THE ROLE OF PRENATAL MATERNAL STRESS AND GENETICS IN CHILDHOOD ASTHMA

1. Meta Hypothesis

Prenatal, maternal stress increases the risk of childhood asthma. Genetic and environmental factors that influence immune development and lung growth/airway inflammation in early life modify the association between maternal psychological stress and the development of asthma.

2. Specific Hypotheses

1. Children of mothers with high psychological stress levels during pregnancy will have an increased risk of asthma/wheezing.

2. Prenatal stress exposure will modify the association of prenatal exposure to oxidative toxins (tobacco smoke and air pollution, in particular) in predicting subsequent risk of asthma.

3. Polymorphisms in maternal genes will interact with prenatal exposures to stress, smoking, and air pollution in predicting subsequent asthma risk.

    3.1 Children with mothers with high stress exposure during pregnancy will vary in their increased risk of asthma/wheezing in part related to variation in genes in the corticosteroid pathway (i.e., beta 11 hydroxylase and the glucocorticoid receptor).

    3.2 Children with mothers with high stress exposure during pregnancy will vary in their increased risk of asthma/wheezing in part related to genetic variation in maternal toxin metabolizing genes (e.g., glutathione S transferase M1, T1, and P1 and Cytochrome P450 1A1).

3. Background and Justification

Asthma prevalence and morbidity has increased in the United States. This disproportionately affects poor minority children living in urban environments and remains a paradox largely unexplained by accepted environmental risk factors. This suggests the existence of as-yet-unidentified factors or unmeasured interactions (Wright, Rodriguez, & Cohen, 1998; Wright, 2005). This paradox, in part, has led to reconsideration of the overlap between biological determinants and psychosocial factors (i.e., life stress). Growing understanding of the complexity of asthma epidemiology underscores the need to examine interactions between established risk factors with genetic factors (Lemanske, 2002) as well as interactions between concurrent exposure to prevalent environmental factors (Cloughterty et al., in press). These hypotheses examine biologic pathways that established environmental risk factors may interact with genetic risk factors and with each other. The interactions, if proven, will help establish the mechanisms by which asthma occurs.

3.1 Public Health Importance

Prevalence

Asthma is the most common chronic illness among children in the United States (National Academy of Sciences, 2000; American Lung Association Epidemiology and Statistics Unit, 2006). In 2004, 6.5 million children younger than 18 had asthma. During the past two decades, asthma prevalence

increased approximately 75 percent among U.S. children. Similar or greater increases in the prevalence of asthma during the second half of the 20th century were reported from other countries (Eder, Ege, & von Mutius, 2006). Though asthma prevalence has plateaued in many groups, black children and adolescents have continued to experience increased prevalence (American Lung Association Epidemiology and Statistics Unit, 2006). Hospitalization and death rates in children have also stabilized (Akinbami, 2001). Physician visits for asthma by children continued to increase after 2000 (Akinbami, 2001), with an increase in the use of preventive (controller) medications such as inhaled corticosteroids (Stafford, Ma, Finkelstein, Haver, & Cockburn, 2003; Suissa, Ernst, Benayoun, Baltzan, & Cai, 2000; Suissa, Ernst, & Kezouh, 2002).

Economic and social burden

In 2004, the direct health care costs of asthma was estimated at $11.5 billion while indirect costs (lost productivity) were estimated at $4.6 billion for a total of $16.1 billion (American Lung Association Epidemiology and Statistics Unit, 2006). The more severe forms of asthma account for a disproportionate amount of the total direct costs. One study estimated that less than 20 percent of asthmatics account for more than 80 percent of the direct costs (Malone, Lawson, & Smith, 2000). Asthma also poses a substantial and increasing public health burden from school absence and restriction of usual physical and social activities (Newacheck & Halfon, 2000).

3.2 Justification for a Large Prospective Longitudinal Study

Prospective studies that incorporate strategies for studying stress reactivity during pregnancy, infancy, and early childhood (De Weeth & Buitellar, 2005; Jones, Holzman, Zanella, Leece, & Rahbar, 2006; Harville et al., 2007; DiPietro, Cotigan, & Gurewitsch, 2003; Egiliston, Maman, & Austin, 2007) are needed to continue to elucidate the mechanisms underlying the links between stress, other vulnerability factors, and asthma development. The prospective nature of the National Children's Study enables this research as stress questionnaires will be administered prior to phenotype expression (childhood asthma), thus limiting the possibility of recall bias. Similar concerns regarding bias in smoking and air pollution exposure measures are mitigated by the prospective design. The size of the cohort will assure sufficient statistical power exists for testing the interaction hypotheses.

Interactions with other factors (e.g., genetic, bioaerosols, lifestyle factors) necessitate a large sample. Accurate exposure and phenotypic data are needed to assess the importance of many asthma and allergy genotypes interacting with many exposures in relation to complex and variable asthma phenotypes. A large sample will help evaluate the susceptibility to the adverse effects of certain pollutants and their sources in specific genotype-phenotype clusters. Also, data are needed from children of different ethnicities living across the United States under varied housing, socioeconomic, and geographic conditions. These factors may be correlated with variations in exposures, and a large sample size will be necessary to allow adjustments to account for this.

3.3 Scientific Merit

Theory and mechanisms

Asthma is a developmental disease with an estimated half of all cases diagnosed by age 3 and two-thirds diagnosed by age 5 (Reed, 2006; Reed, 2006). The early onset of disease suggests adverse early life experiences, including prenatal exposures, are relevant to asthma risk. Despite what is commonly believed, children do not necessarily "grow out of asthma" with a significant reduction in symptoms into adulthood. Almost two-thirds of children diagnosed with asthma continue to have symptoms in puberty and adulthood, and the remaining one-third experiences a persistence of lung

function abnormalities even if they appear to have clinical remission (NIH, 2004). Some prenatal exposures, such as environmental tobacco smoke (ETS), have been demonstrated to increase the risk of childhood asthma, and the greatest risk is among children with a family history of asthma, which suggests a gene-environment interaction (London, James Gauderman, Avol, Rappaport, & Peters, 2001). Stress is a highly prevalent environmental exposure also demonstrated to increase asthma risk (Wright et al., 2002). Several lines of evidence suggest prenatal maternal stress may be particularly relevant to asthma risk. Two potential pathways are proposed which may work in tandem. The first is that prenatal stress will increase polarization of the immune system to the TH2 phenotype via dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis. The second is that stress may enhance airway inflammation through its role as a pro-oxidant.

HPA axis and polarization of the immune system

The HPA axis seems susceptible to early life programming. Maternal and fetal stress stimulates placental secretion of corticotrophin-releasing hormone (CRH), which is elevated in the neonatal circulation (Seckl, 1997, 2001). This may stimulate the fetal HPA axis to secrete glucocorticoids and amplify fetal glucocorticoid excess. These in utero responses may increase the risk of disease in later life (Wellberg & Seckl, 2001). Disturbed regulation of both the HPA axis and the sympathetic adrenomedullary system due to chronic stress suggests immune function, which is modulated by these systems, may also be dysregulated in these individuals. In this context, it is notable that early life caregiver stress has been linked to repeated wheeze (Wright et al., 2002) and dysregulation of immune function in a birth cohort predisposed to atopy (Wright et al., 2004). There is growing evidence these cytokine patterns are already present in the first year of life (Lemanske, 2002) and may have their roots in utero (Miller et al., 2001). Gestational exposure to maternal stress has been shown to alter the development of humoral immunocompetence in offspring as well as their hormonal and immunologic responses to postnatal stress (Barker, 2001; Egiliston et al., 2007). Evidence in rhesus monkeys suggests stress experienced during pregnancy impacts the infant monkeys' response to antigens at birth (Coe, Lubach, & Karaszewski, 1999).

Another study (Wright, Franco-Suglia, Staudenmayer, & Cohen, 2007) examined the relationship between diurnal salivary cortisol expression and total IgE among 89 pregnant mothers enrolled in the Asthma Coalition on Community, Environment, and Social Stress project, a prospective cohort designed to study the effects of in utero and early life stress on urban childhood asthma risk. Higher levels of maternal total IgE were associated with blunted HPA functioning. Other evidence suggests that elevated maternal IgE in utero may potentiate fetal sensitization to allergens and enhance atopic risk in infancy (Liu et al., 2003). Stress-induced altered activity of the maternal HPA axis may have immunomodulatory effects that influence expression of IgE during pregnancy which, in turn, may impact fetal sensitization and childhood allergy and asthma risks. These findings warrant further study taking into account concurrent environmental exposures and genetic susceptibility.

Pro-oxidant properties of stress

Stress may also enhance airway inflammation through its role as a pro-oxidant (for a detailed overview see Wright, Cohen, & Cohen [2004]). Air pollution and ETS are associated with asthma morbidity and have oxidative properties. Stress activates various P450 enzyme systems involved in the biotransformation of environmental oxidative toxins, such as ETS and air pollutants. Several animal and human studies demonstrate chronic stress will produce increased oxidative toxicity due, in part, to a decreased ability to detoxify exogenous toxins.

In a birth cohort in China, women with both occupational stress and benzene exposure had an average reduction of 184 grams in the birth weight of their children. However, subjects with only

stress or benzene exposure had less than a 20-gram reduction in birth weight. Benzene is activated and ultimately detoxified by the hepatic P450 enzyme system in conjunction with phase 2 enzymes. The interaction might therefore be due to induction of benzene activation by maternal stress. In the same cohort, mothers with the P450 system CYP1A1 HincII polymorphism AA and occupational exposure to benzene had a decreased gestational period. CYP1A1 is a phase I enzyme that activates polycyclic aromatic hydrocarbons. The association between the AA genotype and benzene exposure was postulated to be caused by an increased activation of benzene in the AA genotype mothers. The similar interaction between stress and benzene may be due to increased oxidation by stress mediating induction of P450 enzymes.

Other human and animal studies support the concept that psychological stress has pro-oxidant properties and alters biotransformation (Capel, Dorrell, & Smallwood, 1983; Kosugi, Enomoto, Ishizuka, & Kikugawa, 1994; Tomei, Kiecolt-Glaser, Kennedy, & Glaser, 1990; Irie, Asami, Nagata, Miyata, & Kasai, 2000). Spiteri, Bianco, Strange, and Tryer (2000) proposed that differences in host detoxification provide the basis for either resolution or progression of inflammation in individuals with airway hyper-responsiveness after exposure to an environmental trigger (e.g., tobacco smoke, air pollutants) that augments oxidative toxicity.

Maternal genotype

A second innovative approach of these hypotheses is to determine the role of maternal genetics on in utero environmental exposures. While there are no studies of maternal genetic polymorphisms interacting with in utero exposures in predicting respiratory outcomes in children, some studies in birth defects illustrate this concept (Van Rooij et al., 2001; Shi et al., 2007). Because stress is a pro-oxidant, interactions between maternal stress during pregnancy and in utero exposure to ETS and air pollution may be associated with asthma/wheeze in children, similar to the study by Wang et al. (2000). Mechanistically, stress may be is biologically similar to alterations in detoxification produced by genetics and leads to the expression of disease by the same biologic pathway.

3.4 Potential for Innovative Research

Several aspects of these hypotheses are innovative. The role of prenatal stress exposure on subsequent childhood asthma has not been extensively studied. This is important because the role of in utero exposures on the risk of subsequent diseases has been elucidated for environmental toxins (ETS and asthma) and nutrition (decreased fetal nutrition and increased subsequent risk of hypertension and cardiovascular disease). The extension of this critical window concept to a psychosocial exposure, such as psychological stress, may help determine asthma causation.

The concept of critical windows of exposure is well established, but the role of genetic susceptibility during these exposure windows has not been well studied. Reliance on quantitative exposure data without regard to the timing of the exposure may introduce bias into measures of gene-environment interaction. Furthermore, the role of maternal genetic factors that may interact with in utero fetal exposures has been investigated in some fields (e.g., reproductive epidemiology and birth outcomes), but this clearly deserves consideration for health outcomes, such as asthma, in later life.

3.5 Feasibility

While studies of interaction require large sample sizes, these hypotheses can be tested in the National Children's Study. Given the prevalence of the outcome/phenotype (asthma) and the exposures (stress, smoking, air pollution), and the highly polymorphic nature of candidate genes (the glucocorticoid

receptor, 11 beta hydroxylase enzyme, P450 and phase II biotransformation enzymes, and many others), these hypotheses will be testable within the framework of the study.

The data for prenatal exposures and phenotype classification will be collected. All have well established, validated measurement instruments. The frequency of family contact within the framework of the overall study design and protocol will provide opportunities to collect the exposure and outcome data to test these hypotheses. The two pathways proposed in the hypotheses (polarization of immune system by HPA dysregulation and pro-oxidant effects of stress on biotransformation enzymes) can be tested by collection of biomarkers (maternal and cord blood IgE, prenatal maternal salivary cortisol, prenatal maternal CRH, maternal urinary cotinine levels), questionnaires (Perceived Stress Scale, Parenting Stress Index, Family Environment Scale, qualitative and quantitative smoking history), environmental measures (air pollutant exposures), and DNA from both mother and child.

To test these hypotheses, an initial nested case-control design could be employed with random control sampling (matching for age) after the cohort has reached age 3-5 when early childhood wheezing will have been manifested. Nested case-control studies at future times will also be possible when the cohort is older and asthma phenotypes defined by trajectories through time can be used to identify cases.

Ethical considerations pertaining to consent for genetic testing will not differ from standard genetic testing of polymorphisms not specifically causing a known disease (i.e., none of the polymorphisms proposed will be disease-causing genotypes, such as the cystic fibrosis gene, and will not require special genetic counseling).

Important covariates (stress exposure, postnatal smoke exposure, allergen exposure, postnatal air pollution) are likely to be measured as part of the overall cohort goals and will not require additional costs.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Prenatal and postnatal stress

Primary/child

- Prenatal and postnatal stress

4.2 Methods

Primary/maternal

- Biomarkers (maternal blood IgE, prenatal maternal salivary cortisol, prenatal maternal CRH, maternal urinary cotinine levels)
- Questionnaires (Perceived Stress Scale, Parenting Stress Index, Family Environment Scale, qualitative and quantitative smoking history)

- Air pollutant exposures
- Genetic measures

Primary/child

- Biomarkers (cord blood IgE, salivary cortisol, CRH, urinary cotinine levels)
- Household smoking and air pollutant exposures
- Genetic measures

4.3 Life Stages

- Periodic including prenatal, birth, infancy, childhood, adolescence

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Asthma, wheezing, pulmonary function/dysfunction
- Immune system function

5.2 Methods

- Asthma or wheezing: Interview for medical history, medical reports from interactions with the health care system, medication review
- Pulmonary function: Pulmonary function tests and spirometry
- Immune system function: Cytokines, total and specific IgE, possibly lymphocyte subsets

5.3 Life Stage

- Asthma or wheezing: Periodic starting during infancy and throughout the Study
- Pulmonary function: At selected clinic visits starting at approximately age 7; Handheld spirometry may be performed more frequently.
- Immune system function: Starting at birth with cord blood and periodically throughout the study

6. Important Confounders, Mediators, and Effect Modifiers

- Health care access: Poor health care access in inner cities, especially among African Americans and Hispanics, exacerbates the risk of asthma attacks and deaths (Lara et al., 2003; Sin, Bell, & Man, 2004; Wallace, Scott, Klinnert, & Anderson, 2004). This and other disparity factors could be behind the greater asthma prevalence, especially among African Americans. These issues are more thoroughly discussed in hypothesis 4 and in the review by Gold and Wright (2005).

- Lower socioeconomic status and some minority groups such as blacks and Hispanics are likely to live in areas with increased air pollution such as near freeways or industrial sites. Careful assessments of these characteristics will be important to properly control for their influence on outcomes.

- Diet, physical activity, bioaerosols, stress, and infections, which will be the topic of other hypotheses, could interact with or confound associations of asthma onset with air pollutants

- For the analysis of gene-environment interactions, it will be important to include polymorphisms in genes involved in oxidative stress responses to air pollutant exposures and candidate genes

7. Power and Sample Size

The smallest detectable relative risk is approximately 1.2. This power estimate assumes a sample size of 100,000 at age of diagnosis, an asthma incidence of 5 percent and a cutoff value for "high" exposure based on the upper fifth percentile of National Children's Study subjects (i.e., a proportion exposed is 0.05). It assumes only a main effects model based on exposure to a single factor (e.g., a single pollutant) without consideration of interactions with other exposures, genetics, family history, etc. (National Children's Study Interagency Coordinating Committee, 2003).

Assessment of effect modification will be carried out by including multiplicative interaction terms for the potential effect modifier and the pollutant exposure into each regression model. For brevity, we present power analyses for a generic effect modifier. In the following, we assume a total sample size of N = 100,000 and that the incidence of diagnosed asthma by age 6 will be 5 percent. We use a fixed interval proportional hazards model to assess the association between pollutant exposure and the risk of asthma. In this case, minimum detectable hazard ratios associated with the interaction term (multiplicative increase in the effect of the pollutant attributable to the effect modifier) correspond to a specified power point that can be estimated via the usual two-sample proportional hazards formula given by Schmoor et al. (1990). The formula assumes unequal initial sample sizes because sample sizes are determined via the prevalence of the effect modifier. Based upon this formula, Table 1 presents minimal detectable hazard ratios associated with the interaction term corresponding to the 90 percent power point of a level 0.05 two-sided test.

Table 1. Minimum detectable hazard ratios associated with interactions. Estimates correspond to the 90% power point of a level 0.05 two-sided test

Prevalence of effect modifier	Minimum detectable hazard ratio associated with interaction term
2%	1.387
5%	1.234
10%	1.165
20%	1.121
30%	1.105
40%	1.100

8. Other Design Issues

- **Ethical/burden considerations:** The study also will need to have a formal strategy and process to communicate the results of environmental monitoring to the child's parents along with appropriate and feasible recommendations regarding the correction of any unhealthful environmental findings. Repeated tests are potentially burdensome.

- **Cost/complexity of data collection:** Repeated waves of environmental exposure assessment can be costly and burdensome.

- **Need for community involvement:** Daycare and school cooperation would be required for some of the intended measures.

9. References

Akinbami, L., & Centers for Disease Control and Prevention National Center for Health Statistics. (2006, December 12). The state of childhood asthma, United States, 1980-2005. Advance Data, (381), 1-24.

American Lung Association Epidemiology and Statistics Unit, Research and Program Services. (2006). Trends in asthma morbidity and mortality. New York, NY: American Lung Association. Accessed July 2006 from http://www.lungusa.org.

Barker, D.J. (2001). A new model for the origins of chornic disease. *Medicine, Health Care & Philosophy, 4*(1), 31-35.

Capel, I.D., Dorrell, H.M., & Smallwood, A.E. (1983). The influence of cold restraint stress on some components of the antioxidant defense system in the tissues of rats of various ages. *Journal of Toxicology & Environmental Health, 11*(3), 425-436.

Cloughterty, J., Levy, J.I., Kubzansky, L.D., Ryan, B., Franco Suglia, S., Canner Jacobsen, M., et al. (In press). The effect of traffic-related air pollution and exposure to violence on urban asthma. *Environmental Health Perspectives.*

Coe, C.L., Lubach, G.R., & Karaszewski, J.W. (1999). Prenatal stress and immune recognition of self and nonself in the primate neonate. *Biology of the Neonate, 76*(5), 301-310.

De Weeth, C., & Buitellar, J.K. (2005). Physiological stress reactivity in human pregnancy—A review. *Neuroscience & Biobehavioral Reviews*, *29*(2), 295-312.

DiPietro, J.A., Cotigan, K.A., & Gurewitsch, E.D. (2003). Fetal response to induced maternal stress. *Early Human Development, 74*(2), 125-138.

Eder, W., Ege, M.J., & von Mutius, E. (2006). The asthma epidemia. *New England Journal of Medicine, 355*(21), 2226-2235.

Egiliston, K.-A., Maman, C., & Austin, M.-P. (2007). Stress in pregnancy and infant HPA axis function: conceptual and methodological issues relating to the use of salivary cortisol as an outcome measure. *Psychoneruoendocrinology, 32*(1), 1-13.

Gold, D.R., & Wright, R.J., (2005). Population disparities in asthma. *Annual Review of Public Health, 26*, 89-113.

Harville, E.W., Savitiz, D.A., Dole, N., Herring, A.H., Thorp, J.M., & Light, K.C. (2007). Patterns of salivary cortisol secretion in pregnancy and implications for assessment protocols. *Biological Psychiatry, 74*(1), 85-91.

Irie, M., Asami, S., Nagata, S., Miyata, M., & Kasai, H. (2000). Classical conditioning of oxidative DNA damage in rats. *Neuroscience Letters, 288*(1), 13-16.

Jones, N.M., Holzman, C.B., Zanella, A.J., Leece, C.M., & Rahbar, M.H. (2006). Assessing mid-trimester salivary cortisol levels across three consecutive days in pregnant women using an at-home collection protocol. *Paediatric and Perinatal Epidemiology, 20*(5), 425-437.

Kosugi, H., Enomoto, H., Ishizuka, Y., & Kikugawa, K. (1994). Variations in the level of urinary thiobarbituric acid reactant in healthy humans under different physiological conditions. *Biological & Pharmaceutical Bulletin,* 17(12), 1645-1650.

Lara, M., Duan, N., Sherbourne, C., Halfon, N., Leibowitz, A., & Brook. R.H. (2003). Children's use of emergency departments for asthma: Persistent barriers or acute need? *Journal of Asthma, 40*(3), 289-299.

Lemanske, R.F. (2002). Issues in understanding pediatric asthma: Epidemiology and genetics. *Journal of Allergy and Clinical Immunology, 109*(6, Suppl.), S521-S524.

Liu, C.-A., Wang, C.-L., Chiang, H., Ou, C-Y., Hsu, T.-Y., & Yang, K.D. (2003). Prenatal prediction of infant atopy by maternal but not paternal total IgE levels. *Journal of Allergy & Clinical Immunology, 112*(5), 899-904.

London, S.J., James Gauderman, W., Avol, E., Rappaport, E.B., & Peters, J.M. (2001). Family history and the risk of early-onset persistent, early-onset transient, and late-onset asthma. *Epidemiology, 12*(5), 577-83.

Malone, D.C., Lawson, K.A., & Smith, D.H. (2000). Asthma: An analysis of high-cost patients. *Pharmacy Practice Management Quarterly, 20*(1), 12-20.

Miller, R.L., Ginger, L.C., Bell, C.A., Biedermann, S.A., Aggarwal, M., Kinney, P.L., et al. (2001). Prenatal Exposure, Maternal Sensitization, and Sensitization In Utero To Indoor Allergens in an Inner-City Cohort. *American Journal of Respiratory and Critical Care Medicine, 164*(6), 995-1001.

National Academy of Sciences, Institute of Medicine, Divsions of Health Promotion and Disease Prevention. (2000). *Clearing the Air: Asthma and Indoor Air Exposures* (p. 438). Washington, DC: National Academy Press.

National Children's Study Interagency Coordinating Committee. (2003). Supporting documentation for the working list of NCS Core Hypothesis presented at the December, 2002 NCS Study Assembly Meeting–Draft: Rationale Document.

