nebulizer		☐1	or ____ puffs/day	☐3 last week ☐4 last month ☐5 more than 1 month ago		
G237.	**G238.**	**G239.** ☐1 container seen ☐2 container not seen	**G240.** ☐1 pill ☐2 liquid ☐3 inhaler/puffer ☐4 breathing machine or nebulizer	**G241.** ☐1 Yes ☐2 No	**G242.** PRN (as needed) ☐1	____ times/day or ____ puffs/day	**G243.** ☐1 today ☐2 yesterday ☐3 last week ☐4 last month ☐5 more than 1 month ago	**G244.** **R**_____,_____	**G245.** ☐1 Yes ☐2 No
G246.	**G247.**	**G248.** ☐1 container seen ☐2 container not seen	**G249.** ☐1 pill ☐2 liquid ☐3 inhaler/puf	**G250.** ☐1 Yes ☐2 No	**G251.** PRN (as needed) ☐1	____ times/day or	**G252.** ☐1 today ☐2 yesterday ☐3 last week ☐4 last month	**G253.** **R**_____,_____	**G254.** ☐1 Yes ☐2 No

			fer ☐4 breathing machine or nebulizer			____ puffs/day	☐5 more than 1 month ago		

G255.	**G256.**	**G257.** ☐1 container seen ☐2 container not seen	**G258.** ☐1 pill ☐2 liquid ☐3 inhaler/puffer ☐4 breathing machine or nebulizer	**G259.** ☐1 Yes ☐2 No	**G260.** PRN (as needed) ☐1	____ times/day or ____ puffs/day	**G261.** ☐1 today ☐2 yesterday ☐3 last week ☐4 last month ☐5 more than 1 month ago	**G262.** R_____,_____	**G263.** ☐1 Yes ☐2 No
G264.	**G265.**	**G266.** ☐1 container seen ☐2 container not seen	**G267.** ☐1 pill ☐2 liquid ☐3 inhaler/puffer ☐4 breathing machine or nebulizer	**G268.** ☐1 Yes ☐2 No	**G269.** PRN (as needed) ☐1	____ times/day or ____ puffs/day	**G270.** ☐1 today ☐2 yesterday ☐3 last week ☐4 last month ☐5 more than 1 month ago	**G271.** R_____,_____	**G272.** ☐1 Yes ☐2 No

G273. Are there other medications that a doctor or clinic has prescribed for asthma, wheezing, tightness in the chest, shortness of breath, or cough, that you have not bought because of the cost?	☐1 Yes ☐2 No
G274. If yes, do you have the prescription still **[copy name and dose from prescription].**	a. _____ b. _____ c. _____ d. _____ e. _____
G275. If you don't have the prescription, do you remember the name of the medication(s)?	a. _____ b. _____ c. _____ d. _____ e. _____
G276. How much would a one-month supply **[of each medication]** cost?	a. R_____,_____ b. R_____,_____ c. R_____,_____ d. R_____,_____ e. R_____,_____ ☐8 don't know

OTHER MEDICINAL USAGE

The following questions concern **[CHILD]'s** use of medicines, other than those for asthma which we just covered, and certain products the past month.

G277. Has **[CHILD]** taken or used any medicines for which a doctor's or dentist's prescription is needed, in the past month? This includes any products which cannot be obtained without a doctor's or dentist's prescription.	☐1 Yes ☐2 No
IF ANY YES: May I see the containers for all of the prescription medicines **[CHILD]** took in the past month? [**Proceed to table on next page**]	

	MEDICATION 1	MEDICATION 2	MEDICATION 3	MEDICATION 4
Enter the complete name of the medication from the label or probe respondent	**G278.** _____ NAME	**G284.** _____ NAME	**G290.** _____ NAME	**G296.** _____ NAME
Check Item	**G279.** ☐1 container seen ☐2 container not seen, information furnished by respondent	**G285.** ☐1 container seen ☐2 container not seen, information furnished by respondent	**G291.** ☐1 container seen ☐2 container not seen, information furnished by respondent	**G297.** ☐1 container seen ☐2 container not seen, information furnished by respondent
What is the health problem **[CHILD]** had for which he/she took this medication?	**G280.** _____ CONDITION	**G286.** _____ CONDITION	**G292.** _____ CONDITION	**G298.** _____ CONDITION
For how long has **[CHILD]** been taking/using this type of product?	**G281.** _____ number ☐1 days ☐2 weeks ☐3 months ☐4 years ☐8 don't know	**G287.** _____ number ☐1 days ☐2 weeks ☐3 months ☐4 years ☐8 don't know	**G293.** _____ number weeks months ☐1 days ☐2 ☐3 ☐4 years ☐8 don't know	**G299.** _____ number weeks months ☐1 days ☐2 ☐3 ☐4 years ☐8 don't know
How much does a one month supply of [**NAME**] medication [if taken as prescribed] cost you?	**G282.** **R**_____,____	**G288.** **R**_____,____	**G294.** **R**_____,____	**G300.** **R**_____,____

Do you use [**NAME**] medication less often than needed or prescribed because of the cost?	**G283.** ☐1 Yes ☐2 No	**G289.** ☐1 Yes ☐2 No	**G295.** ☐1 Yes ☐2 No	**G301.** ☐1 Yes ☐2 No

	MEDICATION 5	MEDICATION 6	MEDICATION 7	MEDICATION 8
Enter the complete name of the medication from the label or probe respondent	**G302.** _____ NAME	**G308.** _____ NAME	**G314.** _____ NAME	**G320.** _____ NAME
Check Item	**G303.** ☐1 container seen ☐2 container not seen, information furnished by respondent	**G309.** ☐1 container seen ☐2 container not seen, information furnished by respondent	**G315.** ☐1 container seen ☐2 container not seen, information furnished by respondent	**G321.** ☐1 container seen ☐2 container not seen, information furnished by respondent
What is the health problem **[CHILD]** had for which he/she took this medication?	**G304.** _____ CONDITION	**G310.** _____ CONDITION	**G316.** _____ CONDITION	**G322.** _____ CONDITION
For how long has **[CHILD]** been taking/using this type of product?	**G305.** _____ number . ☐1 days ☐2 weeks ☐3 months ☐4 years ☐8 don't know	**G311.** _____ number . ☐1 days ☐2 weeks ☐3 months ☐4 years ☐8 don't know	**G317.** _____ number . weeks months ☐1 days ☐2 ☐3 ☐4 years ☐8 don't know	**G323.** _____ number . ☐1 days ☐2 weeks ☐3 months ☐4 years ☐8 don't know
How much does a one month supply of **[NAME]** medication [if taken as prescribed] cost you?	**G306.** **R**_____,____	**G312.** **R**_____,____	**G318.** **R**_____,____	**G324.** **R**_____,____

Do you use [**NAME**] medication less often than needed or prescribed because of the cost?	**G307.** ☐1 Yes ☐2 No	**G313.** ☐1 Yes ☐2 No	**G319.** ☐1 Yes ☐2 No	**G325.** ☐1 Yes ☐2 No

VITAMIN USAGE

G326. Has **[CHILD]** taken or used any vitamins in the past month? Please include those that are prescribed by a doctor or dentist and those that are not prescribed.	☐1 Yes ☐2 No
IF ANY YES: May I see the containers for all of the vitamins **[CHILD]** took in the past month? [**Proceed to table on next page**]	