Global Initiative for Asthma. (2004). Global strategy for asthma management and prevention [NIH Publication No. 02-3659, Updated 2004]. Bethesda, MD: National Institutes of Health.

Newacheck, P.W., & Halfon, N. (2000). Prevalence, impact, and trends in childhood disability due to asthma. *Archives of Pediatric and Adolescent Medicine, 154*(3), 287-293.

Reed, C.E. (2006). The natural history of asthma. *Journal of Allergy & Clinical Immunology, 118*(3), 543-548.

Schmoor, C., Sauerbrei, W., & Schumacher, M. (2000). Sample size considerations for the evaluation of prognostic factors in survival analysis. Statistics in Medicine, 19(4), 441-452.

Seckl, J. (1997). Glucocorticoids, feto-placental 11 beta-hydroxysteroid dehydrogenase type 2, and the early life origins of adult disease. *Steroids, 62*(1), 89-94.

Seckl, J.R. (2001). Glucocorticoid programming of the fetus: adult phenotypes and molecular mechanisms. *Molecular and Cellular Endocrinology, 185*(1-2), 61-71.

Shi, M., Christensen, K., Weinberg, C.R., Romitti, P., Bathum, L., Lozada, A., et al. (2007). Orofacial cleft risk is increased with maternal smoking and specific detoxification-gene variants. *American Journal of Human Genetics, 80*(1), 76-90.

Sin, D.D., Bell, N.R., & Man, S.F. (2004). Effects of increase primary care access on process of care and health outcome among patient patients with asthma who frequent emergency departments. *American Journal of Medicine, 117*(7), 479-483.

Spiteri, M.A., Bianco, A., Strange, R.C., & Tryer, 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.

Stafford, R.S., Ma, J., Finkelstein, S.N., Haver, K., & Cockburn, I. (2003). National trends in asthma visits and asthma pharmacotherapy, 1978-2002. *Journal of Allergy and Clinical Immunology, 111*(4), 729-735.

Stein, R.T., & Martinez, F.D. (2004). Asthma phenotypes in childhood: lessons from an epidemiological approach. *Paediatric Respiratory Review, 5*(2), 155-161.

Suissa, S., Ernst, P., & Kezouh, A. (2002). Regular use of inhaled corticosteroids and the long term prevention of hospitalization for asthma. *Thorax, 57*(10), 880-884.

Suissa, S., Ernst, P., Benayoun, S., Baltzan, M., & Cai, B. (2000). Low-dose inhaled corticosteroids and the prevention of death from asthma. *New England Journal of Medicine, 343*(5), 332-336.

Tomei, L.D., Kiecolt-Glaser, J.K., Kennedy, S., & Glaser, R. (1990). Psychological stress and phorbol ester inhibition of radiation-induced apoptosis in human peripheral blood leukocytes. *Psychiatry Research, 33*(1), 59-71.

Van Rooij, I.A., Wegerif, M.J., Roelofs, H.M., Peters, W.H., Kuijpers-Jagtman, A.M., Zielhuis, G.A., et al. (2001). Smoking, genetic polymorphisms in biotransformation enzymes, and nonsyndromic oral clefting: a gene-environment interaction. *Epidemiology, 12*(5), 502-507.

Wallace, A., Scott, J., Klinnert, M., & Anderson, M.E. (2004). Impoverished children with asthma: a pilot study of urban healthcare access. *Journal for Specialists in Pediatric Nursing, 9*(2), 50-58.

Wright, R.J. (2005). Stress and atopic disorder. *Journal of Allergy and Clinical Immunology, 116*(6), 1301-1306.

Wright, R.J., Cohen, S., Carey, V., Weiss, S.T., & Gold, D.R. (2002). Parental stress as a predictor of wheezing in infancy: a prospective birth-cohort study. *American Journal of Respiratory & Critical Care Medicine, 165*(3), 358-365.

Wright, R.J., Cohen, R.T., & Cohen, S. (2004). The impact of stress on the development and expression of atopy. *Current Opinions in Allergy and Clinical Immunology, 5*(1), 23-29.

Wright, R.J., Finn, P.W., Contreras, J.P., Cohen, S., Wright, R.O., Staudenmayer, J., et al. (2004). Chronic caregiver stress and IgR expression, allergen-induced proliferation and cytokine profiles in birth cohort predisposed to atopy. *American Academy of Allergy, Asthma and Immunology, 113*(6), 1051-1057.

Wright, R.J., Franco-Suglia, S., Staudenmayer, J.W., & Cohen, S. (2007). Blunted cortisol response predicts increased total IgE in pregnant mother enrolled in an urban asthma birth-cohort: Project ACCESS. International Conference of the American Thoracic Society.

Wright, R.J., Rodriguez, M., & Cohen, S. (1998). Review of psychosocial stress and asthma: an integrated biopsychosocial approach. *Thorax, 53*(12), 1066-1074.

EXPOSURE TO INDOOR AND OUTDOOR AIR POLLUTION, AEROALLERGENS, AND ASTHMA RISK

1. Meta Hypothesis

Exposures to indoor and outdoor air pollution, aeroallergens, and other environmental agents are associated with increased risk of asthma onset and progression in children and modified by genotype and other risk factors.

2. Specific Hypotheses

The following hypotheses address early and later developmental windows of vulnerability to air pollutants, aeroallergens, and other environmental agents and will require evaluation of exposure-outcome relationships at various points during the life of children. In addition, the hypotheses address how exposure to indoor and outdoor air pollution may interact with exposure to aeroallergens and other environmental, genetic, and family factors. Asthma is a complex and multifactorial disease. A myriad of environmental and familial factors are associated with asthma development, progression, and severity in children. Some factors are causative and others are protective, and these may change or vary by age.

The within-child repeated measures in The National Children's Study protocol allow for the use of time-of-exposure as a factor in the analyses of the proposed hypotheses. In Section 7 below, details of statistical tests and power analysis will be described to demonstrate how repeated measures can be used to address interactions with time-of-exposure in the onset of asthma.

1. Exposure to indoor environmental tobacco smoke, outdoor and indoor air pollutants, in-vehicle air pollutants, ozone and related photochemicals will be associated with the development of asthma.

    1.1 Exposure to indoor environmental tobacco smoke (ETS) will be associated with the development of asthma.

    1.2 Exposure to in-vehicle air pollutants will be associated with the development of asthma.

    1.3 Exposure to outdoor and indoor toxic air pollutants will be associated with the development of asthma. Examples of such pollutants include ozone, fine and ultrafine particles, NO2, ozone, volatile organic compounds (VOCs), carbonyl compounds (e.g., formaldehyde), semivolatile organic compounds from outdoor and indoor combustion sources (e.g., polycyclic aromatic hydrocarbons), and from pesticides and home products such as those containing plasticizes (phthalates, phenols).

2. For the development of asthma, a critical window may exist for exposure to indoor and outdoor air pollutants, other chemical exposures, and aeroallergens. Critical windows are age dependent on the development of the immune system and may differ depending on the outcome related to onset of asthma versus the progression and severity of the disease.

    2.1 For many of the above hypothesized associations, the greatest window of vulnerability for the architecture of the disease may be in utero and early life (0-2 years). The progression and severity of asthma, however, may be impacted by exposures throughout childhood.

    2.2 Allergic sensitization to allergens will be enhanced by exposures to air pollutants and will increase the risk of atopic asthma. The expectation is that exposure to specific outdoor and

indoor allergens and endotoxin and the interaction of these aeroallergens with air pollutants will result in varying susceptibility asthma onset and progression depending on the magnitude and timing of exposures during development as well as genetic predisposition.

2.3 The hypothesized associations with air pollutants will be modified by dietary antioxidants (see dietary antioxidants and asthma risk hypothesis), by lower respiratory infections by polymorphisms in genes involved in oxidative stress responses to air pollutant exposures, and by polymorphisms in candidate asthma and allergy genes involved in innate and adaptive immunity.

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

Asthma is the most common chronic illness among children (National Academy of Sciences Institute of Medicine). Data on asthma prevalence from the National Health Interview Survey (NHIS), analyzed recently by the American Lung Association, show the prevalence of asthma increased 85 percent from 1982 through 1996 to an estimated 14.6 million persons (55.2 per 1,000 persons) (American Lung Association Epidemiology and Statistics Unit, 2006). This increase was 76 percent in children younger than 18 years old or 4.43 million in 1996 (62.0 per 1,000 persons). This trend paralleled increasing asthma hospitalization and death rates in children (American Lung Association Epidemiology and Statistics Unit, 2006; Akinbami, 2006). Similar or greater increases in the prevalence of asthma during the second half of the 20th century were reported from other countries (Eder, Ege, & von Mutius, 2006).

Beginning in 1997, when questions addressing asthma in the NHIS were changed, the measures of asthma prevalence were also altered. It was not possible to compare to past trends until 2001, when a key question was added back into the questionnaire. Current asthma prevalence increased until 2001, later reaching a plateau in most groups except Black children and adolescents, who have continued to experience increased prevalence annually (American Lung Association Epidemiology and Statistics Unit, 2006). Hospitalization and death rates in children have also reached a plateau or decreased (Akinbami, 2006). Despite a plateau in prevalence, nonambulatory visits for asthma to physician offices by children have continued to increase after 2000 (Akinbami, 2006), possibly due to increased public and health provider awareness. For instance, it is likely that the main force mitigating increases in hospitalization and mortality have been the dramatic increase in the use of preventive (controller) medications such as inhaled corticosteroids (Stafford, Ma, Finkelstein, Haver, & Cockburn, 2003; Suissa, Ernst, Benayoun, Baltzan, & Cai, 2000; Suissa, Ernst, & Kezouh, 2002).

In 2004, 30.2 million Americans (104.7 per 1,000 persons) had been diagnosed with asthma by a health professional at some time during their lifetime. This estimate included 6.5 million children younger than age 18. Almost 4 million children younger than 18 were estimated to have experienced an asthma attack in 2004. Prevalence data in the United States from both the 12-month prevalence (before 1997) and 12-month attack prevalence of asthma (since 1997) were highest among children aged 5-14 years, Blacks compared with whites, and females compared to males (American Lung Association Epidemiology and Statistics Unit, 2006; Akinbami, 2006). Approximately 38 percent of the asthma hospital discharges in 2004 were in those younger than 15, however, only 21 percent of the U.S. population was that age.

Economic and social burden

In 2004, the total cost of asthma was estimated at $16.1 billion, including $11.5 billion in direct health care costs and $4.6 billion in indirect costs (lost productivity) (American Lung Association Epidemiology and Statistics Unit, 2006). The more severe forms of asthma account for a disproportionate amount of the total direct costs. One study estimated that less than 20 percent of asthmatics account for more than 80 percent of the direct costs (Malone, Lawson, & Smith, 2000). Asthma is also a substantial and increasing public health burden because it contributes to school absence and restriction of usual physical and social activities (Newacheck & Halfon, 2000).

3.2 Justification for a Large Prospective Longitudinal Study

Currently available databases are useful to study acute exacerbations of existing asthma but cannot directly answer questions about asthma incidence and the effects of exposures to chronic outdoor and indoor air pollutants and the interactions with exposures to aeroallergens. These questions require the collection of new data and are best studied in a large prospective cohort study design that collects data in utero, postnatally, through childhood, and into adulthood. A large sample size and prospective approach is needed to accurately assess the timing of exposures, particularly during critical windows of vulnerability (e.g., specific trimester of pregnancy, early vs. later postnatal periods, etc.) on the onset of asthma in childhood. Identification of children at risk for developing the severe forms of asthma will have a clear public health impact. A large prospective study is needed to have sufficient numbers to identify risk factors to determine which children with asthma will develop the severe, persistent variety.

Interactions of air pollutants and aeroallergens with other factors (e.g., genetic, lifestyle factors, etc.) addressed by the other asthma hypotheses contribute to the requirement for a large sample. Accurate exposure and phenotypic data are needed to assess the importance of many asthma and allergy genotypes interacting with many exposures in relation to complex and variable asthma phenotypes. A large sample will be required to evaluate the susceptibility to the adverse effects of certain pollutants and aeroallergens and their sources in specific genotype-phenotype clusters. Also, data are needed from children of different ethnicities living in varied housing, socioeconomic, and geographic conditions. These factors may be correlated with variations in exposures, and a large sample size will be necessary to allow adjustments to account for this.

Landrigan et al. (2006) discuss in more detail the rationale and strengths for such a study, which is expected to direct the development of prevention strategies for both diseases of childhood and chronic diseases of adulthood whose developmental origins likely begin in childhood.

3.3 Scientific Merit

Theory supporting hypothesis

Many organic compounds such as polycyclic aromatic hydrocarbons (PAH) and quinones as well as transition metals are associated with products of fossil fuel combustion and tobacco smoke. PAH and transition metals have adjuvant effects on cytokine-mediated airway inflammation. This occurs, in part, through oxidative stress mechanisms involving the production of reactive oxygen species (ROS) by pollutants and resultant antioxidant, inflammatory, and cytotoxic responses in the human lung (Behndig et al., 2006; Nel, Diaz-Sanchez, & Li, 2001; Li et al., 2003; Xia et al., 2004; Xiao, Wang, Li, Loo, & Nel, 2003). This process has been linked to the enhancement of allergic respiratory responses to airborne allergens and may be involved in the onset of atopy (Diaz-Sanchez, Proietti, & Polosa, 2003).

Evidence for this has come primarily from studies that have used diesel exhaust particles (DEP) as a model exposure since this source is particularly rich in redox cycling compounds (Xiao et al., 2003). However, diesel exhaust in the environment is also comprised of important volatile and semivolatile (nonparticle-phase) organic compounds and metals, and automobile exhaust produces nearly the same set of components, including PAHs, which are semivolatile organic compounds. Emission factor measurements from on-road studies show that gasoline vehicles comprise the majority of mobile source particulate matter (PM) emissions in the United States (Gertler, 2005).

Atopy has repeatedly been shown to be a major risk factor in the development of asthma in children (Holt, Macaubas, Stumbles, & Sly, 1999; Hoffjan, Nicolae, & Ober, 2003; Bel, 2004). In addition to atopy, other identified risk factors include parental asthma, sibling asthma, recurrent chest infections at ages 1 and 2, parental smoking, male gender, obesity, eczema, allergic rhinitis, and outdoor pollutants (Taussig et al., 2003; King, Mannino, & Holguin, 2004; Arshad, Kurukulaaratchy, Fenn, & Matthews, 2005). Sensitization to multiple inhalant allergens (pollen and common environmental) in adults has been shown to increase the risk of having physician-diagnosed asthma, allergic rhinoconjunctivitis, and wheezing in a dose-dependent relationship (Pallasaho, Ronmark, Haahtela, Sovijarvi, & Lundback, 2006). Early (assessed at 6 months) exposure to cats increased the risk of atopic sensitization and the later development of asthma through age 10 in a dose-dependent manner except for the most highly exposed infants. This latter group showed a reduced risk of sensitization and wheezing (Lau et al., 2005). There are age-dependent changes in cytokine production by Th cells, and the blunting of these changes may not occur until age 5. Thus, these exposure/response associations in young children whose immune system is developing complicates the understanding of potential effects that a large prospective study may help resolve. Any investigation into the causative factors of asthma should not ignore the closely related causative factors of atopic sensitization and other atopic diseases as the factors leading to these conditions appear to overlap greatly and could even be independently synergistic with other risk factors for asthma. For example, higher exposures to fine particles may increase the risk of allergic sensitization to common aeroallergens.

The putative role of air pollutants in mixtures such as ETS or vehicular exhaust in allergic sensitization, asthma incidence, and increased asthma severity is hypothesized to be based, in part, on a failure of the T cell population to mature adequately to T helper 1 (T_H1) over T helper 2 (T_H2) subtype early in life. This drives an imbalance toward T_H2 immunity (Macaubas et al., 2000), which has been associated with atopy, asthma, and increased production of proinflammatory cytokines (namely IL-4, IL-5, and IL-13). This process begins prenatally (Macaubas et al., 2000). In experimental studies, increases in cytokines have been consistently linked to airway inflammatory and IgE-mediated allergic responses to DEP (Pandya, Solomon, Kinner, & Balmes, 2002). Experimental data has shown that DEP can induce primary sensitization to an allergen compared with no sensitization upon exposure to the antigen alone (Diaz-Sanchez, Garcia, Wang, Jyrala, & Saxon, 1999). These responses are hallmarks of allergic asthma where eosinophils among other cells play a key role. Effects of air pollutants on innate immunity mechanisms instead of IgE-mediated acquired immunity is largely unknown but may be important in explaining the incidence and ongoing morbidity of nonallergic or non-eosinophilic asthma (Douwes et al., 2006).

The adjuvant effects of air pollutants on shifts in either acquired or innate immune pathways may begin in utero. Continued exposure during early development is expected to increase further the risk of asthma. This is because most human lung development is complete by ages 6-8 (Burri, 1997), and the developing lung is highly susceptible to immunological and structural changes from inhaled toxicants (Finkelstein & Johnston, 2004; Pinkerton & Joad, 2006). In addition, repeated lower respiratory infections during the preschool years have been associated with the onset of asthma, but this is likely modified by genotype and early life immune phenotype (Friedlander et al., 2005).

Current scientific understanding

Asthma is a complex disease of the lower airways variably characterized by reversible airways obstruction, airway inflammation, and airway hyper-responsiveness. This is clearly reflected in the data from gene association studies which indicate a complex inheritance pattern involving perhaps hundreds of genes governing the expression of varying asthma and atopy phenotypes (Ober & Hoffjan, 2006). Asthma phenotypes that emerge from ages 1-6 have been predictive of persistent asthma symptoms and long-lasting decrements in lung function in cohort studies (Stein & Martinez, 2004). However, those studies did not evaluate the influence of air pollutants, aeroallergens, and pollutant-allergen interactions on the expression of these phenotypes or their impact on future morbidity. The National Children's Study will assess the risk of asthma-related phenotypes (e.g., transient early wheeze) and exposure-response relationships as hypothesized for diagnosed asthma. It is important to recognize that the Study's focus is on diagnosed asthma that persists into later childhood.

There is substantial evidence that air pollutants and aeroallergens can exacerbate existing asthma. Air pollutants showing acute effects have included ozone, secondary organic aerosols, and some primary constituents of particulate matter from fossil fuel combustion as well as transition metals (Gilmour, Jaakkola, London, Nel, & Rogers, 2006; Peden, 2005; Trasande & Thurston, 2005). However, the role of these air contaminants in the induction of asthma is less clear primarily due to a paucity of research.

Epidemiologic research to support the putative causal relationship of air pollutants with asthma onset in children is sparse. It is also unclear whether repeated acute biological responses to air pollutants early in life leads to the later development of allergy and asthma and which specific types of acute responses are important. Furthermore, there are major gaps in knowledge concerning the relative toxicity of air pollutant mixtures from various sources, which requires more attention to finer spatial monitoring with source tracers (Schlesinger, 2006). To answer these questions, prospective cohort studies are needed with detailed exposure and response data beginning in pregnancy.

To date, most prospective birth cohort studies of asthma onset have been small and used retrospective recall of exposures during pregnancy and early life, which can lead to exposure error. A pregnancy cohort in New York is the only U.S. study that aims to examine the effects of prenatal and early postnatal air pollution exposures on neurocognitive development, asthma etiology, and cancer risk. Early findings include PAH exposures that have adverse effects on birth weight and head circumference (Perera et al., 2003) and adverse effects on cognitive development (Perera et al., 2006). A study conducted in the Czech Republic evaluated lymphocyte immunophenotypes in cord blood at birth among 1,397 deliveries (Hertz-Picciotto et al., 2005) and suggested that the fetal immune system might be altered by maternal exposure to air pollution. Personal PAH exposure during pregnancy in Krakow, Poland, was positively associated with increased risk of respiratory infections and symptoms in children during the first year of life (Jedrychowski et al., 2005). A birth cohort study in the Netherlands found predicted traffic-related exposure to $PM_{2.5}$, $PM_{2.5}$ absorbance, and NO_2 were associated with incidence by age 2 of wheezing, physician-diagnosed asthma, ear/nose/throat infections, and flu or serious colds (Brauer et al., 2002). These findings were confirmed and strengthened at age 4 (Jedrychowski et al., 2005). They also found a positive association between air pollution and sensitization to food allergens (egg and milk) by specific IgE. Exposure estimations were based on measured pollutants from 40 sites selected on variability in traffic then linked with traffic near subject homes using geographic information systems (GIS) (Brauer et al., 2003). Other studies have used GIS-based exposure model estimates of traffic-related air pollutants and found increased exposures were associated with cough without infection and dry cough at night at age 1 in Munich, Germany (Gehring et al., 2002), with incident cough without a cold at ages 3-5 in Leicester, England (Pierse et al., 2006), and with prevalence of wheezing without a cold in Cincinnati, OH (Ryan et al., 2005). In a Southern California cohort of school children, the prevalence of

asthma in children who had no family history of asthma was significantly increased the closer the residence was to a major road (McConnell et al., 2002). In another cohort of Southern California school children followed for 8 years until age 18, both ambient levels of air pollutants including NO_2, $PM_{2.5}$, and elemental carbon (Gauderman et al., 2004) and proximity to traffic (Gauderman et al., 2007) were associated with reduced lung function. This suggests that air pollutant impacts on lung health may persist into adulthood.

Multiple types of exposures (e.g., aeroallergens, air pollutants, viral infections, diet, etc.) are often not assessed in these cohorts. In addition, most have generally relied on questionnaire-reported prevalence of physician-diagnosed asthma and a varied but incomplete set of data on asthma-related clinical phenotypes collected at one time, usually well past disease onset. Such phenotypes include atopic sensitization, airway inflammation, bronchial hyper-responsiveness, lung function, symptom type and frequency, medication use, etc., all of which will be measured in The National Children's Study. The resulting inaccuracy in exposure assessment and disease characterization can make it difficult to identify susceptible individuals based on genetic or phenotypic characteristics. This is particularly problematic given the likely dependency of susceptibility on the magnitude and developmental timing of environmental exposures (Martinez, 2000; Schwartz, 2006a; Schwartz, 2006b; Zaas & Schwartz, 2003).

There are few examples of studies of asthma or atopy where timing of environmental exposures during early development was fully assessed. Evidence to support the study of exposure to the fetus and to the child during prenatal and postnatal periods comes from studies of ETS. Children prenatally and postnatally exposed to ETS have a significantly higher risk of sensitization to food allergens by the age 3 (Zaas & Schwartz, 2003). A pediatric cohort study in Southern California showed increased risk of asthma and wheezing among school children with a history of in utero exposure to ETS but not later exposure. The association was found only among subjects with a homozygous glutathione S-transferase gene deletion polymorphism, which is expected to reduce the ability of cells to counter the proinflammatory effects of ROS (Gilliland et al., 2002).

3.4 Potential for Innovative Research

The National Children's Study offers the unique opportunity to simultaneously examine multiple risk factors for asthma and to explore possible interactive and synergistic effects between different risk factors. Perhaps the greatest opportunity for innovation would be the examination of genetic or other markers of individual susceptibility to specific environmental agents.

Few cohort studies are examining effects of indoor or ambient air pollution and aeroallergen exposures during pregnancy and early childhood on the occurrence of childhood asthma. The Study will consider these exposures and the impact of regional ambient air pollution (McConnell et al., 2002), traffic-related air pollutants and proximity to roadways (Brauer et al., 2002), and indoor sources of VOCs or semi-VOCs (e.g., cleaning agents, solvents, paints, plastics, cars in garages). The current literature on many of these compounds in relation to asthma in children is limited compared to that on occupational asthma (Delfino, 2002; Dales & Raizenne, 2004; Rumchev, Spickett, Bulsara, Phillips, & Stick, 2004; Jaakkola & Jaakkola, 2006). A recent review identified multiple studies showing associations between asthma diagnosis or other respiratory outcomes and indoor residential chemical emissions (Mendell, in press). Most of these studies were small, not large cohort designs, and not conducted in the United States. Several prospective cohort studies, however, collected data during pregnancy or the postnatal period on exposures to indoor residential emissions.

Indoor and outdoor sources that contribute to levels of a particular chemical will drive concentration of other correlated pollutants that may be unmeasured. For example, findings showing associations of acute asthma outcomes with ambient VOCs (Delfino, Gong, Linn, Hu, & Pellizzari, 2003)

and exhaled breath VOCs (Delfino, Gong, Linn, Pellizzari, & Hu, 2003) have been suggested to serve as surrogate markers for a mixture of more causally active components, particularly from traffic-related sources. These components would include many particle-bound, semivolatile and volatile components, some of which are anticipated to emerge as potentially important casual agents from future toxicological data and can be assessed from archived environmental samples. From the standpoint of asthma prevention, controlling the source will be the key to success.

Traffic is a major source of outdoor air pollution exposure most likely to be encountered on a nearly daily basis by a majority of pregnant women and children in America. Many epidemiologic studies using retrospective data or cross-sectional methods have shown associations between traffic near the home and asthma and atopy prevalence or morbidity (Delfino, 2002; Heinrich & Wichmann, 2004; Sarnat & Holguin, 2007). However, there is limited data on asthma incidence and local air pollution (Brauer et al., 2002). In addition, in-vehicle exposure to toxic air pollutant components may dominate total exposures during the work week of expectant mothers. It has been estimated that more than 50 percent of black carbon and ultrafine particle (UFP) exposures for nonsmoking urbanites in Los Angeles comes from time in vehicles (Fruin, Winer, & Rodes, 2004; Fruin, Westerdahl, Sax, Fine, & Sioutas, 2005).

The potential for assessing individual susceptibility to air pollutants, including genetic, is great with the Study. Most asthma genetic studies employ similar and highly accurate genotyping methods. However, they generally employ widely divergent and generally inaccurate methods of exposure assessment. This has likely been responsible for an inconsistent literature on asthma gene association studies, as well as missed, biased, or ignored gene-environment interactions.

3.5 Feasibility

Outdoor air pollutant exposures at home, work, or preschool during pregnancy and early childhood can be estimated using land use regression and related modeling techniques based on traffic, microenvironmental, neighborhood, and ambient monitoring data (Brauer et al., 2003; Jerrett et al., 2005; Ross et al., 2006). Indoor air pollutant and allergen measurements have been collected successfully in several large surveys and include measures collected by study participants. Personal exposures for most air pollutants and other chemicals are usually correlated with indoor measurements. Outdoor air concentrations and estimates can be compared with measurements made at homes and with information about in-home sources to provide estimates of indoor concentrations over longer time-periods. In larger primary sampling units (PSUs), the data for use in model development to predict outdoor home exposures can be collected at low cost in targeted areas given that the populations will be recruited from relatively small geographic areas (neighborhoods). An air-monitoring network exists across the United States, and populations could be supplemented with community-level outdoor air measurements. The monitoring network can be used to characterize exposure to children who move to another location. Combined with air models, this will allow investigators to characterize a wide range of exposures during prenatal, neonatal, early childhood, and later childhood periods.

The national probability sample for the Study, combined with selection of communities within each PSU, should allow for appropriate gradients in exposure to air pollution on both a regional and local scale. In addition, because pollutants such as ozone vary with climate, temporal variability must be included.

ETS exposure has been successfully assessed using questionnaires validated against particle measurements and cotinine measurements in saliva, urine, or blood plasma. This will continue to be the case in The National Children's Study.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Exposure to indoor and outdoor air pollution and aeroallergens

Primary/child

- Exposure to indoor and outdoor air pollution and aeroallergens

4.2 Methods

Primary/maternal

- Environmental sampling for indoor and outdoor air pollution measures (e.g., PM, elemental carbon, NO2, and O3)
- Time-activity patterns (questionnaires, diaries, or GPS) for use in estimating exposures and contributions to total pollutant exposures from emission sources near the home and work
- Blood samples; urine samples; other physical sampling
- Interview/questionnaire information
- Dust samples will be collected for later measurement of mold, endotoxin, and common environmental antigens

Primary/child

- Environmental air and dust samples inside and outside home, school and daycare (e.g., PM, criteria pollutant gases, allergens, endotoxin, environmental tobacco smoke, VOCs, sVOCs)
- Other exposure information (housing and neighborhood characteristics, product usage in home, child time-activity patterns [questionnaires, diaries, GPS], local agency air quality reports) for use in estimating exposures and pollutant contribution to total exposures from emission sources near the home (mobile sources, including diesel and automobile traffic, and stationary sources, such as industrial emissions)
- Biological samples (exposure markers in blood, urine, etc.)

4.3 Life Stage

Primary/maternal

- Prenatal through birth

Primary/child

- Periodic, birth through year 21

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Airway hyper-reactivity
- Lung function
- Airway inflammation (exhaled NO)
- Immune system function (e.g., lymphocyte subsets, cytokines, total IgE)
- Allergic sensitization (specific IgE)
- Symptom history and medication use
- Respiratory infection history

5.2 Methods

- Examination, interview and testing by medical professional (e.g., history of asthma symptoms, lung function tests, skin prick test, exhaled breath for NO, etc.)
- Medical record reviews
- Cord and blood samples

5.3 Life Stage

- Periodic, birth through year 21

6. Important Confounders, Mediators, and Effect Modifiers

- **Poor health care access in inner cities:** Especially among Blacks and Hispanics, poor health care access exacerbates the risk of asthma attacks and deaths (Lara et al., 2003; Sin, Bell, & Man, 2004; Wallace, Scott, Klinnert, & Anderson, 2004). This and other disparity factors could be behind the greater asthma prevalence, especially among Blacks. These issues are thoroughly discussed in the disparities in asthma hypothesis and in the review by Gold and Wright (2005).
- **Socioeconomic status:** Lower socioeconomic status and some minority groups, such as Blacks and Hispanics, are likely to live in areas with increased air pollution, such as near freeways or industrial sites. Careful assessments of these characteristics will be important to properly control for their influence on outcomes.

- **Environmental exposures:** Diet, physical activity, bioaerosols, stress, and infections, which will be the topic of other hypotheses, could interact with or confound associations of asthma onset with air pollutants (example hypotheses 2.2 and 2.3). Though the following list is not exhaustive, some important factors associated with atopy and asthma include demographic group (age, race, income, or education), familial characteristics (number of siblings, parent and sibling phenotypes for asthma), daycare attendance, pet ownership, living on a farm, body mass index, immunizations, several dietary factors, respiratory infections, and diabetes.

- **Genetics:** For the analysis of gene-environment interactions, polymorphisms in genes involved in oxidative stress responses to air pollutant exposures and candidate asthma and allergy genes will be included (example hypothesis 2.3). Ultimately, gene-environment interactions may dictate the effect any factor has on disease outcome.

7. Power and Sample Size

Power analyses will have a cut-off value for "high" exposure of the pollutant predictor of interest based on the upper fifth percentile of NCS subjects (i.e., a proportion exposed is 0.05). In addition, we assume a total sample size of N = 100,000 and that the incidence of asthma by age 6 will be 5 percent. A fixed interval proportional hazards model was used to assess the association between pollutant exposure and the risk of asthma. The minimal detectable hazard ratios associated with the pollutant corresponding to power points of 80 percent, 90 percent, and 95 percent for a level 0.05 two-sided test are 1.199, 1.234 and 1.264, respectively. The estimates assume only a main effects model based on exposure to a single factor without consideration of interactions with other exposures, genetics, family history, etc. (National Children's Study Interagency Coordinating Committee, 2003).

The overall size of effects of indoor and outdoor air pollution on the onset of asthma may be small due to interactions with time-of-exposure and other factors (e.g., lifestyle and genetic). To exemplify this, in the following we seek to quantify effect modification by subject genotype or co-exposure to endotoxins and indoor allergens. Assessment of effect modification will be carried out by including multiplicative interaction terms for the potential effect modifier and the pollutant exposure into each regression model. For brevity, we present power analyses for a generic effect modifier with varying prevalences and assume the cutoff value for "high" exposure of the predictor of interest is based on the conservative upper fifth percentile of NCS subjects. Again, we assume a total sample size of N = 100,000 and that the incidence of asthma by age 6 will be 5 percent. We used a fixed interval proportional hazards model to assess the association between pollutant exposure and the risk of asthma. In this case, minimum detectable hazard ratios associated with the interaction term (multiplicative increase in the effect of the pollutant attributable to the effect modifier) corresponding to a specified power point that can be estimated via a modification of the usual two-sample proportional hazards formula given by (Schmoor, Sauerbrei, & Schumacher, 2000):

$$\lambda = \exp\left\{ \left(z_{1-\alpha/2} + z_{1-\beta}\right) \left(\sum_{i,j} 1/p_{ij}\right)^{1/2} \Big/ \left[n \Pr(\delta = 1)\right]^{1/2} \right\}.$$

Table 1. Minimum detectable hazard ratios associated with interactions. Estimates correspond to the 80 percent, 90 percent, and 95 percent power points of a level 0.05 two sided test

Prevalence of effect modifier	Minimum detectable hazard ratio associated with interaction term for selected power points		
	80%	90%	95%
2%	3.66	4.49	5.32
5%	2.30	2.62	2.92
10%	1.83	2.02	2.18
20%	1.58	1.69	1.79
30%	1.49	1.58	1.67
40%	1.45	1.54	1.61

8. Other Design Issues

- **Ethical/burden considerations:** The study also will need to have a formal strategy and process for effectively communicating the results of environmental monitoring to the child's parents, along with appropriate and feasible recommendations regarding the correction of any unhealthful environmental findings. Repeated tests are potentially burdensome.

- **Cost/complexity of data collection:** Repeated waves of environmental exposure assessment can be both costly and burdensome, but are necessary to minimize exposure error that would result from reliance on ambient monitoring systems or the administration of larger questionnaires that would be needed to characterize these exposures. The size, intrusiveness, and time needed for indoor measurements will be minimized and complemented by community-level monitoring.

- **Need for community involvement:** Daycare and school cooperation would be required for some of the intended measures.

9. References

Akinbami, L. (2006). The state of childhood asthma, United States, 1980-2005 (pp. 1-24). *Advanced Data from vital and health statistics, no. 381*. Hyattsville, MD: National Center for Health Statistics.

American Lung Association Epidemiology and Statistics Unit, Research and Program Services. (2006) *Trends in asthma morbidity and mortality*. New York: American Lung Association. Retrieved from http://www.lungusa.org

Arshad, S.H., Kurukulaaratchy, R.J., Fenn, M., & Matthews, S. (2005). Early life risk factors for current wheeze, asthma, and bronchial hyperresponsiveness at 10 years of age. *Chest, 127*(2), 502-508.

Behndig, A.F., Mudway, I.S., Brown, J.L., Stenfors, N., Helleday, R., Duggan, S.T., et al. (2006). Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans. *European Respiratory Journal, 27*(2), 359-365.

Bel, E.H. (2004). Clinical phenotypes of asthma. *Current Opinion in Pulmonary Medicine, 10*(1), 44-50.

Brauer, M., Hoek, G., van Vliet, P., Meliefste, K., Fischer, P., Gehring, U., et al. (2003). Estimating long-term average particulate air pollution concentrations: application of traffic indicators and geographic information systems. *Epidemiology, 14*(2), 228-239.

Brauer, M., Hoek, G., Van Vliet, P., Meliefste, K., Fischer, P.H., Wijga, A., et al. (2002). Air pollution from traffic and the development of respiratory infections and asthmatic and allergic symptoms in children. American Journal of Respiratory & Critical Care Medicine, 166(8), 1092-1098.

Burri, P.H. (1997). Postnatal development and growth. In R.G. Crystal, J.B. West, E.R. Weibel, & P.J. Barnes, (Eds.). *The Lung: Scientific Foundations* (Vol. 1, pp. 1013-1026). Philadelphia: Lippincott-Raven Publishers.

Dales, R., & Raizenne, M. (2004). Residential exposure to volatile organic compounds and asthma. *Journal of Asthma, 41*(3), 259-270.

Delfino, R.J., Gong, H., Jr., Linn, W.S., Pellizzari, E.D., & Hu, Y. (2003). Asthma symptoms in Hispanic children and daily ambient exposures to toxic and criteria air pollutants. *Environmental Health Perspectives, 111*(4), 647-656.

Delfino, R.J., Gong, H. Jr., Linn, W.S., Hu, Y., & Pellizzari, E.D. (2003). Respiratory symptoms and peak expiratory flow in children with asthma in relation to volatile organic compounds in exhaled breath and ambient air. *Journal of Exposure Analysis & Environmental Epidemiology, 13*(5), 348-363.

Delfino, R.J. (2002). Epidemiological evidence for asthma and exposure to air toxics: linkages between occupational, indoor, and community air pollution research. *Environmental Health Perspectives, 110*(Suppl. 4), 573-589.

Diaz-Sanchez, D., Garcia, M.P., Wang, M., Jyrala, M., & Saxon, A. (1999). Nasal challenge with diesel exhaust particles can induce sensitization to a neoallergen in the human mucosa. *Journal of Allergy & Clinical Immunology, 104*(6), 1183-1188.

Diaz-Sanchez, D., Proietti, L., & Polosa, R. (2003). Diesel fumes and the rising prevalence of atopy: An urban legend? *Current Allergy and Asthma Reports, 3*(2), 146-152.

Douwes, J., van Strien, R., Doekes, G., Smit, J., Kerkhof, M., Gerritsen, J., et al. (2006). Does early indoor microbial exposure reduce the risk of asthma? The Prevention and Incidence of Asthma and Mite Allergy birth cohort study. *Journal of Allergy and Clinical Immunology, 117*(5), 1067-1073.

Eder, W., Ege, M.J., & von Mutius, E. (2006). The asthma epidemic. *New England Journal of Medicine, 355*, 2226-2235.

Finkelstein, J.N., & Johnston, C.J. (2004). Enhanced sensitivity of the postnatal lung to environmental insults and oxidant stress. *Pediatrics, 113*(4, Suppl.), 1092-1096.

Friedlander, S.L., Jackson, D.J., Gangnon, R.E., Evans, M.D., Li, Z., Roberg, K.A., et al. (2005). Viral infections, cytokine dysregulation and the origins of childhood asthma and allergic diseases. Pediatric Infectious Disease Journal, 24(11, Suppl.), S170-S176, discussion S174-S175.

Fruin, S., Westerdahl, D., Sax, T., Fine, P., & Sioutas, C. (2005). Predictors of In-Vehicle Ultrafine Particulate Matter Concentrations and Other Vehicle-Related Pollutants on Los Angeles Roadways. Paper presented at the AAAR Conference, Austin, TX, October 20, 2005.

Fruin, S.A., Winer, A.M., & Rodes, C.E. (2004). Black carbon concentrations in California vehicles and estimation of in-vehicle diesel exhaust particulate matter exposures. *Atmospheric Environment, 38*(25), 4123-4133.

Gauderman, W.J., Avol, E., Gilliland, F., Vora, H., Thomas, D., Berhane, K., et al. (2004). The effect of air pollution on lung development from 10 to 18 years of age. *New England Journal of Medicine, 351*(11), 1057-1067.

Gauderman, W.J., Vora, H., McConnell, R., Berhane, K., Gilliland, F., Thomas, D., et al. (2007). Effect of exposure to traffic on lung development from 10 to 18 years of age: A cohort study. *Lancet, 369*(9561), 571-577.

Gehring, U., Cyrys, J., Sedlmeir, G., Brunekreef, B., Bellander, T., Fischer, P., et al. (2002). Traffic-related air pollution and respiratory health during the first 2 years of life. *European Respiratory Journal, 19*(4), 690-698.

Gertler, A.W. (2005). Diesel vs. gasoline emissions: Does PM from diesel or gasoline vehicles dominate in the U.S.? *Atmospheric Environment, 39*(13), 2349-2355.

Gilliland, F.D., Li, Y.F., Dubeau, L., Berhane, K., Avol, E., McConnell, R., et al. (2002). Effects of glutathione S-transferase M1, maternal smoking during pregnancy, and environmental tobacco smoke on asthma and wheezing in children. *American Journal of Respiratory & Critical Care Medicine, 166*(4), 457-463.

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

Gold, D.R., & Wright, R.J. (2005). Population disparities in asthma. *Annual Review of Public Health, 26*, 1-25.

Heinrich, J., & Wichmann, H.E. (2004). Traffic related pollutants in Europe and their effect on allergic disease. *Current Opinion in Allergy & Clinical Immunology, 4*(5), 341-348.

Hertz-Picciotto, I., Herr, C.E., Yap, P.S., Dostal, M., Shumway, R.H., Ashwood, P., et al. (2005). Air pollution and lymphocyte phenotype proportions in cord blood. *Environmental Health Perspectives, 113*(10), 1391-1398.

Hoffjan, S., Nicolae, D., & Ober, C. (2003). Association studies for asthma and atopic diseases: A comprehensive review of the literature. *Respiratory Research, 4*(4), 14.

Holt, P.G., Macaubas, C., Stumbles, P.A., & Sly, P.D. (1999). The role of allergy in the development of asthma. *Nature, 402*(6760, Suppl.), B12-B17.

Jaakkola, J.J., & Jaakkola, M.S. (2006). Professional cleaning and asthma. *Current Opinion in Allergy & Clinical Immunology*, *6*(2), 85-90.

Jedrychowski, W., Galas, A., Pac, A., Flak, E., Camman, D., Rauh, V., et al. (2005). Prenatal ambient air exposure to polycyclic aromatic hydrocarbons and the occurrence of respiratory symptoms over the first year of life. *European Journal of Epidemiology, 20*(9), 775-782.

Jerrett, M., Arain, A., Kanaroglou, P., Beckerman, B., Potoglou, D., Sahsuvaroglu, T., et al. (2005). A review and evaluation of intraurban air pollution exposure models. *Journal of Exposure Analysis & Environmental Epidemiology*, *15*(2), 185-204.

King, M.E., Mannino, D.M., & Holguin, F. (2004). Risk factors for asthma incidence. A review of recent prospective evidence. *Panminerva Medica, 46*(2), 97-110.

Kulig, M., Luck, W., Lau, S., Niggemann, B., Bergmann, R., Klettke, U., et al. (1999). Effect of pre- and postnatal tobacco smoke exposure on specific sensitization to food and inhalant allergens during the first 3 years of life. Multicenter Allergy Study Group, Germany. *Allergy, 54*(3), 220-228.

Landrigan, P.J., Trasande, L., Thorpe, L.E., Gwynn, C., Lioy, P.J., D'Alton, M.E., et al. (2006). The National Children's Study: a 21-year prospective study of 100,000 American children. *Pediatrics, 118*(5), 2173-2186.

Lara, M., Duan, N., Sherbourne, C., Halfon, N., Leibowitz, A., & Brook. R.H. (2003). Children's use of emergency departments for asthma: persistent barriers or acute need? *Journal of Asthma, 40*(3), 289-299.

Lau, S., Illi, S., Platts-Mills, T.A., Riposo, D., Nickel, R., Gruber, C., et al. (2005). Longitudinal study on the relationship between cat allergen and endotoxin exposure, sensitization, cat-specific IgG and development of asthma in childhood—Report of the German Multicentre Allergy Study (MAS 90). *Allergy, 60*(6), 766-773.

Li, N., Hao, M., Phalen, R.F., Hinds, W.C., & Nel, A.E. (2003). Particulate air pollutants and asthma: A paradigm for the role of oxidative stress in PM-induced adverse health effects. *Clinical Immunology, 109*(3), 250-265.

Macaubas, C., Prescott, S.L., Venaille, T.J., Holt, B.J., Smallacombe, T.B., Sly, P.D., et al. (2000). Primary sensitization to inhalant allergens. *Pediatric Allergy & Immunology, 11*(Suppl. 13), 9-11.

Malone, D.C., Lawson, K.A., & Smith, D.H. (2000). Asthma: An analysis of high-cost patients. *Pharmacy Practice Management Quarterly, 20*(1), 12-20.

Martinez, F.D. (2000). Context dependency of markers of disease. *American Journal of Respiratory & Critical Care Medicine, 162*(2, Pt. 2), S56-S57.

McConnell, R., Berhane, K., Gilliland, F., London, S.J., Islam, T., Gauderman, W.J., et al. (2002). Asthma in exercising children exposed to ozone: A cohort study. *Lancet, 359*(9304), 386-391.

McConnell, R., Berhane, K., Yao, L., Jerrett, M., Lurmann, F., Gilliland, F., et al. (2006). Traffic, susceptibility, and childhood asthma. *Environmental Health Perspectives, 114*(5), 766-772.

Mendell, M.J. (In press). Indoor residential chemical emissions as risk factors for respiratory and allergic effects in children: A review. *Indoor Air*.

National Academy of Sciences Institute of Medicine, Division of Health Promotion and Disease Prevention. (2000). *Clearing the Air: Asthma and Indoor Air Exposures*. Washington, DC: National Academies Press.

National Children's Study Interagency Coordinating Committee. (2003). Supporting documentation for the working list of NCS Core Hypothesis presented at the December, 2002 NCS Study Assembly Meeting–Draft: Rationale Document.

Nel, A.E., Diaz-Sanchez, D., & Li, N. (2001). The role of particulate pollutants in pulmonary inflammation and asthma: evidence for the involvement of organic chemicals and oxidative stress. *Current Opinion in Pulmonary Medicine, 7*(1), 20-26.

Newacheck, P.W., & Halfon, N. (2000). Prevalence, impact, and trends in childhood disability due to asthma. *Archives of Pediatric and Adolescent Medicine, 154*(3), 287-293.

Ober, C., & Hoffjan, S. (2006). Asthma genetics 2006: the long and winding road to gene discovery. *Genes and Immunity, 7*(2), 95-100.

Pallasaho, P., Ronmark, E., Haahtela, T., Sovijarvi, A.R., & Lundback, B. (2006). Degree and clinical relevance of sensitization to common allergens among adults: A population study in Helsinki, Finland. *Clinical & Experimental Allergy, 36*(4), 503-509.

Pandya, R.J., Solomon, G., Kinner, A., & Balmes, J.R. (2002). Diesel exhaust and asthma: Hypotheses and molecular mechanisms of action. *Environmental Health Perspectives, 110*(Supp. 1), 103-112.