	VITAMIN 1	VITAMIN 2
Enter the complete name of the vitamin from the label or probe respondent	**G327.** _____ NAME	**G333.** _____ NAME
Check Item	**G328.** ☐1 container seen ☐2 container not seen, information furnished by respondent ☐3 product name not on container	**G334.** ☐1 container seen ☐2 container not seen, information furnished by respondent ☐3 product name not on container
[Enter manufacturer's or distributor's name and address (city and province)]	**G329.** _____ NAME _____ CITY _____ PROVINCE	**G335.** _____ NAME _____ CITY _____ PROVINCE
How often did **[CHILD]** take **[product]** in the past month?	**G330.** _____ number of times ☐1 day ☐2 week ☐3 month ☐99 other specify: _____ ☐8 don't know	**G336.** _____ number of times ☐1 day ☐2 week ☐3 month ☐99 other specify: _____ ☐8 don't know

How much **[product]** did **[CHILD]** take each time she/he took it	**G331.** _____ number ☐1 capsules/tablets ☐2 teaspoons ☐3 tablespoons ☐4 ounces ☐5 drops ☐6 packets/packs ☐7 ml ☐9 other specify: _____ ☐8 variable amounts ☐88 don't know	**G337.** _____ number ☐1 capsules/tablets ☐2 teaspoons ☐3 tablespoons ☐4 ounces ☐5 drops ☐6 packets/packs ☐7 ml ☐9 other specify: _____ ☐8 variable amounts ☐88 don't know
For how long has **[child]** been taking this type of product	**G332.** _____ number of times ☐1 less than one month ☐2 months ☐3 years ☐9 other specify: _____ ☐8 don't know	**G338.** _____ number of times ☐1 less than one month ☐2 months ☐3 years ☐9 other specify: _____ ☐8 don't know
	VITAMIN 3	VITAMIN 4
Enter the complete name of the vitamin from the label or probe respondent	**G339.** _____ NAME	**G345.** _____ NAME
Check Item	**G340.** ☐1 container seen ☐2 container not seen, information furnished by respondent ☐3 product name not on container	**G346.** ☐1 container seen ☐2 container not seen, information furnished by respondent ☐3 product name not on container
[Enter manufacturer's or distributor's name and address (city and province)]	**G341.** _____ NAME _____ CITY _____ PROVINCE	**G347.** _____ NAME _____ CITY _____ PROVINCE

How often did **[CHILD]** take **[product]** in the past month?	**G342.** _____ number of times ☐1 day ☐2 week ☐3 month ☐99 other specify: _____ ☐8 don't know	**G348.** _____ number of times ☐1 day ☐2 week ☐3 month ☐99 other specify: _____ ☐8 don't know
How much **[product]** did **[CHILD]** take each time she/he took it	**G343.** _____ number ☐1 capsules/tablets ☐2 teaspoons ☐3 tablespoons ☐4 ounces ☐5 drops ☐6 packets/packs ☐7 ml ☐9 other specify: _____ ☐8 variable amounts ☐88 don't know	**G349.** _____ number ☐1 capsules/tablets ☐2 teaspoons ☐3 tablespoons ☐4 ounces ☐5 drops ☐6 packets/packs ☐7 ml ☐9 other specify: _____ ☐8 variable amounts ☐88 don't know
For how long has **[child]** been taking this type of product	**G344.** _____ number of times ☐1 less than one month ☐2 months ☐3 years ☐9 other specify: _____ ☐8 don't know	**G350.** _____ number of times ☐1 less than one month ☐2 months ☐3 years ☐9 other specify: _____ ☐8 don't know
	VITAMIN 5	VITAMIN 6
Enter the complete name of the vitamin from the label or probe respondent	**G351.** _____ NAME	**G357.** _____ NAME
Check Item	**G352.** ☐1 container seen ☐2 container not seen, information furnished by respondent ☐3 product name not on container	**G358.** ☐1 container seen ☐2 container not seen, information furnished by respondent ☐3 product name not on container
[Enter manufacturer's or distributor's name and address (city and province)]	**G353.** _____ NAME _____ CITY _____ PROVINCE	**G359.** _____ NAME _____ CITY _____ PROVINCE