Peden, D.B. (2005). The epidemiology and genetics of asthma risk associated with air pollution. *Journal of Allergy & Clinical Immunology, 115*(2), 213-219; quiz 220.

Perera, F.P., Rauh, V., Tsai, W.Y., Kinney, P., Camann, D., Barr, D., et al. (2003). Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population. *Environmental Health Perspectives, 111*(2), 201-205.

Perera, F.P., Rauh, V., Whyatt, R.M., Tsai, W.Y., Tang, D., Diaz, D., et al. (2006). Effect of prenatal exposure to airborne polycyclic aromatic hydrocarbons on neurodevelopment in the first 3 years of life among inner-city children. *Environmental Health Perspectives, 114*(8), 1287-1292.

Pierse, N., Rushton, L., Harris, R.S., Kuehni, C.E., Silverman, M., & Grigg, J. (2006). Locally generated particulate pollution and respiratory symptoms in young children. *Thorax, 61*(3), 216-220.

Pinkerton, K.E., & Joad, J.P. (2006). Influence of air pollution on respiratory health during perinatal development. *Clinical & Experimental Pharmacology & Physiology, 33*(3), 269-272.

Ross, Z., English, P.B., Jerrett, M., Scalf, R., Gunier, R., Smorodinsky, S., et al. (2006) Nitrogen dioxide prediction in Southern California using land use regression modeling: Potential for environmental health analyses. *Journal of Exposure Science & Environmental Epidemiology, 16*(2), 106-114.

Rumchev, K., Spickett, J., Bulsara, M., Phillips, M., & Stick, S. (2004). Association of domestic exposure to volatile organic compounds with asthma in young children. *Thorax, 59*(9), 746-751.

Ryan, P.H., LeMasters, G., Biagini, J., Bernstein, D., Grinshpun, S.A., Shukla, R., et al. (2005). Is it traffic type, volume, or distance? Wheezing in infants living near truck and bus traffic. *Journal of Allergy & Clinical Immunology, 116*(2), 279-284.

Sarnat, J.A., & Holguin, F. (2007). Asthma and air quality. *Current Opinion in Pulmonary Medicine 13*(1), 63-66.

Schmoor, C., Sauerbrei, W., & Schumacher, M. (2000). Sample size considerations for the evaluation of prognostic factors in survival analysis. *Statistics in Medicine, 19*(4), 441-452.

Schwartz, D.A. (2006a). The importance of gene-environment interactions and exposure assessment in understanding human diseases. *Journal of Exposure Science & Environmental Epidemiology*, *16*(6), 474-476.

Schwartz, D.A. (2006b). Future of toxicology—A shift in the paradigm: focus on human disease. *Chemical Research in Toxicology, 19*(9), 1121-1124.

Sin, D.D., Bell, N.R., & Man, S.F. (2004). Effects of increase primary care access on process of care and health outcome among patient patients with asthma who frequent emergency departments. *American Journal of Medicine, 117*(7), 479-483.

Stafford, R.S., Ma, J., Finkelstein, S.N., Haver, K., & Cockburn, I. (2003). National trends in asthma visits and asthma pharmacotherapy, 1978-2002. *Journal of Allergy and Clinical Immunology, 111*(4): 729-735.

Stein, R.T., & Martinez, F.D. (2004). Asthma phenotypes in childhood: Lessons from an epidemiological approach. *Paediatric Respiratory Review, 5*(2), 155-161.

Suissa, S., Ernst, P. Benayoun, S., Baltzan, M., & Cai, B. (2000). Low-dose inhaled corticosteroids and the prevention of death from asthma. *New England Journal of Medicine 343*(5), 332-336.

Suissa, S., Ernst, P., & Kezouh, A. (2002). Regular use of inhaled corticosteroids and the long term prevention of hospitalization for asthma. *Thorax 57*(10), 880-884.

Taussig, L.M., Wright, A.L., Holberg, C.J., Halonen, M., Morgan, W.J., & Martinez, F.D. (2003). Tucson Children's Respiratory Study: 1980 to present. *Journal of Allergy and Clinical Immunology, 111*(4), 661-75; quiz 676.

Trasande, L., & Thurston, G.D. (2005). The role of air pollution in asthma and other pediatric morbidities. *Journal of Allergy & Clinical Immunology, 115*(4), 689-699.

Wallace, A., Scott, J., Klinnert, M., & Anderson, M.E. (2004). Impoverished children with asthma: a pilot study of urban healthcare access. *Journal for Specialists in Pediatric Nursing, 9*(2), 50-58.

Xia, T., Korge, P., Weiss, J.N., Li, N., Venkatesen, M.I., Sioutas, C., et al. (2004). Quinones and aromatic chemical compounds in particulate matter induce mitochondrial dysfunction: implications for ultrafine particle toxicity. *Environmental Health Perspectives, 112*(14), 1347-1358.

Xiao, G.G., Wang, M., Li, N., Loo, J.A., & Nel, A.E. (2003). Use of proteomics to demonstrate a hierarchical oxidative stress response to diesel exhaust particle chemicals in a macrophage cell line. *Journal of Biological Chemistry, 278*(50), 50781-50790.

Zaas, D., & Schwartz, D.A. (2003). Genetics of environmental asthma. *Seminars in Respiratory & Critical Care Medicine, 24*(2), 185-196.

DIETARY ANTIOXIDANTS AND ASTHMA RISK

1. Meta Hypothesis

Intake of antioxidants in diet affects the risk of asthma.

2. Specific Hypotheses

1. Maternal intake of antioxidants during pregnancy reduces the incidence of asthma in their offspring.

2. Intake of antioxidants during childhood will reduce the frequency and severity of asthma in children by age 5.

3. Genes involved in immune pathways will interact with childhood antioxidant intake to produce gene-environment interactions.

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

Asthma is the most common chronic illness among children. (National Academy of Sciences Institute of Medicine, 2000). U.S. data on asthma prevalence from the National Health Interview Survey (NHIS), The Centers for Disease Control and Prevention's [CDC] National Center for Health Statistics (NCHS), show the prevalence of current asthma increased 85 percent from 1982 through 1996 to an estimated 14.6 million people (5.52 percent of the U.S. population) according to the American Lung Association (American Lung Association Epidemiology and Statistics Unit, 2006). This increase was 76 percent in children younger than age 18 or 4.43 million children in 1996 (6.20 percent).

Between 1997 and 2000, the NHIS used a different definition of current asthma prevalence, which suggested an abrupt decrease, but this difference made comparison to past trends inappropriate. To rectify this, in 2001 a question was added back to restate the prevalence of current asthma regardless of attack occurrence in the past year, which made comparisons to years before 1997 appropriate. It was then apparent that current asthma prevalence had continued to increase until 2001, later reaching a plateau in most groups except Black children and adolescents, who have continued to experience increased prevalence annually (American Lung Association Epidemiology and Statistics Unit, 2006).

In 2004, 30.2 million Americans (10.47 percent of the population) had been diagnosed with asthma by a health professional. This estimate included 6.5 million children younger than 18. Almost 4 million children younger than 18 were estimated to have experienced an asthma attack in 2004. U.S. prevalence data from both the 12-month prevalence (before 1997) and 12-month attack prevalence of asthma (since 1997) were highest among children ages 5-14, Blacks compared with whites, and females (American Lung Association Epidemiology and Statistics Unit, 2006; Akinbami, 2006).

Hospitalization and death rates in children have also reached a plateau or decreased (Akinbami, 2006). Despite a plateau in prevalence in 2001, nonambulatory visits for asthma to physician offices by children have continued to increase (Akinbami, 2006), possibly due to increased public and

health provider awareness, which improved treatment. For example, there was a dramatic increase in the use of preventive (controller) medications, chiefly inhaled corticosteroids (Stafford et al., 2003; Suissa et al., 2000, 2002), which may have mitigated increases in hospitalization and mortality.

In the United States, approximately 38 percent of the asthma-related hospital discharges in 2004 were children younger than age 15, even though only 21 percent of the U.S. population was younger than that age. This trend paralleled increasing asthma hospitalization and death rates in children (American Lung Association Epidemiology and Statistics Unit, 2006; Akinbami, 2006).

Similar or greater increases in the prevalence of asthma during the second half of the 20th century were reported from other countries (Eder, Ege, & von Mutius, 2006) and increases may still be occurring, as seen in a recent birth cohort study in Oslo, Denmark, where every fifth child is now affected by asthma. (Lodrup Carlsen et al., 2006).

Economic and social burden

In 2004, the total cost of asthma was estimated at $16.1 billion, including $11.5 billion in direct health care costs and $4.6 billion in indirect costs (lost productivity)(American Lung Association Epidemiology and Statistics Unit, 2006). The more severe forms of asthma account for a disproportionate amount of the total direct costs. Malone, Lawson, and Smith (2000) estimated that less than 20 percent of asthmatics account for more than 80 percent of the direct costs. Asthma also poses a substantial and increasing public health burden from school absence and restriction of usual physical and social activities (Newacheck & Halfon, 2000).

3.2 Justification for a Large Prospective Longitudinal Study

Opinions differ on which study designs are ideal to use to address complex common disorders such as asthma. In a recent literature debate, Willett et al. (2007) supported the retrospective use of available databases to study common disorders such as asthma, while Collins and Manolio (2007) supported a new large cohort study mainly of adults (Manolio, Bailey-Wilson, & Collins, 2006). The commentary in both articles covered the strengths and weaknesses of both approaches. Willet et al. and Collins and Manolio praised the strengths of a design based on a large birth cohort, but they questioned the feasibility of a large prospective cohort study design that incorporates in utero and early postnatal data as well as data from stages of development from infancy through childhood and into adulthood. However, the NCS could provide valuable data that could be used to accurately assess the timing of exposures, particularly during critical windows of vulnerability (e.g., specific trimester of pregnancy, early vs. later postnatal periods, etc.) on the onset of asthma in childhood and persistence into adulthood.

Accurate exposure and phenotypic data are needed to assess the importance of many asthma and allergy genotypes interacting with numerous exposures in relation to complex and variable asthma phenotypes. Interactions of maternal and fetal genotypes contribute to the requirement for a large sample. A large sample will be required to evaluate the susceptibility to the adverse effects of certain exposures and their sources in specific genotype-phenotype clusters.

Also, data are needed from children of different ethnicities living in varied housing, socioeconomic, and geographic conditions. These factors may account for some of the ethnic disparities related to asthma, which may be correlated with variations in exposures. A large sample size will be necessary to allow for statistical adjustments to account for this. Landrigan et al. (2006) discuss the rationale and strengths for a large prospective study, which is expected to direct the development of prevention strategies for both diseases of childhood and chronic diseases of adulthood whose developmental origins likely begin in childhood.

3.3 Scientific Merit

Seaton et al., (1994) proposed that the increases in asthma occurring at that time might be due to changes in diet (i.e., decreased vegetable consumption) rather than an increase in air pollution as others had proposed. They reasoned that decreased antioxidant consumption had increased population susceptibility and had contributed to the increases in asthma incidence and prevalence, and proposed a mechanism that related decreased dietary antioxidant intake to reduced lung antioxidant defenses, which increased airway susceptibility to oxidant damage and resulted in airway inflammation and asthma. Support for this hypothesis has grown (Kirkham and Rahman, 2006; Rahman, Biswas, & Kode, 2006; Romieu et al., 2006; Romieu et al., 2002).

Interest in the relation between dietary antioxidant consumption and risk of asthma arises from several sources. One hypothesis proposed that the effect of antioxidants on asthma is related to an effect on the developing immune system of the fetus (Cunningham-Rundles, McNeeley, & Moon, 2005). Another hypothesis proposes that antioxidants protect against exposure that leads to oxidative stress and its adverse effects on lung function (Romieu et al., 2002). General population-based health surveys have demonstrated modest direct associations between pulmonary function and antioxidant consumption and serum levels (Schunemann et al., 2002; Schwartz & Weiss, 1990). Also, oxidative damage markers are found at higher concentrations in the exhaled breath condensate of children with asthma compared to those without asthma, while markers of antioxidant status are higher among children without asthma (Corradi et al., 2003). Deleterious pulmonary inflammatory response to ozone exposure, thought to be mediated at least in part by oxidative damage to airway tissues, may be modified by oral intake of vitamins C and E (Samet et al., 2001; Romieu et al., 1998).

Little direct evidence exists for the relationship between antioxidant consumption and the development of asthma, especially in relation to exposure to potential oxidative stressors such as ozone, nitrous oxides, or environmental tobacco smoke. Although the mechanisms that might link oxidative insult with the onset of asthma have not been fully elucidated, there is some evidence that pollution plays a role, either as gas (e.g., ozone) or as ultrafine particles (Elder, Gelein, Finkelstein, Cox, & Oberdorster, 2000; Kim & Nadel, 2004; Klebanoff et al., 2005; Frampton et al., 2006).

Examination of the temporal relationship between antioxidant consumption and subsequent outcome and assessment of genetic variation in immunologic response to oxidative stress and the potential modifying influence of antioxidant exposure will be important in understanding potential intervention techniques, whether related to asthma incidence or treatment (National Children's Study Interagency Coordinating Committee, 2003). A recent review (Kalantar-Zadeh, Lee, & Block, 2004) echoed this with a recommendation for more epidemiological studies and a specific proposal to evaluate the intake of antioxidants in children ages 2-6, interactions with genetic and environmental risk factors that may lead to the development and persistence of asthma, and the relationship between degree of antioxidant deficiency and the course of asthma and atopy.

Recent cohort studies of maternal intake of antioxidants during pregnancy

Devereux and Seaton (2005) reviewed a decade of research on the evidence of diet as a risk factor for asthma and dietary interventions. They noted that studies of lung function in adults (e.g., Hu & Cassano, 2000) and children (e.g., Gilliland et al., 2003) showed independent effects of positive association of antioxidant nutrients [including vitamins C and E] and measures of lung function (forced expiratory volume). However, intervention studies with children were mostly negative, and increases in antioxidants in the diets of children did not reduce the risk for asthma. The authors concluded dietary intake of antioxidants most likely were exerting effects during pregnancy or early childhood.

The theory of developmental origins of health and disease, suggesting that fetal effects could have profound effects on later outcomes, contributed to a recent change in the design of studies investigating the impact of antioxidants on asthma (Devereux & Seaton, 2005). Instead of focusing just on the effects of child intake of antioxidants, recent studies have focused on maternal intake of antioxidants during pregnancy and later effects on the risk of childhood asthma in the offspring. For example, multiple reports about maternal intake of antioxidants on childhood wheeze and asthma have been published based on birth cohorts established in Aberdeen, United Kingdom (Martindale et al., 2005; Devereux et al., 2006, 2007) and in Boston, MA (Litonjua et al., 2006; Camargo et al., 2007). These studies have focused specifically on two components of maternal antioxidant intake during pregnancy (vitamins E and D).

Based on the Aberdeen cohort, Martindale et al., (2005) evaluated 2,000 women and followed 1,924 children born to the cohort at 6, 12, and 24 months of age. Maternal vitamin E intake during pregnancy was negatively associated with wheeze (in the absence of "cold") and eczema (in atopic mothers) in 1,253 offspring at 2 years of age, and vitamin C intake was positively associated with wheeze during childhood. Devereux et al. (2006) evaluated 1,120 children born to this cohort at age 5 and reported a continuation of the negative association of vitamin E intake during pregnancy with wheeze in the offspring. This research also showed a similar negative association of vitamin E intake during pregnancy with several other phenotypes in offspring, including the persistent wheeze-without-cold phenotype and doctor-confirmed asthma and asthma plus wheeze at age 5. Maternal intake of zinc during pregnancy was also negatively associated with asthma and eczema at age 5. An additional report by Devereux et al. (2007) on outcome at age 5 revealed that increased maternal intake of vitamin D was associated with a decrease in the risk for wheezing in childhood, but another report of asthma at age 5 showed no association with maternal intake of vitamin E, vitamin D, or zinc that was suggested by the earlier reports (Willers et al., 2007).

Based on the VIVA cohort in Boston, Litonjua et al. (2006) evaluated 1,290 mother-child pairs in a cohort study of maternal diet and reported a negative association of maternal intake of vitamin E and zinc with wheezing in their offspring at age 2. In a separate report on this cohort, Camargo et al. (2007) documented a negative association of maternal intake of vitamin D with recurrent wheeze at age 3. Analysis of both child and maternal intake of vitamin D revealed that a child's intake was not related to recurrent wheezing in childhood, and the protection provided by high maternal intake of vitamin D was present for low and for high vitamin D intake of the child.

A mitochondrial antioxidant hypothesis of asthma

The focus on antioxidants suggests an involvement of the mitochondria, which produce reactive oxygen species (ROS) as a byproduct of cellular energy production. Wallace, Lott, and Procaccio (2007) developed a variant of the antioxidant hypothesis of asthma based on the involvement of mitochondria (see Appendix A). They contrasted two perspectives about the pathophysiology of asthma (the beta adrenergic theory [Israel et al., 2004], and the ROS-inflammatory theory [Corradi et al., 2003; Kinnula and Crapo, 2003]), that are consistent with the mechanism of action of common pharmacologic treatments of asthma, such as long-acting beta agonists and inhaled corticosteroids (Sin et al., 2004). Wallace, Lott, and Procaccio reasoned that these different pathophysiological mechanisms converge on a single disease process that leads to asthma. They proposed this "third factor" was the mitochondrion, which is shared by the beta adrenergic and ROS-inflammatory systems of the lung and affected by antioxidants (Shoffner & Wallace, 1994; Wallace, 2005). This innovative theory directs the search for genetic contributions to asthma and for evaluation of the effects of environmental factors (antioxidant intake) on the incidence and prevalence of asthma.

An evaluation of variation of mtDNA and the risk for asthma is lacking in the literature, but Wallace, Lott, and Procaccio hypothesized that common variants in mtDNA may predispose an individual to asthma. They reviewed the sources of genetic variation in mtDNA and identified three classes of mutation: maternally inherited deleterious mutations, ancient adaptive polymorphisms, and somatic mutations. The maternally inherited and ancestral mutations that have emerged in the human species require a search for thrifty genotypes, which have resulted from positive selection in former environments that now increase risk for asthma due to "environmental mismatch." The mtDNA haplotypes that emerged in evolutionary history and adaptation to cold climates (Wallace, 2005) produce clear haplogroups, and the most prevalent haplogroup (designated as H) is carried by about 45 percent of individuals with European heritage. In the NCS sample, it would be possible to evaluate whether this common haplogroup is associated with asthma in the context of the planned evaluations of variation in nuclear DNA using the nested case-control approach.

Evaluations of somatic mutations that occur in mtDNA and accumulate during a person's life require a different strategy. In the NCS, repeated samples will be obtained, and epithelial cells can be obtained from urine samples collected at each visit. Cells from these samples can be assayed to determine the percentage of somatic mutations at each point in time. Typically, somatic mutations are investigated for diseases in the elderly. As a result of more mutations accumulating through time, little information exists about variation in children that may be associated with common diseases. Since somatic mutations are clearly related to environmental exposures and conditions that increase ROS, a high load may predispose to asthma. Most of the endogenous ROS is from mitochondrial oxidation of calories via oxidative phosphorylation, and antioxidants affect ROS production. The mitochondrial antioxidant hypothesis of asthma would suggest that antioxidant deficiency may result in an increased rate of somatic mutations that would contribute to asthma. A simple test of this would be to determine in the case-control design whether somatic mutations in mtDNA are elevated in cases with asthma. Based on the mitochondrial antioxidant hypothesis of asthma, the intake of antioxidants may have an effect on the rate of accumulation of somatic mutation over time, and the repeated samples specified in the NCS would allow for the test of this hypothesis of an interaction of an environmental exposure (antioxidant intake) and a mitochondrial genetic factor (somatic mutation).

3.4 Potential for Innovative Research

Vulnerability to particular risk factors is determined not only by the genome acquired at conception but by the nature of the environmental influences during critical periods of development. As a result, a longitudinal assessment of the environment from before conception through pregnancy, fetal life, birth, and infancy could provide an opportunity to understand time-dependent susceptibility to asthma. For example, the review by Saglani and Bush (2006) emphasized the importance of the first three years of life and concluded that fetal and maternal genotype interact with environmental exposures to establish a risk phenotype, which interacts with antenatal exposures to set by age 3 the life-long course of chronic asthma and lung impairment.

The recent studies of maternal intake of antioxidants and the effects on the risk of asthma in their offspring based on the Aberdeen cohort (Martindale et al., 2005; Devereux et al., 2006, 2007) and the VIVA cohort (Litonjua et al., 2006; Camargo et al., 2007) indicate that fetal exposure to vitamins E and D has an impact on asthma in childhood. However, the VIVA and Aberdeen cohorts are relatively small (approximately 1,000) and were probably underpowered to address the effects on rare outcomes (e.g., asthma rather than wheeze) and interactions of different antioxidants (e.g., separate and combined effects of vitamins C, D, and E) and combination of maternal and child dietary intake of antioxidants. The sample participating children of 100,000, collected by the National Children's Study, will provide an opportunity to test hypotheses derived from these groundbreaking studies.

Also, the interplay between genes and environment is characterized by dynamic modifications to the genome, such as epigenetic modifications to nuclear and mitochondrial DNA that have been hypothesized as likely mechanisms for gene-environment interactions. The consideration of mitochondrial involvement in asthma (Wallace et al., 2007) offers an innovative approach to the investigation of genetic and environmental etiologies of asthma that may be relevant to the understanding of variability in response to common treatments (Sin et al., 2004) and to rare side effects of treatment (Israel, 2005).

3.5 Feasibility

The NCS protocol specifies the collection of biological samples that could be the source of DNA for traditional genetic analysis of candidate genes and for gene discovery based on genome-wide association scans. The anticipation of chip-based genotyping of all participants based on current technology (with an estimated cost of $30 million to $100 million), or complete sequencing of all individuals at some point in the future (at an expected cost of less than $1,000 per person), will provide extraordinary detail about genetic variation of nuclear DNA. The inclusion of markers for mtDNA would add little to the cost of this genotyping. Along with the planned definition of cases based on asthma phenotypes, this provides an opportunity to use the efficient nested case-control design for subsets of the sample or the proportional hazard design for the entire sample to evaluate effects of genes, environments (including intake of antioxidants), and their interaction.

The NCS protocol also specifies collection of biological samples at multiple points through time, which provides the opportunity to evaluate epigenetic changes proposed to be the molecular mechanisms of some gene-environment effects (Gluckman & Hanson, 2005). The epigenetic assays for environmental effects of methylation and chromatin status are rapidly evolving, and current methods will improve as a result of extensive current (Callinan & Feinberg, 2006) and future work (NIH Epigenetics Workshop, 2007).

Finally, the mitochondrial antioxidant hypothesis of asthma (Wallace et al., 2007) offers innovative and highly feasible approaches due in part to the small number of mitochondrial genes compared to the number of human genes. The assessment of mtDNA haplogroups in a case-control design would add little to the cost of the genomewide scans proposed for nuclear DNA. The methods for assessment of somatic mutations in mtDNA over time are already in place (Wallace, 2005).