How often did **[CHILD]** take **[product]** in the past month?	**G354.** _____ number of times ☐1 day ☐2 week ☐3 month ☐9 other specify: _____ ☐8 don't know	**G360.** _____ number of times ☐1 day ☐2 week ☐3 month ☐9 other specify: _____ ☐8 don't know
How much **[product]** did **[CHILD]** take each time she/he took it	**G355.** _____ number ☐1 capsules/tablets ☐2 teaspoons ☐3 tablespoons ☐4 ounces ☐5 drops ☐6 packets/packs ☐7 ml ☐9 other specify: _____ ☐8 variable amounts ☐8 don't know	**G361.** _____ number ☐1 capsules/tablets ☐2 teaspoons ☐3 tablespoons ☐4 ounces ☐5 drops ☐6 packets/packs ☐7 ml ☐9 other specify: _____ ☐8 variable amounts ☐8 don't know
For how long has **[child]** been taking this type of product	**G356.** _____ number of times ☐1 less than one month ☐2 months ☐3 years ☐9 other specify: _____ ☐8 don't know	**G362.** _____ number of times ☐1 less than one month ☐2 months ☐3 years ☐9 other specify: _____ ☐8 don't know

D. CAREGIVER'S QUALITY OF LIFE

Now, I am going to ask you some similar questions about how your child's asthma has affected you and also some questions about your health.

G363. In the past 3 months, how often did you wake up or lose sleep because of **[child's]** asthma? would you say you woke up or lost sleep……	☐1 most nights ☐2 more than 2 times per week ☐3 more than 2 times per month ☐4 1 or 2 times per month ☐5 no nights
G364. Is there a particular season or month when you wake up or lose sleep most because of **[child's]** asthma?	☐1 YES ☐2 NO **[SKIP TO G366]**
G365. During what season or month do you wake up or lose sleep most because of **[child's]** asthma? **[CHECK ONE]**	☐1 Spring (September, October, November) ☐2 Summer (December, January, February) ☐3 Autumn (March, April, May) ☐4 Winter (June, July, August)
G366. During the last **[USE ANSWER FROM G365]**, how often did you wake up or lose sleep because of **[child's]** asthma?	☐1 Most nights ☐2 More than 2 times per week ☐3 More than 2 times per month ☐4 1 or 2 times per month ☐5 No nights
G367. In the past 3 months, how often did you have to change your daytime or evening plans because of [**child's**] asthma? Would you say it was…..	☐1 Most days/evenings ☐2 More than 2 times per week ☐3 More than 2 times per month ☐4 1 or 2 times per month ☐5 No days/evenings
G368. Is there a particular season or month when you have to change your daytime or evening plans most because of **[child's]** asthma?	☐1 YES ☐2 NO **[SKIP TO G371]**
G369. During what season or month do you have to change your daytime or evening plans most because of **[child's]** asthma? **[CHECK ONE]**	☐1 Spring (September, October, November) ☐2 Summer (December, January, February) ☐3 Autumn (March, April, May) ☐4 Winter (June, July, August)
G370. During the last **[USE ANSWER FROM G369]**, how many days or nights per week do you have to change your daytime or evening plans because of **[child's]** asthma? Would you say it was…	☐1 Most days/evenings ☐2 More than 2 times per week ☐3 More than 2 times per month ☐4 1 or 2 times per month ☐5 No days/evenings

E. HOUSEHOLD ENVIRONMENTAL CHECKLIST

G371. Do you rent or own your home or neither?	☐1 rent ☐2 own **[skip to G373]** ☐3 neither **[skip G373]**
G372. In general, how easy or difficult would you say that it is to get your landlord to make repairs when they are needed? Would you say . . . **[READ CHOICES]**	☐1 Very easy ☐2 Somewhat easy ☐3 Neither easy or difficult ☐4 Somewhat difficult ☐5 Very difficult
At any time during the year is there standing water or puddles located in. . . **[READ EACH CHOICE]**	
G373. [Child's] sleeping room?	☐1 Yes ☐2 No
G374. The sitting room?	☐1 Yes☐2 No ☐3 No sitting room
G375. The kitchen?	☐1 Yes☐2 No
G376. Another place I did not specify?	☐1 Yes (**specify**_____) ☐2 No
In the past year have you had any other problem with water damage or leaking water in your home, such as from a leaking roof or leaky plumbing	
G377. [Child's] sleeping room?	☐1 Yes ☐2 No
G378. The sitting room?	☐1 Yes☐2 No ☐3 No sitting room
G379. The kitchen?	☐1 Yes☐2 No
G380. Another place I did not specify?	☐1 Yes (**specify**_____) ☐2 No
G381. How many pets of each type come inside the home?	Dog? _____ Cat? _____ Other pets (**SPECIFY:** _____) ☐1 No pets in the house **[GO TO G384]**
[IF ANY CATS OR DOGS OR OTHER PETS WITH FUR ARE PRESENT, ASK G382. AND C383.]	
G382. Do any of these pets spend any time in child's bedroom?	☐1 Yes ☐2 No ☐8 Don't Know
G383. Are the pets put out of the house at night?	☐1 Yes ☐2 No ☐3 Sometimes ☐8 Don't Know
G384. Are there cockroaches in your	☐1 Yes

Home?	☐2 No ☐8 Don't know
G385. Have you had any problems with cockroaches in your home during the past year?	☐1 Yes ☐2 No ☐8 Don't know
G386. Have you or someone else (your landlord, another family member, a professional) treated your home for cockroaches in the past year?	☐1 Yes ☐2 No **[GO TO G389]** ☐8 Don't know **[GO TO G389]**
G387. When was the last time it was treated ?	☐1 Within last month ☐2 1 to 3 months ago ☐3 3 to 6 months ago ☐4 6 to 12 months ago ☐5 More than 12 months ago ☐8 Don't know
G388. What was used to treat your home for roaches? **[READ EACH CHOICE AND CHECK ALL THAT APPLY]**	☐1 Dry powder ☐2 Spraying ☐3 Gel ☐4 Roach bait trap (**SPECIFY:** _____) ☐5 Boric acid ☐6 Other (**SPECIFY:** _____) ☐8 Don't know
G389. Have you had any problems with mice or rats in your home during the past year?	☐1 Yes ☐2 No ☐8 Don't know
G390. Have you or someone else (your landlord, another family member, a professional) treated your home for rats or mice in the past year?	☐1 Yes ☐2 No **[GO TO G393]** ☐8 Don't know **[GO TO G393]**
G391. When was the last time?	☐1 Within last month ☐2 Between 2 and 6 months ago ☐3 Between 6 and 12 months ago ☐4 More than 12 months ago ☐8 Don't know
G392. How is your home treated for rats or mice? **[READ EACH CHOICE AND CHECK ALL THAT APPLY]**	☐1 Spring traps ☐2 Glue traps ☐3 Poison ☐4 Other (**SPECIFY:** _____) ☐8 Don't know

F. BEHAVIOR CHANGE TO REDUCE ENVIRONMENTAL HAZARDS

The purpose of these questions is to look at the environment in your home and how it relates to your child's asthma as well as the health of other household members.