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Antioxidant consumption and antioxidant levels

Primary/child

- Antioxidant consumption and antioxidant levels

4.2 Methods

Primary/maternal

- Interviews/questionnaires (diet/nutrition assessment, vitamin use): Standard food frequency questionnaires (FFQs) will be adjusted to account for the substantial addition of antioxidants to common foods such as fruit juices and cereals. Unless this is done, the FFQ tool will miss a sizeable source of antioxidants in daily diet from foods readily available in most supermarkets.
- Blood samples
- Urine samples
- Breast milk

Primary/child

- Urine samples
- Blood samples
- Interviews/questionnaires (diet/nutrition assessment, vitamin use)

4.3 Life Stage

Primary/maternal

- Prenatal through infancy

Primary/child

- Periodic, 0-3 months to year 21

5 Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

Primary

- Decrease the risk of asthma measured via allergy; child asthma index; airway reactivity

5.2 Methods

Primary

- Direct observation by medical professionals; medical record reviews; interviews/questionnaires; blood samples; urine samples

5.3 Life Stage

Primary

- Periodic, birth to year 21

6. Important Confounders, Mediators, and Effect Modifiers

- **Smoking** which is related to higher levels of oxidative stress, may reduce the effect of an antioxidative diet (Romieu & Trenga, 2001; Farchi et al., 2003).

- **Consumption of flavinoids** in red wine and apples has been shown to be negatively associated with asthma severity (OR = 0.89). This may suggest a protective effect of flavonoids (Shaheen et al., 2001).

- **Everyday foods not measured**: Unless specifically accounted for, the addition of large sources of antioxidants to everyday foods, such as juices and cereals, will be missed by the FFQ.

7. Power and Sample Size

Several methods are available for the evaluation of the antioxidant hypothesis of asthma. A standard approach is to use the nested case-control design with affected cases defined by asthma phenotype and matching to select control subjects (Gauderman, 2002). An alternative would be to use the proportional hazard method of analysis, which uses all cases, and the time when the case is identified as well as the number of cases analyzed. The power and sample size of each of these methods is provided below and described in Section 10 of the NCS Research Plan.

For the nested case-control design, if we assume the initial cohort size is 100,000, the study period is 5 years, there is an annual incidence rate of 2.5 percent and a prevalence by age 6 of 13.1 percent , then we would expect about 11,300 cases. The minimum detectable odds ratio for a genetic main effect and a gene-environment effect (using 1.5 and 10.0 controls per case) is:

Disease	Genetic Main Effect		Gene-environment Interaction	
Incidence (%)	1:1.5	1:10	1:1.5	1:10
2.50	1.16	1.13	1.52	1.36

For the proportional hazard model we present power analyses with a cut-off value for “high” exposure of the predictor of interest (intake of antioxidants) based on the upper fifth percentile of NCS subjects (i.e., a proportion exposed is 0.05). A total sample size will be N = 100,000 and the incidence of asthma by age 6 will be 5 percent. A fixed interval proportional hazards model can be used to assess the association between exposure (history of respiratory viral infection) and the risk of asthma. For a two-sided test at 0.05, the minimal detectable hazard ratios associated with the exposure for power of 80 percent, 90 percent , and 95 percent are 1.199, 1.234 and 1.264, respectively. The estimates assume only a main effects model based on exposure to a single factor without consideration of interactions with other exposures (National Children’s Study Interagency Coordinating Committee, 2003).

8. Other Design Issues

- Ethical considerations are associated with genetic tests, but such considerations underlie the overall NCS and would not be unique to this specific hypothesis.
- For nongenetic factors, the study will need to have a formal strategy and process for effectively communicating results of physiological and biochemical measures to the child's parents and a responsible health care provider.
- Study will need to have a formal strategy and process for effectively communicating results of environmental monitoring to the child's parents along with appropriate and feasible recommendations regarding the correction of any unhealthful environmental findings. In addition, a mechanism needs to be identified to ensure that children found to have asthma through this study can receive adequate medical care for their illness.
- Repeated assessments can be burdensome, and the total respondent burden must be evaluated and be reasonable.
- Blood studies, especially fasting, in younger children will require careful attention.

9. References

Akinbami, L. (2006). *The state of childhood asthma, United States, 1980-2005* (pp. 1-24). Advanced Data from vital and health statistics, no. 381. Hyattsville, MD: National Center for Health Statistics.

Al-Jamal, R., Wallace, W.A., & Harrison, D.J. (2005). Gene therapy for chronic obstructive pulmonary disease: Twilight or triumph? *Expert Opinion on Biological Therapy, 5*(3), 333-346.

American Lung Association Epidemiology and Statistics Unit, Research and Program Services. (2006). *Trends in asthma morbidity and mortality*. New York: American Lung Association. Retrieved from http://www.lungusa.org

Barker, D.J.P. (2001). A new model for the origins of chronic disease. *Medicine, Health Care & Philosophy, 4*(4), 31-35.

Barker, D.J.P., Gluckman, P.D., Godfrey, K.M., Harding, J.E., Owens, J.A., & Robinson, J.S. (1993). Fetal nutrition and cardiovascular disease in adult life. *Lancet, 341*(8850), 938-941.

Baudouin, S.V., Saunders, D., Tiangyou, W., Elson, J.L., Poynter, J., Pyle, A., et al. (2005). Mitochondrial DNA and survival after sepsis: a prospective study. *Lancet, 366*(9503), 2118-2121

Bellomo, F., Piccoli, C., Cocco, T., Scacco, S., Papa, F., Gaballo, A., et al. (2006). Regulation by the cAMP cascade of oxygen free radical balance in mammalian cells. *Antioxidants & Redox Signaling, 8*(3-4), 495-502

Bjornsson, H.T., Fallin, M.D., & Feinberg, A.P. (2004). An integrated epigenetic and genetic approach to common human disease. *Trends in Genetics, 20*(8), 350-358.

Bousquet, J., Jacot, W., Yssel, H., Vignola, A.M., & Humbert, M. (2004). Epigenetic inheritance of fetal genes in allergic asthma. *Allergy, 59*(2), 138-147.

Brown, M.D., Starikovskaya, E., Derbeneva, O., Hosseini, S., Allen, J.C., Mikhailovskaya, I.E., et al. (2002). The role of mtDNA background in disease expression: A new primary LHON mutation associated with Western Eurasian haplogroup. *Journal of Human Genetics, 110*(2), 130-138

Callinan, P.A., & Feinberg, A.P. (2006). The emerging science of epigenomics. *Human Molecular Genetics, 15*(Special 1), R95-R101.

Camargo, Jr., C.A., Rifas-Shiman, S.L., Litonjua, A.A., Rich-Edwards, J.W., Weiss, S.T., Gold, D.R., et al. (2007). Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 t of age. *American Journal of Clinical Nutrition, 85*(3), 788-795.

Carroll, W.D., Lenney, W., Child, F., Strange, R.C., Jones, P.W., & Fryer, A.A. (2005). Maternal glutathione S-transferase GSTP1 genotype is a specific predictor of phenotype in children with asthma. *Pediatric Allergy & Immunology, 16*(1), 32-39.

Collins, F.S., & Manolio, T.A. (2007). Merging and Emerging Cohorts: Necessary but not sufficient. Nature, 445(7125), 259.

Comhair, S.A., Ricci, K.S., Arroliga, M., Lara, A.R., Dweik, R.A., Song, W., et al. (2005). Correlation of systemic superoxide dismutase deficiency to airflow obstruction in asthma. *American Journal of Respiratory and Critical Care Medicine, 172*(3), 306-313

Cook, D.G., & Strachan, D.P. (1997). Health effects of passive smoking. 3. Parental smoking and prevalence of respiratory symptoms and asthma in school age children. *Thorax, 52*(12), 1081-1094.

Cookson, W. (2002) Genetics and genomics of asthma and allergic diseases. *Immunological Reviews, 190*, 195-206.

Corradi, M., Folesani, G., Andreoli, R., Manini, P., Bodini, A., Piacentini, G., et al. (2003). Aldehydes and glutathione in exhaled breath condensate of children with asthma exacerbation. *American Journal of Respiratory and Critical Care Medicine, 167*(3), 395-399.

Culpitt, S.V., Rogers, D.F., Fenwick, P.S., Shah, P., De Matos, C., Russell, R.E., et al. (2003). Inhibition by red wine extract, resveratrol, of cytokine release by alveolar macrophages in COPD. *Thorax, 58*(11), 942-946

Cunningham-Rundles, S., McNeeley, D.F., & Moon, A. (2005). Mechanism of nutrient modulation of the immune response. *Journal of Allergy & Clinical Immunology, 115*(6), 1119-1128; quiz 1129.

Custovic, A., & Simpson, A. (2006). What are we learning from genetic cohort studies? *Paediatric Respiratoiry Reviews, 7*(Suppl. 1), S90-S92.

Devereux, G., & Seaton, A. (2005). Diet as a risk factor for atopy and asthma. *Journal of Allergy & Clinical Immunology, 115*(6), 1109-1117; quiz 1118.

Devereux, G., Litonjua, A.A., Turner, S.W., Craig, L.C.A., McNeil, G., Martindale, S., et al. (2007). Maternal vitamin D intake during pregnancy and early childhood wheezing. *American Journal of Clinical Nutrition, 85*(3), 853-859.

Devereux, G., Turner, S.W., Craig, L.C.A., McNeill, G., Martindale, S., Harbour, P.J., et al. (2006). Low maternal vitamin E intake during pregnancy is associated with asthma in 5-year-old children. *American Journal of Respiratory & Critical Care Medicine, 174*(5), 499-507.

Dey, A.N., Schiller, J.S., & Tai, D.A. (2004, March). Summary Health Statistics for U.S. Children: National Health Interview Survey, 2002. *Vital and Health Statistics, Series 10, Data from the National Health Survey*, (221), 1-78.

Donnelly, L.E., Newton, R., Kennedy, G.E., Fenwick, P.S., Leung, R.H., Ito, K., et al. (2004). Anti-inflammatory effects of resveratrol in lung epithelial cells: Molecular mechanisms. *American Journal of Physiology: Lung Cellular and Molecular Physiology, 287*(4): L774-L783.

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

Eder, W., Ege, M.J., & von Mutius, E. (2006). The asthma epidemic. *New England Journal of Medicine, 355*(21), 2226-2235.

Elder, A.C., Gelein, R., Finkelstein, J.N., Cox, C., & Oberdorster, G. (2000). *Inhalation Toxicology, 12*(Suppl. 4)., 227-246.

Ercan, H., Birben, E., Dizdar, E., Keskin, O., Karaaslan, C., Soyer, O.U., et al. (2006). Oxidative stress and genetic and epidemiologic determinants of oxidant injury in childhood asthma. *Journal of Allergy & Clinical Immunology, 118*(5). 1097-1104.

Farchi, S., Forastiere, F., Agabiti, N., Corbo, G., Pistelli, R., Fortes, C., et al. (2003). Dietary factors associated with wheezing and allergic rhinitis in children. *European Respiratory Journal, 22*(5), 772-780.

Frampton, M.W., Stewart, J.C., Oberdörster, G., Morrow, P.E., Chalupa, D., Pietropaoli, A.P., et al. (2006). Inhalation of ultrafine particles alters blood leukocyte expression of adhesion molecules in humans. *Environmental Health Perspectives, 114*(1), 51-58.

Gauderman, W.J. (2002). Sample size requirements for matched case-control studies of gene-environment interaction. *Statistics in Medicine, 21*, 35-50.

Gauderman, W.J., & Morrison, R. (2007). QUANTO 1.2: A computer program for power and sample size calculations for genetic-epidemiologic studies [Computer software]. Retrieved from http://hydra.usc.edu/gxe

Gilliland, F.D., Berhane, K.T., Li, Y.F., Gauderman, W.J., McConnel, R., & Peters, J. (2003). Children's lung function and antioxidant vitamin, fruit, juice, and vegetable intake. *American Journal of Epidemiology, 158*(6), 576-584.

Gilliland, F.D., Li, Y.F., Saxon, A., Diaz-Sanchez, D. (2004). Effect of gluthathione-S-tranferase M1 and P1 genotypes on xenobiotic enhancement of allergic responses: randomized placebo-controlled crossover trial. *Lancet, 363*(9403), 119-125.

Gluckman, P.D., & Hanson, M.A. (2004). Living with the past: Evolution, development, and patterns of disease. *Science, 305*(5691), 1733-1736.

Hoffjan, S., Nicolae, D., Ostrovnaya, I., Roberg, K., Evans, M., Mirel, D.B., et al. (2005). Gene-environment interaction effects on the development of immune responses in the 1st year of life. *American Journal of Human Genetics, 76*(4), 696-704.

Hu, G., & Cassano, P.A. (2000). Antioxidant nutrients and pulmonary function: the third national health nutrition examination survey (NHANES III). *American Journal of Epidemiology, 151*(10), 975-981.

Israel, E. (2005). Genetics and the variability of treatment response in asthma. *Journal of Allergy & Clinical Immunology, 115*(4, Suppl.), S532-S538.

Kalantar-Zadeh, K., Lee, G.H., & Block, G. (2004). Relationship between dietary antioxidants and childhood asthma: more epidemiological studies are needed. *Medical Hypotheses, 62*(2), 280-290.

Kinnula, V.L., & Crapo, J.D. (2003). Superoxide dismutases in the lung and human lung diseases. *American Journal of Respiratory & Critical Care Medicine, 167*(12), 1600-1619.

Kirkham, P., & Rahman, I. (2006). Oxidative stress in asthma and COPD: Antioxidants as a therapeutic strategy. *Pharmacology & Therapeutics, 111*(2), 476-494.

Landrigan, P.J., Schechter, C.B., Lipton, J.M., Fahs, M.C., & Schwartz, J. (2002). Environmental pollutants and disease in American children: estimates of morbidity, mortality, and costs for lead poisoning, asthma, cancer, and developmental disabilities. *Environmental Health Perspectives 110*(7), 721-728.

Landrigan, P.J., Trasande, L., Thorpe, L.E., Gwynn, C., Lioy, P.J., D'Alton, M.E., et al. (2006). The National Children's Study: A 21-year prospective study of 100,000 American children. *Pediatrics, 118*(5), 2173-2186.

Lemanske, Jr., R.F. (2002). Issues in understanding pediatric asthma: Epidemiology and genetics. *Journal of Allergy & Clinical Immunology, 109*(6, Suppl.), S521-S524.

Li, H., Romieu, I., Wu, H., Sienra-Monge, J.-J., Ramirez-Aguilar, M., Rio-Navarro, B.E., et al. (2007). Genetic polumorphisms in transforming growth factor beta-1 (TGFB1) and childhood asthma and atopy. Human Genetics, 121(5), 529-538.

Li, N., Hao, M., Phalen, R.F., Hinds, W.C., & Nel, A.E. (2003). Particulate air pollutants and asthma: A paradigm for the role of oxidative stress in PM-induced adverse health effects. *Clinical Immunology, 109*(3), 250-265.

Litonjua, A.A., Carey, V.J., Burge, H.A., Weiss, S.T., & Gold, D.R. (1998). Parental history and the risk for childhood asthma. American Journal of Respiratory & Critical Care Medicine, 158 (1), 176-181.

Litonjua, A.A., Rifas-Shiman, S.L., Ly, N.P., Tantisira, K.G., Rich-Edwards, J.W., Camargo, Jr., C.A., et al. (2006). Maternal oxidant intake in pregnancy and wheezing illnesses in children at 2 y of age. *American Journal of Clinical Nutrition, 84*(4), 903-911.

Lødrup Carlsen, K.C., Håland, G., Devulapalli, C.S., Munthe-Kaas, M., Pettersen, M., Granum, B., et al. (2006). Asthma in every fifth child in Oslo, Norway: a 10 year follow-up of a birth cohort study. *Allergy, 61*(4), 454-460.

London, S.J., James Gauderman, W., Avol, E., Rappaport, E.B., & Peters, J.M. (2001). Family history and the risk of early-onset persistent, early-onset transient, and late-onset asthma. *Epidemiology, 12*(5), 577-83.

Lowe, L.A., Simpson, A., Woodcock, A., Morris, J., Murray, C.S., Custovic, A., et al. (2005). Wheeze phenotypes and lung function in preschool children. *American Journal of Respiratory and Critical Care Medicine, 171*(3), 231-237.

Malone, D.C., Lawson, K.A., & Smith, D.H. (2000). Asthma: An analysis of high-cost patients. *Pharmacy Practice Management Quarterly, 20*(1), 12-20.

Manolio, T.A., Bailey-Wilson, J.E., & Collins, F.S. (2006). Opinion: Genes, environment and the value of prospective cohort studies. *Nature Reviews Genetics, 7*(10), 812-820.

Martindale, S., McNeill, G., Devereux, G., Campbell, D., Russell, G., & Seaton, A. (2005). Antioxidant Intake in Pregnancy in Relation to Wheeze and Eczema in the First Two Years of Life. *American Journal of Respiratory & Critical Care Medicine, 171*(2), 121-128.

Mishmar, D., Ruiz-Pesini, E., Mondragon-Palomino, M., Procaccio, V., Gaut, B., & Wallace, D.C. (2006). Adaptive selection of mitochondrial complex I subunits during primate radiation. *Gene 378*, 11-18

Morgan, W.J., Stern, D.A., Sherrill, D.L., Guerra, S., Holberg, C.J., Guilbert, T.W., et al. (2005). Outcome of asthma and wheezing in the first 6 years of life: Follow-up through adolescence. *American Journal of Respiratory and Critical Care Medicine, 172*(10), 1253-1258.

National Academy of Sciences Institute of Medicine, Division of Health Promotion and Disease Prevention. (2000). *Clearing the air: Asthma and indoor air exposures*. Washington, DC: National Academies Press.

National Children's Study Interagency Coordinating Committee. (2003). Supporting documentation for the working list of NCS Core Hypothesis presented at the December, 2002 NCS Study Assembly Meeting–Draft: Rationale Document.

Newacheck, P.W., & Halfon, N. (2000). Prevalence, impact, and trends in childhood disability due to asthma. *Archives of Pediatric and Adolescent Medicine, 154*(3), 287-293.

O'Donnell, A.R., Toelle, B.G., Marks, G.B., Hayden, C.M., Laing, I.A., Peat, J.K., et al. (2004). *American Journal of Respiratory & Critical Care Medicine, 169*(5), 615-622.

Ober, C., & Hoffjan, S. (2006). Asthma genetics 2006: the long and winding road to gene discovery. *Genes and Immunity, 7*(2), 95-100.

Ober, C., & Thompson, E.E. (2005). Rethinking genetic models of asthma: The role of environmental modifiers. *Current Opinion in Immunology, 17*(6), 670-678.

Papa, S., Scacco, S., Sardanelli, A.M., Petruzzella, V., Vergari, R., Signorile, A., et al. (2002). Complex I and the cAMP cascade in human physiopathology. *Bioscience Reports 22*(1): 3-16

Parker, E.A., Baldwin, G.T., Israel, B., & Salinas, M.A. (2004). Application of health promotion theories and models for environmental health. *Health Education & Behavior, 31*(4),491-509.

Peters, J.M., Avol, E., Gauderman, W.J., Linn, W.S., Navidi, W., London, S.J., et al. (1999). A study of 12 Southern California communities with different levels and types of air pollution. II. Effects on pulmonary function. *American Journal of Respiratory and Critical Care Medicine, 159*(3), 768-775.

Rahman, I., Biswas, S.K., & Kode, A. (2006). Oxidant and antioxidant balance in the airways and airway diseases. *European Journal of Pharmacology, 533*(1-3), 222-239.

Romieu, I., & Trenga, C. (2001). Diet and obstructive lung diseases. *Epidemiological Reviews, 23*(2), 268-287.

Romieu, I., Mannino, D.M., Redd, S.C., & McGeehin, M.A. (2004). Dietary intake, physical activity, body mass index, and childhood asthma in the Third National Health And Nutrition Survey (NHANES III). *Pediatric Pulmonology, 38*(1), 31-42

Romieu, I., Meneses, F., Ramirez, M., Ruiz, S., Perez-Padilla, R., Sienra, J.J., et al. (1998). Antioxidant supplementation and respiratory functions among workers exposed to high levels of ozone. *American Journal of Respiratory and Critical Care Medicine, 158*(1), 226-232.

Romieu, I., Sienra-Monge, J.J., Ramirez-Aguilar, M., Tellez-Rojo, M.M., Moreno-Macias, H., Reyes-Ruiz, N.I., et al. (2002). Antioxidant supplementation and lung functions among children with asthma exposed to high levels of air pollutants. *American Journal of Respiratory and Critical Care Medicine 166*(5), 703-709.

Romieu, I., Varraso, R., Avenel, V., Leynaert, B., Kauffmann, F., & Clavel-Chapelon, F. (2006). Fruit and vegetable intakes and asthma in the E3N study. *Thorax, 61*(3), 209-215.

Ruiz-Pesini, E., & Wallace, D.C. (2006). Evidence for adaptive selection acting on the tRNA and rRNA genes of the human mitochondrial DNA. *Human Mutation, 27*(11), 1072-1081.

Ruiz-Pesini, E., Mishmar, D., Brandon, M., Procaccio, V., & Wallace, D.C. (2004). Effects of purifying and adaptive selection on regional variation in human mtDNA. *Science, 303*(5655), 223-226.

Saglani, S., & Bush, A. (2007). The early-life origins of asthma. *Current Opinion in Allergy & Clinical Immunology, 7*(1), 83-90.

Samet, J.M., Hatch, G.E., Horstman, D., Steck-Scott, S., Arab, L., Bromberg, P.A., et al. (2001). Effect of antioxidant supplementation on ozone-induced lung injury in human subjects. *American Journal of Respiratory and Critical Care Medicine, 164*(5), 819-825.

Sanders, V.M. (2006). Epigenetic regulation of Th1 and Th2 cell development. *Brain, Behavior, and Immunity, 20*(4), 317-324.

Schunemann, H.J., McCann, S., Grant, B.J., Trevisan, M., Muti, P., & Freudenheim, J.L. (2002). Lung function in relation to intake of carotenoids and other antioxidant vitamins in a population-based study. *American Journal of Epidemiology, 155*(5), 463-471.

Schwartz, J., & Weiss, S.T. (1990). Dietary factors and their relation to respiratory symptoms, The Second National Health and Nutrition Examination Survey. *American Journal of Epidemiology, 132*(1), 67-76.

Schwartz, S. (2006). Modern epidemiological approaches to interaction: Applications to the study of genetic interactions. In L.M. Hernandez & D.G. Blazer, (Eds.). Genes, Behavior, and the Social Environment: Moving Beyond the Nature/Nurture Debate (Appendix E, pp. 310-337). Washington, DC: National Academies Press.

Seaton, A., Godden, D.J., & Brown, K. (1994). Increase in asthma: a more toxic environment or a more susceptible population? *Thorax, 49*(2), 171-174.

Shaheen, S.O., Sterne, J.A., Thompson, R.L., Songhurst, C.E., Margetts, B.M., & Burney, P.G. (2001). Dietary antioxidants and asthma in adults: population-based case-control study. American Journal of Respiratory & Critical Care Medicine, 164(10, Pt. 1), 1823-1828.