G393. Is there anyone whose paying job is working around chemicals (such as pesticides, paints) or dust living in the home?	☐1 Yes ☐2 No ☐8 Don't know
G394. If yes, do they usually wear their work clothes home?	☐1 Yes ☐2 No ☐8 Don't know
G395. Is there anyone whose informal job (includes working with chemicals) is in or near the home?	☐1 Yes ☐2 No ☐8 Don't know
G396. During the last 2 weeks, how many times was the room in which **[child]** sleeps dusted?	☐1 None ☐2 1 ☐3 2 ☐4 3 ☐5 4 or more ☐8 Don't know
G397. What do you use when you dust?	☐1 Dry cloth ☐2 Damp cloth ☐9 Other (**SPECIFY:** _____)
G398. During the last 2 weeks, how many times were other rooms in the house dusted?	☐1 None ☐2 1 ☐3 2 ☐4 3 ☐5 4 or more ☐8 Don't know
G399. How often do you change the **[child's]** Bedding? **[DO NOT READ RESPONSE CATEGORIES TO RESPONDENT; CHOOSE CATEGORY WHICH FITS RESPONSE]**	☐1 once a week or more ☐2 more than every two weeks ☐3 more that once a month ☐4 once a month or less ☐9 Other (**SPECIFY:** _____) ☐8 Don't know
G400. When the child's bedding are machine washed, what temperature is usually used for the wash cycle? **[WE ARE INTERESTED IN THE WASH CYCLE ONLY. THIS IS THE FIRST CYCLE OF THE WASHING MACHINE. EXAMPLE: SOMEONE USES THE WARM-COLD SETTING, YOU WOULD**	☐1 Hot ☐2 Warm ☐3 Cold ☐4 Not applicable [**DO NOT READ**] ☐8 Don't know

RECORD WARM.]	
G401. When the child's bedding is hand washed, what temperature is usually used for the wash?	☐1 Hot ☐2 Warm ☐3 Cold ☐4 Not applicable [**DO NOT READ**] ☐8 Don't know
G402. Do you or any member of your family add anything to the wash to help get rid of dust mites? **[PROMPT: "Such as eucalyptus oil."]**	☐1 Yes (**SPECIFY:** _____) ☐2 No ☐8 Don't know
G403. How often does the cover on your child's bed get washed (i.e. bedspreads/comforters)? **[RECORD THE CATEGORY CLOSEST TO THE RESPONSE. IF RESPONDENT UNSURE, READ RESPONSES]**	☐1 Once a week or more often ☐2 More than once a month ☐3 More often that every 3 months (4 times a year) ☐4 More often that every six months (2 times a year) ☐5 Less often than every six months ☐8 Don't know
G404. Does **[child]** have stuffed animals in his or her bedroom?	☐1 Yes ☐2 No **[GO TO G407]** ☐8 Don't know
G405. Do **[child's]** stuffed animals get washed?	☐1 Yes ☐2 No **[GO TO G407]** ☐8 Don't know or not applicable **[GO TO G407]**
G406. How many times per year do **[child's]** stuffed animals get washed? **[DO NOT READ RESPONSE CATEGORIES TO RESPONDENT; CHOOSE CATEGORY WHICH FITS RESPONSE]**	☐1 Once a week ☐2 Once a month ☐2 Every three months (4 times a year) ☐3 Every six months (2 times a year) ☐4 Less than once a year ☐5 Once a year ☐9 Other (**SPECIFY:** _____) ☐8 Don't know
Have you done any of the following things around the house because of **[child's]** asthma?	
G407. Removed visible mold growth?	☐1 Yes ☐2 No
G408. Removed pets from the home?	☐1 Yes ☐2 No ☐3 Never had pets
G409. Changed cigarette-smoking rules in the home?	☐1 Yes, no one allowed to smoke ☐2 Yes, reduced amount of smoking ☐3 Yes, limited smoking to one room. ☐4 No ☐5 Never any smokers