Sherriff, A., Farrow, A., Golding, J., & Henderson, J. (2005). Frequent use of chemical household products is associated with persistent wheezing in preschool age children. *Thorax, 60*(1), 45-49.

Shoffner, J.M., & Wallace, D.C. (1994). Oxidative phosphorylation diseases and mitochondrial DNA mutations: Diagnosis and treatment. *Annual Review of Nutrition, 14*, 535-568

Sin, D.D., Man, J., Sharpe, H., Gan, W.Q., & Man, S.F.P. (2004). Pharmacological management to reduce exacerbation in adults with asthma. *Journal of the American Medical Association, 292*(3), 367-376.

Singh, A.M., Moore, P.E., Gern, J.E., Lemanske, Jr., R.F., & Hartert, T.V. (2007). Bronchiolitis to asthma: a review and call for studies of gene-virus interactions in asthma causation. *American Journal of Respiratory & Critical Care Medicine, 175*(2), 108-119.

Smit, H.A., Grievink, L., & Tabak, C. (1999). *The Proceedings of the Nutrition Society, 58*(2): 309-319.

Stafford, R.S., Ma, J., Finkelstein, S.N., Haver, K., & Cockburn, I. (2003). National trends in asthma visits and asthma pharmacotherapy, 1978-2002. *Journal of Allergy and Clinical Immunology, 111*(4): 729-735.

Stein, R.T., & Martinez, F.D. (2004). Asthma phenotypes in childhood: Lessons from an epidemiological approach. *Paediatric Respiratory Review, 5*(2), 155-161.

Suissa, S., Ernst, P. Benayoun, S., Baltzan, M., & Cai, B. (2000). Low-dose inhaled corticosteroids and the prevention of death from asthma. *New England Journal of Medicine 343*(5), 332-336.

Suissa, S., Ernst, P., & Kezouh, A. (2002). Regular use of inhaled corticosteroids and the long term prevention of hospitalization for asthma. *Thorax 57*(10), 880-884.

Talati, M., Meyrick, B., Peebles, Jr., R.S., Davies, S.S., Dworski, R., Mernaugh, R., et al. (2006). Oxidant stress modulates murine allergic airway responses. Free Radical Biology and Medicine, 40(7), 1210-1219

Valle, D. (2004). Genetics, individuality, and medicine in the 21st century. *American Journal of Human Genetics, 74*(3), 374-381.

Van der Walt, J.M., Dementieva, Y.A., Martin, E.R., Scott, W.K., Nicodemus, K.K., Kroner, C.C., et al. 2004. Analysis of European mitochondrial haplogroups with Alzheimer disease risk. *Neuroscience Letters 365*(1), 28-32

Vercelli, D., & Martinez, F. (2006). The Faustian bargain of genetic association studies: Bigger might not be better, or at least it might not be good enough. *Journal of Allergy & Clinical Immunology, 117*(6), 1303-1305.

Wallace, D.C. (2005a). A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annual Review of Genetics, 39, 359-407

Wallace, D.C. (2005b). The mitochondrial genome in human adaptive radiation and disease: On the road to therapeutics and performance enhancement. *Gene, 354*, 169-180

Wallace, D.C., Lott, M.T., & Procaccio, V. (2007). Mitochondrial Genes in Degenerative Diseases, Cancer and Aging. In D.L. Rimoin, J.M. Connor, R.E. Pyeritz, & B.R. Korf (Eds.). *Emery and Rimoin's Principles and Practice of Medical Genetics* (5th ed., pp. 194-298). Philadelphia: Churchill Livingstone Elsevier

Weiss, K.B. (2001). The health economics of asthma and rhinitis. I. Assessing the economic impact. *Journal of Allergy & Clinical Immunology 107*(1): 3-8.

Willers, S., Devereux, G., Craig, L., McNeill, G., Wijga, A., El-Magd, W.A., et al. (2007). Maternal food consumption during pregnancy and asthma, respiratory and atopic symptoms in 5 year-old children. *Thorax*, (epublication ahead of print).

Willett, W.C., Blot, W.J., Colditz, G.A., Folsom, A.R., Henderson, B.E., & Stampfer J. (2007). Not worth the wait. *Nature, 445*(7125), 257-258.

Yeatts, K., Sly, P., Shore, S., Weiss, S., Martinez, F., Geller, A., et al. (2006). A brief targeted review of susceptibility factors, environmental exposures, asthma incidence, and recommendations for future asthma incidence research. *Environmental Health Perspectives, 114*(4), 634-640.

SOCIAL ENVIRONMENTAL INFLUENCES ON ASTHMA DISPARITIES

1. Meta Hypothesis

Disparities in the incidence, prevalence, severity, and effective management of asthma by race and socioeconomic status are significantly associated and modified by social-environmental factors and processes that influence exposure to physical environmental risk factors, psychosocial stress, and health-related behaviors.

2. Specific Hypotheses

1. The relationship between socioeconomic status, race/ethnicity, and asthma incidence, morbidity, and mortality is significantly modified by socially influenced differential exposure to physical environmental risk factors (e.g., local-source air pollutants, allergens) and psychosocial stress (e.g., poverty, community disorganization, crime and violence, social incivility, noise, crowding, hazardous conditions, physical decay, and unemployment). These effects are moderated by policies and programs that buffer the effects of economic disadvantage on families.

2. Economic, cultural, and social features of the local area are significantly associated with: (1) exposure to stressful life conditions and events; (2) the availability of social ties that provide informational, emotional, and instrumental resources to individuals and families. These factors, in turn, influence outcomes, including immunological function in the child, the likelihood that the child will develop asthma, and asthma severity and management.

3. Background and Justification

3.1 Public Health Importance

Disparities

Many studies and reviews have documented systematic differences or disparities in asthma prevalence, severity, and mortality by race/ethnicity and socioeconomic status that are not fully explained by analysis of individual-level risk factors (Shanawani, 2006; Ford & McCaffrey, 2006; Gold & Wright, 2005; McDaniel, Paxson, & Waldfogel, 2006). Asthma severity and death rates have been highest in areas with higher concentrations of poor people and minority residents, particularly African Americans (Lang & Polansky, 1994; Bai, Hillemeier, & Lengerich, 2007; Lane, Newman, Edwards, & Blaisdell, 2006). In national surveys, African Americans report higher rates of asthma and a higher increase during time (McDaniel et al., 2006). Prevalence rates vary among Hispanic populations, with Mexican-American children in the Southwest experiencing a low prevalence and Puerto Rican children in the East high prevalence (National Academy of Sciences Institute of Medicine, 2000).

National Health Interview Survey (NHIS) data reveal a higher prevalence of asthma in urban areas, but evidence suggests rates can be as high in some rural areas (National Academy of Science Institute of Medicine, 2000). Asthma mortality and hospitalization vary significantly among large cities and neighborhoods within cities (Wright & Fisher, 2003; Byrd & Joad, 2006). Severe asthma demonstrates a graded association with socioeconomic status in the United States, but the precise role is not clear because of complex interactions with race/ethnicity and other factors (Shanawani, 2006; McDaniel et al., 2006). One report suggested that the most significant race/ethnic differences in asthma prevalence are observed among the poor (Smith, Hatcher-Ross, Wertheimer, & Kahn, 2005). The

associations between race/ethnicity, socioeconomic status, and other factors seem to differ for different asthma outcome measures: incidence or risk, prevalence, severity, and mortality.

Economic and social burden

In 2004, the total cost of asthma was estimated at $16.1 billion, including $11.5 billion in direct health care costs and $4.6 billion in indirect costs (lost productivity) (American Lung Association Epidemiology and Statistics Unit, 2006). The more severe forms of asthma account for a disproportionate amount of the total direct costs. One study estimated that less than 20 percent of asthmatics account for more than 80 percent of the direct costs (Malone, Lawson, & Smith, 2000). Asthma also poses a substantial and increasing public health burden from school absence and restriction of usual physical and social activities (Newacheck & Halfon, 2000).

3.2 Justification for a Large Prospective Longitudinal Study

Multiple aspects of the social environment influence asthma etiology and management. Although asthma is common, elucidating the complex pathways that contribute to asthma will require a large sample because interactions will be common. For example, we anticipate interactions between family economic disadvantage and social policy variables, such as the generosity of welfare programs and the design and enforcement of public housing programs and codes.

A large prospective study is needed to adequately represent a range of geographic, environmental, economic, cultural, and policy variability among neighborhoods and communities to select enough cases within neighborhoods and communities to permit estimation of neighborhood effects and to model social and physical environmental influences.

Residential mobility is a critical problem in studies of neighborhoods and neighborhood effects. A prospective longitudinal study will be able to follow respondents through time and monitor residential mobility in order to assess residential change or mobility and the role it plays in health disparities by socioeconomic status, race, and geography.

Collection of retrospective data on variables such as family stress, parenting, and cultural beliefs is susceptible to various types of information bias. These factors must be observed prospectively.

Landrigan et al. (2006) discuss the rationale and strengths for such a study, which is expected to direct the development of prevention strategies for both diseases of childhood and chronic diseases of adulthood whose developmental origins likely begin in childhood.

3.3 Scientific Merit

Current scientific understanding

The existence of social environmental determinants of asthma etiology, severity, and management is evident from the strong differentials in asthma and asthma mortality noted above. The social factors and mechanisms involved are only beginning to be studied. Wright and Fisher (2003) suggest that differentials are likely to occur via four pathways: environmental exposures, stress, health behaviors and psychological factors, and access to health care. Other investigators have emphasized similar factors that contribute to disparities in asthma, including systematic differences in outdoor and indoor pollution, traffic, allergen exposure, violence, segregation, culturally influenced health behaviors, psychosocial stress, asthma knowledge, and access to care (Shanawani, 2006; Byrd & Joad, 2006; Morello-Frosch, & Lopez, 2006; Ford & McCaffrey, 2006).

Minorities and economically disadvantaged populations are more likely to experience unhealthy environmental exposures, including antigens and pollutants that are linked to asthma. This occurs in part because social, economic, and political disadvantage increases the probability that such groups will reside in impoverished urban environments where the levels are high; and in part because the same disadvantages reduce their ability to escape such areas or improve their physical environment through collective action (Massey & Denton, 1993; Byrd & Joad, 2006; Gold & Wright, 2005). Public policies and programs may differentially buffer these effects. The concentration of minority populations in unhealthy environments is exacerbated by discriminatory housing practices (Massey & Denton, 1993) and segregation (Morello-Frosch & Lopez, 2006), theoretically governed by public policies. Studies have clearly documented linkages between low income, substandard housing, and exposure to asthma-triggering factors such as smoke, dust mites, cockroaches, rodents, dry heat, and lack of heat (Boston Medical Center and Children's Hospital Doc4Kids Project, 1998). In the Moving to Opportunity study, a housing-mobility experiment in which a random sample of families in public housing were offered the opportunity to move to low-poverty neighborhoods, the incidence of asthma attacks was reduced among the group that moved compared to those not given the opportunity to move (Katz, Kling & Liebman, 2001). It is plausible that public policies influencing housing conditions as well as housing location may also influence exposure to asthma-causing antigens. Exposure to environmental tobacco smoke also occurs disproportionately in disadvantaged populations because of the higher prevalence of smoking in low-status groups. Physical exposures such as these affect asthma etiology prenatally and throughout childhood.

Research has linked stress to both the etiology and management of asthma (Wright, Rodriguez, & Cohen, 1998; Wright, Cohen, Carey, Weiss, & Gold, 2002). Stress contributes to asthma etiology directly by impairing immune function and indirectly through its effect on smoking. This occurs both prenatally (via maternal stress and smoking) and during childhood. Stress complicates effective asthma management by reducing the psychological resources families coping with asthma have to seek care and comply with preventive measures. Few studies have documented those aspects of the social environment that create harmful levels of stress could underlie socioeconomic and racial disparities in asthma. As conceptualized here, stress is inherently a property of an individual, the result of exposure to experiences and events with which the individual cannot readily cope (Herbert & Cohen, 1993). The social environment may contribute to stress by affecting the individual's exposure to experiences and events or by affecting the individual's ability to cope. Furthermore, the cumulative balance during development between chronic and acute stress and stress-buffering supports is consequential for health (Singer & Ryff, 1997).

Economic disadvantage is associated with greater levels of psychosocial stress (Baum, Garofalo, & Yali, 1997). Children growing up in poverty show early signs of elevated allostatic load, including elevated secretion of cortisol and epinephrine and higher resting blood pressure. Children who live in sustained poverty and those who become poor are more likely to be exposed to family violence and inadequate parenting (Repetti, Taylor, & Seeman, 2002). In turn, parenting problems and family stress have been linked to both wheeze and asthma in children (Wright et al., 2002). Social service programs that alleviate poverty may influence stress levels experienced by families and children, as well as the resources available to families for coping with stress. Relevant programs include income transfer programs and programs that deliver in-kind resources and services (e.g., school lunch, housing, and home-visiting programs). Specific impacts of such programs on asthma etiology have not been assessed.

Health behaviors and psychological factors are closely associated with stress pathways. Mental disorders may be influenced by genetic risk and triggered by stressful events produced by the mechanisms elaborated above. Perceptions of control over the environment and related factors, such as self-efficacy, etc., are in part a function of the experience of unpredictable and stressful environments (e.g., violence) (Carter, Perzanowksi, Raymond, & Platts-Mills, 2001; Wright, 2006). Thus, as noted

above, individual factors and stressful environments reinforce each other in the etiology of asthma. Health behaviors may be in part a response to stress (e.g., substance use). Such behaviors are also, however, influenced by health information and community norms.

Many environmental conditions have been hypothesized to contribute to the experience of stress by the residents of urban communities. These include poverty, community disorganization, crime and violence, social incivility, noise, crowding, hazardous conditions, physical decay, and unemployment (Wright & Steinbach, 2001; Ewart & Suchday, 2002). Aspects of the social environment hypothesized to influence individuals' ability to cope with stressful events include community economic resources, social cohesion, and social support networks. The presence and integration of community institutions that provide resources and services and reinforce social ties also contribute to resiliency in the face of stress. Suggestive evidence of the value of social support comes from a study of asthma management in inner-city children. In this study, two groups received home visits but in only one were asthma prevention measures actually implemented. In both groups acute visits for asthma were reduced relative to the control group that received no home visits, but no difference existed between the two home visit groups (Carter et al., 2001).

Access to health care is relevant to both asthma etiology and management. Although asthma is more prevalent in geographic areas with a high density of health care providers (Sly & O'Donnell, 1992), access depends as much on social, cultural, and economic constraints as on geographic ones. Access issues may affect the etiology of asthma via the timeliness of prenatal care and health care during infancy and childhood. Asthma management practices are strongly related to asthma mortality and hospitalization; poor management of asthma (delays in seeking care, under use of anti-inflammatory medications) is in turn associated with a variety of social, cultural, and economic barriers to care (Wright & Fisher, 2003).

Research that identifies the nature of the social processes that contribute to these pathways is not well developed, but the pathways are likely to be multiple and complex. These processes also must be considered at multiple levels, including individual, family, and neighborhood. Contextual-level stress may be conceptualized as a neighborhood disadvantage that is characterized by factors including poverty, limited social capital, segregation, and high crime/violence rates) (Attar, Guerra, & Tolan, 1994; Goldin & Katz, 1998; Kawachi, Kennedy, Lochner, & Prothrow-Stith, 1997; Kawachi & Kennedy, 1997; Kawachi, 1999; Wilson, 1987; Woolcock, 1998; Morello-Frosch & Lopez, 2006; Ford & McCaffrey, 2006). Research has focused on individual-level risks and limits our understanding of disease causation (O'Neill et al., 2003; Strunk, Ford, & Taggart, 2002). Drawing from economics, epidemiology, sociology, and urban studies (Brock, 2001; Brooks-Gunn, Duncan, & Aber, 1997; Galster, 2001a; Galster, 2001b; Galster & Killen, 1995), asthma researchers have brought the concept of contextual (neighborhood) effects to recent study designs. There is a need to understand how both the physical and psychological demands of living in a deprived neighborhood may increase an individual's susceptibility, including genetic predisposition (Wright et al., 2004; Wright, 2006; Sandel & Wright, 2006; Wright & Fisher, 2003).

These economic, cultural, and social conditions are interrelated and feed on each another. For example, violent crime is reduced in neighborhoods with high levels of collective efficacy (Wright & Fisher, 2003). These interrelationships and pathways are better understood in the context of urban environments. Very little research has examined pathways between the social environment and health in suburban or rural areas.

3.4 Potential for Innovative Research

Research that links the characteristics and dynamics of the social environment to biological pathways resulting in the development and severity of asthma is in its infancy. A study of the scope of the National Children's Study is necessary to adequately model the contributions of various social environmental domains and factors to the behavioral, psychological, and biological processes that lead to asthma and complicate its management. Opportunities for innovation include:

- The opportunity to further develop research regarding the environmental determinants of stress-induced changes in physiological functioning. Current theory points to a variety of environmental factors but does not adequately integrate existing social science theory on the social, economic, political, and cultural processes that shape the environments that individuals occupy.

- The opportunity to extend social science and examine the relevance of existing and potentially new models of the dynamics of disadvantaged urban and rural neighborhoods. These models will be able to incorporate specific health processes and outcomes using biomarker data from mothers and infants as well as direct measurement of social environmental processes.

3.5 Feasibility

To accomplish objectives laid out within this hypothesis, the study sample should include a diversity of living units (e.g. an urban neighborhood or rural community) that are broadly representative of the United States and that are widely varied with regard to race/ethnic composition, socioeconomic status, rural/suburban/urban location, proximity to health services, public policy environment, and physical environmental exposures. Within the selected communities, it is recommended that in-depth studies be conducted to provide richer community-level data on the social dynamics, cultural norms affecting asthma management, and the impact of public policy.

The study must also make an effort to track families (focal child plus primary caretaker) to new residences and must also track the circumstances prompting such moves. Also it is necessary to get adequate samples within all areas in order to obtain the desired multilevel modeling of community effects. This is necessary because of the need to have measures of all potential exposures during pregnancy, early infancy of the child, and every two or three years during childhood.

The primary focus of measuring asthma symptoms in the participating child must be the severity and also the biological pathways underlying the etiology of disease, as described in other NCS hypotheses that focus on asthma as an outcome. It is also important to look at the social aspects of the asthma, with respect to the mother's attitudes towards symptoms, health care contacts, medication (specific prescription, dose, and frequency of administration), and compliance with recommended treatment.

Since the current hypothesis is analyzing the association between asthma and social influences such as socioeconomic status and race/ethnicity, there are many factors at the community-level that are necessary to examine for possible mediating or confounding effects. The level of neighborhood poverty, racial/ethnic composition of the area, racial or ethnic segregation, and job market characteristics are all aspects of the child's social and community environment that may play a mediating role in overall asthma risk. In addition, chemical exposures, neighborhood pollutants, and physical housing characteristics, along with the proximity of and access to health care systems also play a significant role.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Exposure to community and household factors that have the potential to influence asthma symptoms in offspring (e.g., violence, stress); access to health care; mother's attitudes/actions towards symptoms and treatment

Secondary/maternal

- Socioeconomic factors (e.g., social networks and support for mother; enrollment in welfare)

Primary/child

- Exposure to community and household factors with potential to influence asthma symptoms (e.g., violence, stress, environmental pollutants); access to health care

4.2 Methods

Primary/maternal

- Administrative record reviews
- Interviews/questionnaires

Secondary/maternal

- Interviews/questionnaires; secondary data collection on social environment

Primary/child

- Administrative record reviews
- Household environmental samples
- Interviews/questionnaires

4.3 Life Stage

Primary/maternal

- Periodic, prenatal through early childhood

Secondary/maternal

- Periodic, prenatal through early childhood

Primary/child

- Periodic, birth through year 21

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

Primary

- Asthma incidence, prevalence and severity:
  - Airway hyperreactivity
  - Lung function
  - Airway inflammation (exhaled NO)
  - Immune system function (e.g., lymphocyte subsets, cytokines, total IgE)
  - Allergic sensitization (specific IgE or allergy skin prick testing)
  - Symptom history and medication use
  - Respiratory infection history
- Asthma management
  - Number of trips to hospital
  - Health care contacts, quality, medications, compliance
  - Number of attacks

5.2 Methods

- Questionnaire of mother
- Examination, interview and testing by medical professional (e.g., history of asthma symptoms, lung function tests, specific IgE, exhaled breath for NO (eNO can be measured prior to age 5)
- Cord and blood samples of IgE and IgA and cytokines related to the allergic/asthma cascade
- Medical record reviews

5.3 Life Stage

Primary/child

- Age 2 to 21 (specific measures depend on age)

6. Important Confounders, Mediators, and Effect Modifiers

Social isolation: Social isolation, or lack thereof, has the ability to modify the effect of physical environment risk factors on asthma-related outcomes in children. The impact of physical environment risk factors may be reduced in a child that has a strong family and social network. For example, one investigation revealed African Americans in education programs had stronger views that asthma could be managed (partial correlation = 0.27) (Fisher, Strunk, Sussman, Sykes, & Walker, 2004; Schmaling, McKnight, & Afari, 2002).

Parental health attitudes: The parental care received by children has the potential to be both a confounder or effect modifier within the association between physical environmental risk factors, psychosocial stress, and health related outcomes and risk of asthma in children. Research shows that parents did a poorer job of caring for a child with asthma if they had feelings of uncertainty, helplessness, and guilt (Annett, Bender, DuHamel, & Lapidus, 2003; Trollvik & Severinsson, 2004).

Health care access: Poor health care access in inner cities, especially among African Americans and Hispanics, exacerbates the risk of asthma attacks and deaths (Lara et al., 2003; Sin, Bell, & Man, 2004; Wallace, Scott, Klinnert, & Anderson, 2004). This and other disparity factors could be behind the greater asthma prevalence, especially among Blacks. These issues are more thoroughly discussed in the disparities in asthma hypothesis and in the review by Gold and Wright (2005).

Lower socioeconomic status and some minority groups such as Blacks and Hispanics are likely to live in areas with increased air pollution, such as near freeways or industrial sites. Careful assessments of these characteristics will be important to properly control for their influence on outcomes.

Diet, physical activity, exposure to bioaerosols, stress, and infections, which will be the topic of other hypotheses, could interact with or confound associations of asthma onset. Though the following is not exhaustive, some important factors associated with atopy and asthma include: demographic group (age, race, income or education), familial characteristics (number of siblings, parent and sibling phenotypes for asthma), daycare attendance, pet ownership, living on a farm, body mass index, immunizations, dietary factors, respiratory infections, and diabetes.

Gene-environment interactions: Polymorphisms in genes involved in oxidative stress responses to air pollutant exposures and candidate asthma and allergy genes will be included. Ultimately gene-environment interactions may dictate the effect of any given factor has on disease outcome.

7. Power and Sample Size

In this example, power analyses with a cutoff value for high-exposure of the predictor is based on a conservative cutoff value for a key determinant of disparity, low income, based on the lower fifth percentile of NCS subjects (i.e., a proportion exposed is 0.05). A total sample size will be N = 100,000 and the incidence of asthma by age 6 will be 5 percent. A fixed interval proportional hazards model was used to assess the association of low income and the risk of asthma. The minimal detectable hazard ratios associated with the disparity, for power points of 80 percent, 90 percent, and 95 percent for a level 0.05 two-sided test, are 1.199, 1.234, and 1.264, respectively. The estimates assume only a main effects model based on exposure to a single factor (low income) without consideration of interactions with other exposures, genetics, family history, etc. (National Children's Study Interagency Coordinating Committee, 2003).

Effect modification of the relationship between measures of social disparity, such as income and asthma incidence, can result from environmental exposures, such as presence of a high level of psychosocial stress, living within 100 meters of a freeway or major highway, high indoor levels of allergens such as cockroach antigens, or the presence of policies and programs that buffer the effects of economic disadvantage on families. Assessment of effect modification will be carried out by including multiplicative interaction terms for the potential effect modifier and low income into each regression model. For brevity, we present power analyses for a generic effect modifier with varying prevalences and assume as above that the cutoff value for low income is based on the conservative lower fifth percentile of NCS subjects. Again, a total sample size is assumed to be N = 100,000 and the incidence of asthma by age 6 will be 5 percent. A fixed interval proportional hazards model assesses the association between low income and the risk of asthma. In this case, minimum detectable hazard ratios associated with the interaction term (multiplicative increase in the effect of low income attributable to the effect modifier) corresponding to a specified power point that can be estimated via a modification of the usual two-sample proportional hazards formula given by Schmoor, Sauerbrei, & Schumacher, 2000:

$$\lambda = \exp\left\{ \left(z_{1-\alpha/2} + z_{1-\beta}\right)\left(\sum_{i,j} 1/p_{ij}\right)^{1/2} \Big/ \left[n \Pr(\delta = 1)\right]^{1/2} \right\}.$$

In this case, λ denotes the minimum detectable hazard ratio associated with the interaction term under the alternative hypothesis, n denotes the number of subjects available in the study, $\Pr(\delta = 1)$ denotes the probability of observing an event for a typical subject in the sample over the course of follow-up (assumed to be 5 percent), p_{ij}, i,j = 0,1 denotes the probability of being in level i of the exposure (0 = low, 1 = high) and level j of the effect modifier (0 = not present, 1 = present), α denotes the type I error, 1-β denotes the power and $z_{1-\gamma}$ denotes the 1-γ quantile of the standard normal distribution. We assume that the effect modifier and low income are independent so that $p_{ij} = p_i \times p_j$, that is, the joint probability of falling into one of the four groups is equal to the product of the conditional probabilities for each covariate. Based upon this formula, Table 1 presents minimal detectable hazard ratios associated with the interaction term corresponding to power points of 80 percent, 90 percent, and 95 percent for a level 0.05 two-sided test.

Table 1. Minimum detectable hazard ratios associated with interactions. Estimates correspond to the 80%, 90%, and 95% power points of a level 0.05 two sided test

Prevalence of effect modifier	Minimum detectable hazard ratio associated with interaction term for selected power points		
	80%	90%	95%
2%	3.66	4.49	5.32
5%	2.30	2.62	2.92
10%	1.83	2.02	2.18
20%	1.58	1.69	1.79
30%	1.49	1.58	1.67
40%	1.45	1.54	1.61

8. Other Design Issues

- **Ethical/burden considerations:** General issues include the need to protect privacy of individuals and communities and when to intervene in families to protect children's health.

- **Need for community involvement:** Daycare and school cooperation would be required for some of the intended measures. Other social environmental factors may be tied to neighborhoods or communities.

9. References

American Lung Association Epidemiology and Statistics Unit, Research and Program Services. (2006). *Trends in asthma morbidity and mortality*. New York: American Lung Association. Retrieved from http://www.lungusa.org

Annett, R.D., Bender, B.G., DuHamel, T.R., & Lapidus, J. (2003). Factors influencing parent reports on quality of life for children with asthma. *Journal of Asthma, 40*(5), 577-587.

Attar, B., Guerra N., & Tolan, P. (1994). Neighborhood disadvantage, stressful life events and adjustment in urban elementary-school children. *Journal of Clinical Child Psychology, 23*(4), 391-400.

Bai, Y., Hillemeier, M.M., & Lengerich, E.J. (2007). Racial/ethnic disparities in symptom severity among children hospitalized with asthma. *Journal of Health Care for the Poor & Underserved, 18*(1), 54-61.

Baum, A., Garofalo, J.P., & Yali, A.M. (1997). Socioeconomic status and chronic stress: Does stress account for SES effects on health? *Annals of the New York Academy of Sciences, 896*, 131-144.

Boston Medical Center and Children's Hospital, Doc4Kids Project. (1998). Not safe at home: How america's housing crisis threatens the health of its children. Boston: Author. Retreived May 25, 2007, from http://www.bmc.org/pediatrics/research/Research/Doc4Kids/docs4kids_report.pdf

Brock, D.W. (2001). Children's rights to health care. *Journal of Medicine & Philosophy, 26*(2), 163-177.

Brooks-Gunn, J., Duncan, G., & Aber, J., (Eds.). (1997). *Neighborhood poverty: Context and consequences for children*. New York: Russell Sage Foundation.

Byrd, R.S., & Joad, J,P. (2006). Urban asthma. *Current Opinion in Pulmonary Medicine, 12*(1), 68-74.

Carr, W., Zeitel, L., & Weiss, K. (1992). Variations in asthma hospitalizations and deaths in New York City. *American Journal of Public Health, 82*(1), 59-65.

Carter, M.C., Perzanowksi, M.S., Raymond, A., & Platts-Mills, T.A.E. (2001). Home intervention in the treatment of asthma among inner-city children. *Journal of Allergy & Clinical Immunology, 108*(5), 732-737.

Ewart, C.C.K., & Suchday, S. (2002). Discovering how urban poverty and violence affect health: Development and validation of a neighborhood stress index. *Health Psychology, 21*(3), 254-262.

Fisher, E.B., Strunk, R.C., Sussman, L.K., Sykes, R.K., & Walker, M.S. (2004). Community organization to reduce the need for acute care for asthma among African American children in low-income neighborhoods: The Neighborhood Asthma Coalition. *Pediatrics, 114*(1), 116-123.

Ford, J.G., & McCaffrey, L. (2006). Understanding disparities in asthma outcomes among African Americans. *Clinics in Chest Medicine, 27*(3), 423-430.

Galster, G. (2001). On the nature of neighborhood. *Urban Studies, 38*(12), 2111–2124.

Galster, G. (2001, November). *Investigating place and opportunity: Needs in data and analytic strategies.* Paper presented at National Academy of Sciences' Workshop on Equality of Opportunity: The Importance of Place, Washington, DC.

Galster, G., & Killen, S. (1995). The geography of metropolitan opportunity: A reconnaissance and conceptual framework. *Housing Policy Debate, 6*(1), 7-43.

Gold, D.R., & Wright, R.J. (2005). Population disparities in asthma. *Annual Review of Public Health, 26*, 1-25.

Goldin, C., & Katz, L. (1998. Human capital and social capital: The rise of secondary schooling in America, 1910 to 1940. *National Bureau of Economic Research Working Papers*, *6439*.

Herbert, T.B., & Cohen, S. (1993). Stress and immunity in humans: A meta-analytic review. *Psychosomatic Medicine, 55*(4), 364-379.

Katz, L.F., Kling, J.R., & Liebman, J.B. (2001). Moving to opportunity in Boston: Early results of a randomized mobility experiment. *Quarterly Journal of Economics, 116*(2), 607-654.

Kawachi, I. (1999). Social capital and community effects on population and individual health. *Annals of the New York Academy of Sciences, 896*, 120-130.

Kawachi, I., & Kennedy, B. (1997). Health and social cohesion: Why care about income inequality? *British Medical Journal, 314*(7086), 1037-1040.

Kawachi, I., Kennedy, B., Lochner, K., & Prothrow-Stith, D. (1997). Social capital, income inequality, and mortality. *American Journal of Public Health, 87*(9), 1491-1498.

Landrigan, P.J., Trasande, L., Thorpe, L.E., Gwynn, C., Lioy, P.J., D'Alton, M.E., et al. (2006). The National Children's Study: A 21-year prospective study of 100,000 American children. *Pediatrics, 118*(5), 2173-2186.

Lane, W.G., Newman, D., Edwards, M., & Blaisdell, C. (2006). Disparities in the circumstances of asthma deaths in Maryland. *Journal of Asthma, 43*(10), 777-782.

Lang, D.M., & Polansky, M. (1994). Patterns of asthma mortality in Philadelphia from 1969 to 1991. *New England Journal of Medicine, 331*(23), 1542-1546.

Lara, M., Duan, N., Sherbourne, C., Halfon, N., Leibowitz, A., & Brook. R.H. (2003). Children's use of emergency departments for asthma: Persistent barriers or acute need? *Journal of Asthma, 40*(3), 289-299.

Malone, D.C., Lawson, K.A., & Smith, D.H. (2000). Asthma: An analysis of high-cost patients. *Pharmacy Practice Management Quarterly, 20*(1), 12-20.

Massey, D., & Denton, N. (1993). *American apartheid. segregation and the making of the underclass*. Cambridge, MA: Harvard University Press.

McDaniel, M., Paxson, C., & Waldfogel, J. (2006). Racial disparities in childhood asthma in the United States: Evidence from the National Health Interview Survey, 1997 to 2003. *Pediatrics, 117*(5), e868-e877.

Morello-Frosch, R., & Lopez, R. (2006). The riskscape and the color line: examining the role of segregation in environmental health disparities. *Environmental Research, 102*(2), 181-196.

National Academy of Sciences Institute of Medicine, Division of Health Promotion and Disease Prevention. (2000). *Clearing the air: Asthma and indoor air exposures*. Washington, DC: National Academies Press.

National Children's Study Interagency Coordinating Committee. (2003). Supporting documentation for the working list of NCS Core Hypothesis presented at the December, 2002 NCS Study Assembly Meeting–Draft: Rationale Document.

Newacheck, P.W., & Halfon, N. (2000). Prevalence, impact, and trends in childhood disability due to asthma. *Archives of Pediatric and Adolescent Medicine, 154*(3), 287-293.

O'Neill, M.S., Jerrett, M., Kawachi, I., Levy, J.I., Cohen, A.J., Gouveia, N., et al. (2003). Health, wealth, and air pollution: Advancing theory and methods. *Environmental Health Perspectives, 111*(16), 1861-1870.

Repetti, R.L., Taylor, S.E., & Seeman, T.E. (2002). Risky families: Family social environments and the mental and physical health of offspring. *Psychological Bulletin, 128(2)*, 330-366.

Sampson, R.J., Raudenbush, S.W., & Earls, F. (1997). Neighborhoods and violent crime: A multilevel study of collective efficacy. *Science, 277*(5328), 918-924.

Sandel, M., & Wright, R.J. (2006). When home is where the stress is: Expanding the dimensions of housing that influence asthma morbidity. *Archives of Disease in Childhood, 91*(11), 942-948.

Schmaling, K.B., McKnight, P.E., & Afari, N. (2002). A prospective study of the relationship of mood and stress to pulmonary function among patients with asthma. *Journal of Asthma, 39*(6), 501-510.

Schmoor, C., Sauerbrei, W., & Schumacher, M. (2000). Sample size considerations for the evaluation of prognostic factors in survival analysis. *Statistics in Medicine, 19*(4), 441-452.

Shanawani, H. (2006). Health disparities and differences in asthma: concepts and controversies. *Clinics in Chest Medicine, 27*(1), 17-28.

Sin, D.D., Bell, N.R., & Man, S.F. (2004). Effects of increase primary care access on process of care and health outcome among patient patients with asthma who frequent emergency departments. *American Journal of Medicine, 117*(7), 479-483.

Singer, B., & Ryff, C.D. (1997). Hierarchies of life histories and associated health risks. *Annals of the New York Academy of Sciences, 896*, 96-115.

Sly, R., & O'Donnell, R. (1992). Association of asthma mortality with medical specialist density. *Annals of Allergy, 68*, 340-344.

Smith, L.A., Hatcher-Ross, J., Wertheimer, R., & Kahn, R. (2005). Rethinking race/ethnicity, income, and childhood asthma: Racial/ethnic disparities concentrated among the very poor. *Public Health Reports, 120*(2), 109-116.

Strunk, R.C., Ford, J.G., & Taggart, V. (2002). Reducing disparities in asthma care: Priorities for research—National Heart, Lung, and Blood Institute workshop report. *Journal of Allergy & Clinical Immunology, 109*(2), 229-237.

Trollvik, A., & Severinsson, E. (2004). Parents' experiences of asthma: Process from chaos to coping. *Nursing & Health Sciences, 6*(2), 93-99.

Wallace, A., Scott, J., Klinnert, M., & Anderson, M.E. (2004). Impoverished children with asthma: A pilot study of urban healthcare access. *Journal for Specialists in Pediatric Nursing, 9*(2), 50-58.

Wilson, J. (1987). *The truly disadvantaged: The inner city, the underclass and public policy*. Chicago: University of Chicago Press.

Woolcock, M. (1998). Social capital and economic development: Toward a theoretical synthesis and policy framework. *Theory and Society, 27*(2), 151-208.

Wright, R., & Steinbach, S.F. (2001). Violence: An unrecognized environmental exposure that may contribute to greater asthma morbidity in high risk inner-city populations. *Environmental Health Perspectives, 109*(10), 1085-1089.

Wright, R., Rodriguez, M., & Cohen, S. (1998). Review of psychosocial stress and asthma: An Integrated biopsychosocial approach. *Thorax, 53*(12), 1066-1074.

Wright, R.J. (2006). Health effects of socially toxic neighborhoods: The violence and urban asthma paradigm. *Clinics in Chest Medicine, 27*(3), 413-421.

Wright, R.J., & Fisher, E.B. (2003). Putting asthma into context: Community influences on risk, behavior, and intervention. In I. Kawachi & L.F. Berkman, (Eds.). *Neighborhoods and Health.* New York: Oxford University Press.

Wright, R.J., Cohen, S., Carey, V., Weiss, S.T., & Gold, D.R. (2002). Parental stress as a predictor of wheezing in infancy: A prospective birth-cohort study. *American Journal of Respiratory & Critical Care Medicine, 165*(3), 358-365.

Wright, R.J., Mitchell, H., Visness, C., Cohen, S., Stout, J., Evans, R., et al. (2004). Community violence and asthma morbidity in the Inner-City Asthma Study. *American Journal of Public Health, 94*(4), 625-632.

EARLY EXPOSURE TO STRUCTURAL COMPONENTS AND PRODUCTS OF MICROORGANISMS DECREASES THE RISK OF ASTHMA

1. Meta Hypothesis

Early exposure to heterologous structural components and products of biologics (microorganisms, e.g., viruses, bacteria, fungi, parasites, and common indoor aeroallergens) significantly decreases or increases the risk of asthma and other atopic diseases (e.g., eczema, allergic rhinoconjunctivitis), and/or this will be mediated by genetic and other risk factors.

2. Specific Hypotheses

1. Exposure to microbial structural components or products, indoor or outdoor allergens, viral and/or bacterial infections in utero (maternal exposure) or in early childhood decreases the risk of atopy (atopic sensitization with or without symptoms), asthma, eczema, and allergic rhinitis later in childhood.

2. Immunologic outcomes measured in those children demonstrating reduced or enhanced risk of developing asthma or atopy will reflect varying profiles of upregulation or downregulation of Th1 and Th2 lymphocytes as well as other significant immunologic consequence(s), such as the development of regulatory T lymphocytes (e.g., antigen specific suppressor T-regulatory cells).

3. Significant interactions between the host, environmental exposures (including in utero), and genetics will determine the magnitude and direction of associations in hypotheses 1-2.

3. Background and Justification

The human innate and adaptive immune systems can mount responses to heterologous structural components and metabolic products of various species of living organisms (biologics) during exposure in an environment rich in these immunoreactive factors. These microscopic and macroscopic and ogranisms include bacteria, viruses, fungi, parasites, animals, insects, and plants. The determinants of the exact nature of a particular response in a given circumstance are complex and not well understood. Almost two decades ago, Strachan (1989) proposed that reduced exposure to microbial contaminants early in life as a result of improved "hygiene" and reduced infection rates in developed countries increased the prevalence of atopy, asthma, and other allergic diseases. This hygiene hypothesis has steadily garnered scientific support (Gehring et al., 2001; Braun-Fahrlander, 2003; Liu, 2004; De Meer, Janssen, & Brunekeef, 2005; Douwes et al., 2006; Ege et al., 2006; Perzanowski et al., 2006). Some investigations produced conflicting results (Platts-Mills, Erwin, Heymann, & Woodfolk, 2005; Liu & Leung, 2006; Schaub, Lauener, & von Mutius, 2006; Vercelli, 2006). The meta hypothesis proposed above seeks to clarify the validity of the broad hygiene hypothesis and, wherever possible, elucidate the underlying causes and susceptibility factors.

3.1 Public Health Importance

Prevalence/incidence

Among children, asthma is the most common chronic illness (National Academy of Sciences Institute of Medicine, 2000). Asthma prevalence in the United States, estimated from the National Health Interview Survey (NHIS) by the American Lung Association (American Lung Association Epidemiology

and Statistics Unit, 2006), shows the prevalence of current asthma increased 85 percent from 1982 through 1996 to an estimated 14.6 million persons (55.2 per 1,000 persons). This increase was 76 percent in children younger than 18 years or 4.43 million in 1996 (62.0 per 1,000 persons). This trend paralleled increasing asthma hospitalizations and death rates in children (Akinbami, 2006; American Lung Association Epidemiology and Statistics Unit, 2006). Similar or greater increases in the prevalence of asthma during the second half of the 20th century were reported from other countries (Eder et al., 2006). Asthma prevalence increased until 2001 and plateaued in most groups except African-American children and adolescents, who have continued to experience increased prevalence annually (American Lung Association Epidemiology and Statistics Unit, 2006). Hospitalization and death rates in children have also reached a plateau or declined (Akinbami, 2006). Despite the plateau in prevalence, nonambulatory visits for asthma to physician offices by children increased after 2000 (Akinbami, 2006). Explanations could include increased public and health provider recognition of this disorder and earlier and more effective treatment. For example, it is likely the main factor mitigating increases in hospitalization, and mortality has been the dramatic increase in the use of preventive (controller) medications, chiefly inhaled corticosteroids (Suissa, Ernst, Benayoun, Baltzan, & Cai, 2000; Suissa, Ernst, & Kezouh, 2002; Stafford, Ma., Finkelstein, Haver, & Cockburn, 2003).

In 2004, the prevalence of doctor-diagnosed asthma reached 30.2 million Americans (104.7 per 1,000 persons), including 6.5 million children younger than 18. An estimated 4 million children younger than 18 experienced an asthma attack in 2004. Prevalence data in the United States both from the 12-month prevalence (before 1997) and 12-month attack prevalence of asthma (since 1997) were highest among those ages 5-14, Blacks compared with whites, and females (Akinbami, 2006; American Lung Association Epidemiology and Statistics Unit, 2006). Approximately 38 percent of the asthma hospital discharges in 2004 were children younger than 15, although only 21 percent of the U.S. population was younger than 15 years old.

Eder et al. (2006) report the prevalence of atopy (from skin tests or specific IgE) has increased in some populations, whereas, in others there has been a decrease or a plateau in prevalence since 1990. However, there is little data prior to 1990. Other epidemiologic data show the prevalence of atopy-related illnesses such as allergic rhinitis have increased in many industrialized nations during the 20th century (Diaz-Sanchez, Proietti, & Polosa, 2003).

Economic and/or social burden

In 2004, the direct health care costs of asthma were estimated at $11.5 billion while indirect costs (lost productivity) were estimated at $4.6 billion for a total of $16.1 billion (American Lung Association Epidemiology and Statistics Unit, 2006). The more severe forms of asthma account for a disproportionate amount of the total direct costs. One study estimated that less than 20 percent of asthmatics account for more than 80 percent of the direct costs (Malone, Lawson, & Smith, 2000). Asthma also poses a substantial, increasing public health burden from school absenteeism and restriction of usual physical and social activities (Newacheck & Halfon, 2000).

3.2 Justification for a Large Prospective Longitudinal Study

Currently available databases, although useful for the study of acute exacerbations of existing asthma, are not able to directly answer questions about the effects of chronic microorganism exposure on asthma incidence. These questions require the collection of new data. They can best be studied in a large prospective cohort study design incorporating data from preconception into adulthood. A large sample size and prospective approach is needed to accurately assess the effect of timing and dose of biologic exposures, particularly during critical windows of vulnerability (e.g., specific trimester of pregnancy, early vs. later postnatal periods, etc.), on the development of childhood asthma. Identification

of children at risk for developing the severe forms of asthma would have clear public health impact. A large prospective study can provide sufficient numbers to identify risk factors and determine which children with asthma develop the severe, persistent variety.

Distinguishing the effects and interactions of biologics with other potential causative factors (e.g., genetic, air pollution, lifestyle factors, addressed by the other asthma hypotheses) requires a large sample. Accurate exposure and phenotypic data are needed to assess the significance of various asthma and allergy genotype-complex exposure interactions occurring during the development of the child that result in several different asthma phenotypes (Taussig et al., 2003; Bel, 2004). A large sample will be required to evaluate the susceptibility to the adverse effects of certain pollutants and their sources in specific genotype-phenotype clusters. Also, data are needed from children of different ethnicities living in varied housing, socioeconomic, and geographic conditions. These factors may be correlated with variations in exposures and possibly genotypes. A large sample size will be necessary to allow adjustments to account for this.

3.3 Scientific Merit

Current scientific understanding

Biologics can be parts of living organisms (e.g., animal dander, cell wall lipoproteins) or may refer to the entire organism (e.g., viruses, bacteria). Exposure to specific biologics can be episodic with periodic exposure through vector transmission, immunization, or contact with these substances in our everyday environment. Exposure also may be continuous (such as bacteria colonizing the skin and alimentary tract). These biologics or their components can induce immunologic responses which are protective, though some misdirected responses become pathologic. These responses can be identified through changes in immunologic status measured in the laboratory or as phenotypic expression.

Pathologic perturbations of the immune system control the development of atopy and atopic diseases. The primary function of this organ system is to prevent injury from foreign organisms. A complex armamentarium of cells (e.g., neutrophils and mononuclear cells); receptors (e.g., pattern recognition receptors [PRR] such as toll-like receptors [TLRs]); and soluble agents (e.g., complement, soluble cluster of differentiation molecule 14 [sCD14]), provide immediate recognition and response to proteins, lipoproteins, lipopolysaccharides, and other substances recognized as nonself via the innate immune system. Previously programmed adaptive immune system components can also initiate and execute reactions to neutralize and remove foreign substances from the body (e.g., mononuclear cells and lymphocytes [memory cells], intracellular and extracellular receptors, and soluble molecules, such as specific antibodies, interleukins, and interferons). Other environmental exposures, such as airborne pollutants, can induce or augment immunological reactions. These responses by the immune system are modulated by factors such as genetic makeup, presence of stimulatory or inhibitory cofactors, prior immunologic conditioning, and current state and level of maturity of the immune system. Not all immunologic reactions are healthy. For example, heterologous substances such as endotoxin have the potential to induce or inhibit various pathways of immunological defense thereby augmenting or diminishing a host's effective response (Vercelli, 2006). In experimental models, higher doses of endotoxin can induce immunologic tolerance, whereas low doses may augment the development of atopic sensitization. Introduced before allergen exposure, endotoxins can inhibit sensitization. However, endotoxin exposure some time after induction of sensitization can enhance the hyperreactive airway response to antigen exposure. These dose-dependent immunologic consequences depend upon different pathways. In one study, low-dose endotoxin induction of allergen sensitization and Th2 skewing was found to be independent of myeloid differentiation primary response protein 88 (MyD88), while high-dose endotoxin induction toward a Th1 profile required the presence MyD88 (Eisenbarth et al., 2002). In a murine model, however, the Th2 adjuvant activity of house dust extract was dependant upon the

presence of MyD88, indicating the requirement of TLRs for this activity (Ng, Lam, Paulus, & Horner, 2006).