G410. Attempted to control or eliminate cockroaches?	☐1 Yes ☐2 No ☐3 Never a problem
G411. Attempted to control or eliminate mice or rats?	☐1 Yes ☐2 No ☐3 Never a problem
G412. Did you do any other things around the house because of [**child's**] asthma?	(**SPECIFY:** _____)
Now I'm going to ask you a few questions about smoking. These questions concern smoking of cigarettes.	
G413. How many people who live in [child's] home smoke? **[INCLUDE RESPONDENT IF SMOKER.]**	_____ people ☐1 None **[GO TO G418]**
G414. Do you smoke cigarettes, even occasionally?	☐1 Yes ☐2 No **[GO TO G418]**
G415. About how many cigarettes a day do you now smoke?	_____ cigarettes
G416. How often do you go outside the home to smoke?	☐1 Always ☐2 Sometimes ☐3 Rarely ☐4 Never ☐8 Don't know
G417. Does **[child]** smoke cigarettes?	☐1 Yes ☐2 No ☐8 Don't know
G418. Do any frequent visitors smoke?	☐1 Yes ☐2 No ☐8 Don't know
G419. Many people have difficulties keeping their children away from cigarette smoke. Do you have problems keeping **[child]** away from people who are smoking?	☐1 Yes ☐2 No
G420. How frequently is your child around people who are smoking? Would you say. . . . **[READ CHOICES]**	☐1 Daily ☐2 Several times a week ☐3 Several times a month ☐4 Never ☐8 Don't know

Thank you for your time for these questions.

We may need to contact you again to obtain additional information. Please give me the name, address and telephone number of two relatives or friends who would know where you could be reached in case we have difficulty in contacting you.

Name of first contact person:

_____

Telephone number of first contact person: _____

Address of first contact person:

_____ _____

House No. Road/Street

_____ _____

Suburb/Township Postal Code

Relationship of contact person to you: _____

Name of second contact person:

_____

Telephone number of second contact person: _____

Address of second contact person:

_____ _____

House No. Road/Street

_____ _____

Suburb/Township Postal Code

Relationship of contact person to you: _____

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE!

END: Thank you for helping us!

Interview completed at: Time: _ _:_ _ am / pm

APPENDIX 3.5

Parent Informed Consent

Informed Consent Form for participant's parent / legal guardian

Please note: this form is to be read, or read to, and signed by the child's parent or legal guardian and an adult witness

Your consent is required so that your child
_____ can participate in a **FIRST NAME** **SURNAME**
study of the health effects of air pollution at the _____School.

If you have any queries after reading this consent form, kindly call Ms. Poovie Reddy @ 204 2082 (b/h).

The study is being conducted in conjunction with the Nelson R. Mandela Medical School. The purpose of the study is to find out whether health problems (like asthma, bronchitis, reactive airway disease, pneumonia and asthmatic bronchitis) experienced by learners at the school, are caused by air pollution. This study will determine whether certain genes are involved in your child's response to air pollution. The study has the support of the school staff, community groups in South Durban and the City Health Department.

If you agree to participate, your child will be required to give a sample of blood which will be taken by trained medical personnel.

Risks:

⇒ There are no risks from taking the samples of blood, but should any medical emergency arise, there will be trained medical personnel on site to render assistance.

Confidentiality:

The results and information that we collect from you and your child is ***completely confidential***. Other than the study personnel, this information will never be seen by anyone without your written consent. The results of the overall study will be made available to the school and the community and will be presented so as to protect the identity of individual participants.

Costs to you resulting from participation in the study:

The study is offered at ***no cost*** to you. If a problem is discovered and you wish to consult a doctor, we will recommend a doctor. However, the study cannot pay for these additional medical visits or treatments.

Voluntary nature of participation:
You and your child are free to decline to participate or to withdraw from the study at any time without suffering any penalty or disadvantage.

Consent:
I understand the meaning of the information given above.

I hereby consent to having my child

_____ _____

FIRST NAME **SURNAME**
participate in the study.

Documentation of consent:

The child's parent or legal guardian and an adult witness should sign and date both copies of this document. You or your child should return ONE copy to your child's teacher at his/her School by _____.

It will be given to the research staff and kept with the records of the study. The other copy is for you to keep.

_____ _____

Printed name of child's parent / legal guardian **Signature**

_____ _____

Printed name of witness **Signature**

Date: _____

APPENDIX 3.6

Child Informed Assent

IMPORTANT:

1) If you do not understand any words, please ask for an explanation before giving assent.

INFORMED ASSENT FORM

Title of research project:
Epidemiologial and genetic risk factors associated with asthma among children in the South Durban Region, KwaZulu Natal.

Please circle the appropriate answer:

1.	Have you understood the subject information sheet?	YES/NO
2.	Did you discuss the study with anyone?	YES/NO
3.	Who did you discuss it with? _____	
4.	Do you have any questions about the study or about your role in the study?	YES/NO
5.	Are you worried about any part of this study?	YES/NO
6.	Have you received enough information about this study?	YES/NO
7.	Do you understand how you will be involved in this study?	YES/NO
8.	Do you understand that you are free to withdraw from this study:	
	(a) at any time and;	
	(b) without having reason to withdraw	YES/NO
9.	Do you agree to voluntarily take part in this study?	YES/NO

If you have answered **NO** to any of the above questions, please obtain the necessary information **BEFORE** signing.

I, _____ hereby give assent for the proposed procedures to

SUBJECT'S NAME
be performed on me as part of the above mentioned project.

_____ _____
(PRINTED NAME OF WITNESS) **(SIGNATURE)**

APPENDIX 3.7

Information Sheet

IMPORTANT

1) If you do not understand any words, please ask for an explanation before giving assent.

SUBJECT INFORMATION SHEET

Title of research project:
Epidemiological and genetic risk factors associated with asthma among children in South Durban Region, KwaZulu Natal.

I am requesting your permission to participate in a study of the health effects of air pollution in the eThekwini Municipality. I would like to describe to you the purpose of the study, what you would be asked to do if you agree to participate, and what the risks and benefits of participating in the study are. If you have any questions or if something I say is not clear to you, please stop me and let know right away.

The study has the support of the local industry, community groups concerned about these sorts of health problems, and the City Health Department. We are studying this community because of its location near sources of air pollutants such as oil refineries and because of health concerns expressed by teachers and students at the school and by the larger community. The purpose of the study is to figure out whether any health problems are being caused by air pollution in the community, and, if so, to make recommendations to improve the situation.

If you agree to participate, you and your parent will be interviewed, you will be asked to complete baseline breathing tests, and, each day for a period of two or three weeks, fill out a daily log about activities and any breathing problems, and blow into a handheld device several times a day to further test your breathing, and also have a blood test conducted.

Baseline breathing tests. You will be asked to blow several times into a machine which measures how well your lungs are working. You will be asked to repeat the breathing test after you first breathe in a small amount of a chemical subsistence (either methacholine or histamine). This test helps up find out if you may have a breathing problem like asthma. You may be asked to breathe in this substance and then blow into the machine a few times.

Blood Tests. Trained technicians will take samples of blood from you. These tests will determine if you have genes that put you at greater risk of getting respiratory problems.

Only specified tests will be conducted on this blood sample, and the remainder will be destroyed immediately after. You WILL NOT BE INJECTED WITH ANY SUBSTANCE/MEDICATION.

Risks. There are no risks from the interview, keeping the daily logs, blowing into the handheld device, or the baseline simple breathing test. The breathing test using the chemical substance can cause chest tightness, hoarse voice or a sore throat for a short time in some people. This can be treated immediately with a different medication, which you breathe in. You will only be given the chemical substance if the simple breathing test is normal. This greatly reduces the chance of having a serious problem.

Expected benefits to you and others. Your parents will be given a written copy of all your child's test results along with an explanation of what they mean. What we learn from this study may help to protect people in South Africa and other parts of the world from problems caused by air pollution.

Confidentiality. The interview, diary, and breathing test information we collect about you is completely confidential and will never be seen by anyone other than the personnel conducting the study without your without your written consent. The results of the overall study, which will be made available to the local government and the community, will be presented so as to protect the identity of individual participants.

Voluntary nature of participation. You are free to refuse to participate or to withdraw from the study at any one time without suffering any penalty or disadvantage.

Contact person. You may contact **MS POOVIE REDDY** (telephone no.: **2042082**) or **DR RAJEN NAIDOO** (telephone no.: **2604385**) for answers to further questions about the research.