Effects on immunologic function can be quantified by measurements of immunologic expression, such as the relative proportions or quantities of lymphocyte subsets, usually defined by their receptor expression or function; the type of cellular recruitment; and the cytokine profile. Th1 induction generally results in the recruitment of lymphocytes and other mononuclear cells, upregulation of TLRs, and the secretion of cytokines such as IL6, IL12, and IFN-γ, and specific antibody of the IgG class, especially of the IgG4 subclass. On the other hand, Th2 induction usually is associated with the recruitment of eosinophils, downregulation of TLRs, the secretion of IL4, IL5, IL9, IL13, and IFN-α, and by specific antibody production of IgG2 (Jabara & Vercelli, 1994; Martinez & Holt, 1999; Woodfolk, 2006). Immunologic consequences also may be assessed by measuring relevant physiologic reactions affected by regional inflammatory or immune response, such as airway hyperreactivity or obstruction and in vivo (skin testing) and in vitro (ELISA) assays for specific IgE.

The immune system of the fetus or newborn is not fully mature and demonstrates a Th2-dominance. Over the ensuing months, the infant's immune system acquires Th1-dominance, unless this natural process is interrupted by genetic, environmental, or other factors. The development of asthma and atopy is thought to result from the persistence of, or return to, a Th2-dominant phenotype from a Th1 dominant phenotype. As noted, biologics such as endotoxin induce a Th1 response under one set of conditions, a Th2 response under another, and inhibit both classes of response in other circumstances (Braun-Fahrlander, 2003). This has led to the concept of T regulatory lymphocytes ultimately controlling Th1 and Th2 activity, thereby connecting the innate with the adaptive immune system (Eder & von Mutius, 2004). Consequently, factors other than the nature of the heterologous substance to which we are exposed contribute to the ultimate effects of the response. These factors include genetic differences (e.g., single nucleotide polymorphisms, inherited atopic/asthmatic genotype); exposure characteristics (e.g., critical windows in time, temporal sequence of exposure with a second factor such as allergen, dose); epigenetic changes (e.g., DNA methylation); in utero maternal exposures (smoking, stable/barn animals); frequency, type and duration of infection, colonization, or exposure (e.g., bacterial, parasites, viruses, antibiotics, probiotics, immunizations, hygiene); air pollution exposure (e.g., ozone, particulate matter); and presence of stimulatory cofactors.

Several studies have suggested in utero or perinatal influences determine allergic disease outcomes. Jones et al. (2002) found reduced levels of sCD14 in the fetal and neonatal gastrointestinal tract (demonstrated in amniotic fluid and breast milk colostrums) were associated with an increased prevalence of atopic sensitization and eczema, although the presence of eczema was independent of atopic sensitization. TLR2 and TLR4 transcripts were identified in extracts of fetal gut and skin of these fetuses at all ages of gestation examined. The authors concluded this regulatory effect of sCD14 likely requires the interaction and binding of TLR4 and its ligand, sCD14, both of which are present in utero, and that this immunologic effect is endotoxin independent as previously described for inhibition of IgE production by sCD14 (Arias et al., 2000). However, they did not measure endotoxin in breast milk. Ege et al. (2006) discovered an inverse dose-dependent relationship between maternal exposure to farm animals and a risk of atopy in offspring, accompanied by upregulation of Th1 genetic markers (TLR2, TLR4, and CD14) in the children.

Fetuses demonstrate peripheral blood mononuclear cell proliferative responses from 22 weeks gestation, with increased responses at birth to specific allergens to which the mother was exposed during gestation (Warner, Jones, Jones, & Warner, 2000). Immunologic memory appears to begin developing in utero as a result of the transplacental transfer of small amounts of antigens, with subsequent induction of T regulatory cells that determine whether tolerance or allergic sensitization to these antigens will occur (Holt & Macaubas, 1997). This early activation of both innate and adaptive immunity could

explain the presence of critical windows of exposure to biologics early in life, even in the prenatal period, that help determine the type and predominance of immune responses that will characterize each human being, at least for infancy and childhood. The immunologic status of the newborn may, similarly, already be headed in a particular direction at birth (e.g., Th1 or Th2 predominance) with further influence by other postnatal factors that can impact the direction of immune system maturation.

Of all of the biologics, endotoxin has received the most attention. Endotoxin, a lipopolysaccharide component of gram negative bacteria cell walls, is found routinely in house dust, and, in increasing amounts, in the presence of animals. In a prospective birth cohort study, Simpson et al. (2006) reported a marked reduction in atopic sensitization and eczema with increasing endotoxin exposure (measured in living room dust), and an increased risk of nonatopic wheeze, but not atopic wheeze, in children at 5 years of age. However, this protective effect for atopy was seen only in children homozygous for the CC genotype in a promoter region of the CD14 gene (CD14/−159). Similar findings have been described elsewhere, particularly for children in the first 2-5 years of life (Gehring et al., 2001; Perzanowski et al., 2006). Diametrically opposite effects have been reported in studies under different conditions, with possession of the T allele of CD14/−159 serving to reduce, not enhance, the IgE response (Vercelli, 2003). These opposing effects prompted Kabesch (2006) to question the validity of a causative role for endotoxin, raising the possibility that endotoxin is actually a surrogate for some other, as yet, undefined immunomodulator. Vercelli, however, suggests the response to endotoxin may be bimodal or even trimodal with Th2 responses at low and very high concentrations of endotoxin exposure and Th1 responses at high concentrations. Polymorphisms in other regions of the CD14 gene can create contradictory effects on total and specific IgE production in children exposed to varying levels of endotoxin and different animal exposures, i.e., pet versus farm animals (Eder et al., 2005). Lau et al. (2005) found cat allergen exposure in infancy to be a risk factor for childhood asthma. However, very high allergen exposures early in life was associated with a reduced rate of sensitization and wheezing and increased levels of cat-specific IgG (Lau et al., 2005). A reduced prevalence of infantile wheezing has been reported with exposures to high levels of endotoxin in the presence of multiple dogs (Campo et al., 2006). Several other previously established risk factors, such as maternal smoking and daycare attendance, were also identified, however, no reduction in specific allergen sensitization was noted at 1 year of age.

Some (Braun-Fahrlander, 2003; Eder & von Mutius, 2004; Liu & Leung, 2006; Schaub et al., 2006; Vercelli, 2006) but not all (Platts-Mills et al., 2005; Racila & Kline, 2005) recent reviews have argued for the probable validity of the hygiene hypothesis construct, although not necessarily in its original form. Some studies have demonstrated an inverse relationship between early-life farm animal exposure as a surrogate for microbial biocontaminant exposure (likely to represent, at least, endotoxin) and the development of atopy, asthma, and allergic rhinitis, including some in a dose-dependent manner (Von Ehrenstein et al., 2000; Downs et al., 2001; Riedler et al., 2001). Leynaert et al. (2006) found the greatest protection in children with both early farm exposure and possession of the CD14-159TT genotype. Lauener et al. (2002) demonstrated higher amounts of CD14 and TLR2 (a receptor for peptidoglycans in gram-positive bacterial cell walls) in farmers' versus non-farmers' children. In a western European study, the development of atopy and asthma was negatively associated with farm stable exposure and, independently, with the consumption of farm milk in the first year of life (Riedler et al., 2001). These studies suggest a critical window of prenatal and early childhood exposure to produce a protective response. Windows of exposure have been described for other environmental elements (e.g., smoking) associated with the induction of asthma (Strachan & Cook, 1998). In a prospective birth cohort study of Dutch children followed for four years, endotoxin and EPS levels expressed as units per square meter (but not in units per gram of dust) in floor dust demonstrated a significant inverse association with doctor-diagnosed asthma, and, in addition, with persistent wheeze but only for extracellular polysaccharides of fungi (EPS) (Douwes et al., 2006). A weak correlation was found with biocontaminant levels and atopy. However, the authors point out the study design may have

impeded finding a positive relationship. EPS exposure, used as a marker for total fungal exposure, has been significantly positively associated with home dampness as well as respiratory symptoms in 6- to 12-year-old children (Douwes et al., 2006). Gehring et al. (2001) found exposure to higher levels of endotoxin in urban homes reduced the risk of developing atopic eczema in infants in the first 6 months, with an increased risk of experiencing respiratory infections with cough and wheezing that increased over the first year of life. Perzanowski et al. (2006) reported similar results. A number of studies have demonstrated reduced risks for the development of allergic diseases and atopic sensitization in children growing up in close proximity to farm animals (Alfven et al., 2006), particularly with early life exposure.

Attending day care between birth and 4 years of age and having a dog or cat indoors in the first 2 years of life have been shown to reduce the risk of atopic sensitization and atopic symptoms (De Meer et al., 2005). However, no reduced risk was seen when older siblings were present, contrary to one of the predictions of the original hygiene hypothesis. Ball et al. (2000) did find a significantly reduced risk of asthma in children with exposure to older siblings and also associated with attendance at day care in the first 6 months of life. They also reported increased wheezing in children with more exposure to other children at age 2 but a reduced risk of wheezing from ages 6-13 years. Significant dose-dependent relationships have been reported for the lack of vaccination and the risk of self-report of atopic diseases in children, especially when there was no family history of atopic disease (Enriquez et al., 2005).

Bidirectional gene-environment interactions can affect the resultant asthma phenotype. Only certain polymorphisms in TLR2, (e.g., carrying a T allele in TLR2/-16934), modulate the protective effect provided by the farm environment (Eder et al., 2004). Non-mutational epigenetic changes in gene expression caused by varying environmental exposures could also help explain the opposite effects of identical genotypes or discrepant results for similar exposures. NOD1 is an intracellular PPR with a leucine-rich domain whose ligand is the diaminopimelic acid (iE-DAP) found in PGN. iE-DAP is found on the surfaces of bacteria such as gram-negative bacilli and certain gram-positive bacilli such as *Bacillus subtilis* and *Listeria monocytogenes*. Activation of NOD1 induces NF-κB activation resulting in the release of pro-inflammatory cytokines. Certain polymorphic variants in the NOD1 gene have shown variable association with asthma and total serum IgE (independent of allergen-specific mechanisms), with one SNP in particular, ND+32656*2 indel strongly associated with both (OR = 6.30 for asthma and 18.4 for IgE above the 90th percentile) (Hysui et al., 2005). In another study of the NOD1 gene, central European children homozygous for the T allele in the caspase recruitment domain protein (CARD) 4 portion of NOD1-21596 (CARD4/-21596) and living on farms demonstrated reduced frequencies of atopic sensitization, allergic rhinitis and atopic asthma compared with children not living on farms. This protective effect was not seen, however, in children with the C allele (Eder et al., 2006). The complexities of gene-environment interactions reflect the fact that asthma is a complex disease of the lower airways variably characterized by reversible airways obstruction, airway inflammation, and airway hyperresponsiveness. Gene association studies indicate a complex inheritance pattern involving perhaps hundreds of genes governing the expression of varying asthma and atopy phenotypes (Ober & Hoffjan, 2006). Confounding factors can make the job of identifying truly causative genotypes more difficult. Associations may not be reproducible because of inter-study differences in definitions of phenotypes (e.g., criteria used to identify subjects with “asthma” or “atopy”). Some genetic effects may only be demonstrable when combined with other genes or environmental conditions, and associations with single nucleotide polymorphisms (SNPs) or various haplotypes likely differ between populations, which may conceal true causative associations. Despite this complexity, eight genes associated with the asthma phenotype, and frequently with atopy, have been identified in five or more studies (Hoffjan et al., 2003).

Bacteria and their components in the intestinal tract are another source of constant exposure to microbial products, and, thereby, the potential for induction of immunologic effects. *Bifidobacterium thermophilum*, a commensal bacterium of the gut, fed orally to mice enhanced the cytotoxic activity of their lymphocytes (Sasaki, Fukami, & Namioka, 1994). They are also an excellent source of antioxidants

(Pyo & Lee, 2005). Yurong et al. (2005) found significant enhancement of intestinal mucosal immunity in chickens fed a probiotics mixture of microbial agents, including *Lactobacillus acidophilus*, *Bacillus subtilis*, and *Candida utilis* early in life. Fecal short chain fatty acid patterns more often associated with the presence of *Clostridium difficile* were predominant in allergic Swedish infants (defined by at least one positive prick skin test and symptoms of asthma or eczema). Nonallergic infants demonstrated fecal patterns resembling those seen with normal intestinal bacterial flora (Bottcher, Nordin, Sandin, Midtvedt, & Bjorksten, 2000). Similar findings have been reported by Sepp in Swedish infants who demonstrated significantly more allergy and the presence of *C. difficile* vs. Estonian infants showing much less allergy and more often the normal gastrointestinal commensals *Lactobacilli* and *Eubacteria* (Sepp et al., 1997). This has also been shown to be true for 2-year-olds in these two countries (Bjorksten, Naaber, Sepp, & Mikelsaar, 1999). Certain species of *Lactobacilli* may inhibit allergen-induced IgE production by murine splenocytes, suggesting the GI bacterial flora may play a role in modulating Th1/Th2 balance (Shida et al., 1998). Other studies have shown immunomodulatory effects in animals exposed to common commensal GI bacteria (e.g., *B. subtilis*) (Panigrahi et al., 2007) or their metabolic products (Sung et al., 2005). Finally, feeding *Lactobacillus GG* to mothers prenatally and to infants in their first 6 months reduced the development of atopic diseases through the first 2 years of life (Kalliomaki et al., 2001).

We are constantly exposed to biologics and their immunoreactive components before birth, and these are only one class of many types of contaminants and other environmental exposures we encounter daily. The dual human immune system is relentlessly reacting to and protecting us from the potential harmful effects of these biologics as they come in contact with our bodies. Some of these immunologic reactions result in pathologic effects. Among these are the development of atopy, asthma, and other allergic disorders. Investigative studies directed toward elucidating the causal factors in the development of asthma continue to provide support for an updated hygiene hypothesis that explains how relatively recent changes in hygiene and reductions in biologics exposure account for the increase in asthma and atopy in developed and developing countries during the past three decades.

3.4 Potential for Innovative Research

The NCS provides the opportunity to collect longitudinally a broad range of data thought to affect the development of atopy and asthma. This includes genotyping; serologic reflections of immunologic status (specific and total and specific IgE, cytokines, lymphocytes); and environmental exposures (e.g., animal dander, dust mite, cockroach). This also allows us to examine the relationships of multiple potential causes and precipitating factors of asthma, both allergic and nonallergic, using standardized definitions for disease, atopy, and environmental exposures. By collecting data in a standardized manner and using nurses or doctors to evaluate study-defined parameters, investigators can compare and combine data across the study centers in a scientifically valid manner, contrary to what has often been done in reviews of the literature. As the study progresses, one will be able to observe the effectiveness of both proactive and reactive modalities for promoting good health, (e.g., effectiveness of smoking cessation for relevant house members; or regular vs. intermittent vs. no care by a primary care physician vs. a specialist in allergy/immunology or pulmonary medicine).

3.5 Feasibility

The probability sampling design of the NCS will allow for variability in birth order, number of siblings, family size, time spent in day care, and opportunity for contact with sources of infection and endotoxin. Sampling should include exposure to farm animals (urban/rural gradient), household pets, other sources of fecal material (such as soiled diapers or compost in the home), and socioeconomic status. The collection of specimens for genotyping or epigenetic changes are possible. Obtaining blood for serum total and specific IgE is feasible, but assays are expensive but necessary to determine atopic status and other immunological markers. Spirometry is practical after 4-5 years of age. Repeated household

measurements of allergens and other biocontaminants from dust and air are feasible and can be collected during home visits and by study participants. Analyses of these samples are expensive, yet are needed to provide evidence of exposures at specified windows of vulnerability. The study needs to track respiratory infections clearly associated with increased incidence of early wheezing illness. However, confirmation of the type of respiratory infection will be difficult and limited to a subset of confirmed viral titers, such as Vericella. Therefore, data regarding episodes of infections will be largely based on parental recall of the frequency and severity of infection. High-throughput genotyping methods and genome-wide scans will characterize and potentially discover asthma and atopy genes that interact with the exposures of interest.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Prenatal exposure to bacteria and microbial products through CBC of blood samples and Immunoassay
- Prenatal and postnatal ETS exposure

Primary/child

- Exposure to bacteria and microbial products via indoor measurements and other infection measures
- Day care history
- Family member number and history (e.g., concomitant upper respiratory infections)

4.2 Methods

Primary/maternal

- Interview/questionnaire
- Blood samples
- Urine samples
- Other physical sampling
- Household environmental survey and endotoxin and allergen sampling prenatally

Primary/child

- Household environmental endotoxin and allergen sampling
- Blood samples
- Urine samples

- Other physical sampling
- Spirometry and exhaled NO
- Interview/questionnaire to assess health history, diet, daycare attendance, exposure to pets, etc.

4.3 Life Stage

Primary/maternal

- Repeated, birth through year 5

Primary/child

- Repeated, birth through year 5

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Decreased risk of asthma measured via allergy, asthma in index child, airway reactivity

5.2 Methods

- Direct observation by medical professional
- Interview/questionnaire
- Blood samples
- Urine samples
- Other physical sampling

5.3 Life Stage

- Repeated, birth to year 21

6. Important Confounders, Mediators, and Effect Modifiers

- **Infection:** Site, type of prior infection (e.g., respiratory, gastrointestinal) does not change likelihood of protective effect.
- **Medication use:** Antibiotic or paracetamol use may increase the risk of asthma.
- **Living conditions:** Living in uncrowded conditions and in higher socioeconomic status reduces risk of asthma.
- **Location of contaminant collection:** (e.g., living room vs. bedroom, floor vs. mattress, carpeted vs. non-carpeted); units of contaminant (e.g., units/area vs. units/gram of dust); previous efforts to reduce exposure (e.g., bedding encasings) (Dillon et al., 1999; Douwes et al., 2006).
- **Use of air conditioners**
- **Obesity**
- **Lifestyle:** Diet, antioxidants, breast milk; smoking.
- **Timing of exposure:** Critical windows; before, with, or after allergen or other potential factor.
- **Lung growth:** (e.g., low-birth weight).
- **Genetics of asthma and atopy:** (Ober & Hoffjan, 2006), see NCS environmental exposures and genetic variation interactions and asthma hypothesis.

7. Power and Sample Size

The smallest detectable relative risk is approximately 1.2. This power estimate assumes a sample size of 100,000 at age of diagnosis, an asthma incidence of 5 percent, and a cutoff value for "high" exposure based on the upper fifth percentile of NCS subjects (i.e., a proportion exposed of 0.05). It assumes only a main effects model based on exposure to a single factor (e.g., early exposure to bacterial and microbial products) without consideration of interactions with other exposures, genetics, family history, etc.

8. Other Design Issues

The multifactoral determination of immunologic responses contributes to the difficulty in identifying causative factors of asthma and atopy. Although the risk of developing asthma is significantly greater in atopic children, the majority of children with asthma in the first 5 years of life is not atopic. What conditions determine whether the child develops atopic sensitization, asthma, or other allergic diseases, or both? What data must be collected to discover risk factors for each? The study design must control for non-measured potentially causative factors while assuring accurate assessment of dependent variables and accurate measurement of independent variables. For instance, the locations of sampling, previous or ongoing anti-allergy measures in affected children's homes, and assays utilized to evaluate atopy or biocontaminant concentrations can influence study results and lead to misleading conclusions. Additionally, operational definitions of disease states (e.g., asthma) must be similar to allow for

comparison of data from comparative studies. Study design should differentiate incident asthma from prevalent asthma, which can be difficult with older children.

9. References

Akinbami, L. (2006). The state of childhood asthma, United States, 1980-2005 (pp. 1-24). *Advanced data from vital and health statistics, no. 381*. Hyattsville, MD: National Center for Health Statistics.

Alfven, T., Braun-Fahrlander, C., Brunekreef, B., von Mutius, E., Riedler, J., Scheynius, A., et al. (2006). Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle–the PARSIFAL study. *Allergy, 61*(4), 414-421.

American Lung Association Epidemiology and Statistics Unit, Research and Program Services. (2006) *Trends in asthma morbidity and mortality*. New York: American Lung Association. Accessed at http://www.lungusa.org

Arias, M.A., Rey Nores, J.E., Vita, N., Stelter, F., Borysiewicz, L.K., Ferrara, P., et al. (2000). Cutting edge: Human B cell function is regulated by interaction with soluble CD14: Opposite effects on IgG1 and IgE production. *Journal of Immunology, 164*(7), 3480-3486.

Ball, T.M., Castro-Rodriguez, J.A., Griffith, K.A., Holberg, C.J., Martinez, F.D., & Wright, A.L. (2000). Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. *New England Journal of Medicine, 343*(8), 538-543.

Bel, E.H. (2004). Clinical phenotypes of asthma. *Current Opinion in Pulmonary Medicine, 10*(1), 44-50.

Bjorksten, B., Naaber, P., Sepp, E., & Mikelsaar, M. (1999). The intestinal microflora in allergic Estonian and Swedish 2-year-old children. *Clinical Experimental Allergy, 29*(3), 342-346.

Bottcher, M.F., Nordin, E.K., Sandin, A., Midtvedt, T., & Bjorksten, B. (2000). Microflora-associated characteristics in faeces from allergic and nonallergic infants. *Clinical Experimental Allergy, 30*(11), 1590-1596.

Braun-Fahrlander, C. (2003). Environmental exposure to endotoxin and other microbial products and the decreased risk of childhood atopy: evaluating developments since April 2002. *Current Opinion in Allergy and Clinical Immunology, 3*(5): 325-329.

Campo, P., Kalra, H.K., Levin, L., Reponen, T., Olds, R., Lummus Z.L., et al. (2006). Influence of dog ownership and high endotoxin on wheezing and atopy during infancy. *Journal of Allergy and Clinical Immunology, 118*(6), 1271-1278.

De Meer, G., Janssen, N.A., & Brunekreef, B. (2005). Early childhood environment related to microbial exposure and the occurrence of atopic disease at school age. *Allergy, 60*(5), 619-625.

Diaz-Sanchez, D., Proietti, L., & Polosa, R. (2003). Diesel fumes and the rising prevalence of atopy: An urban legend? *Current Allergy and Asthma Reports, 3*(2), 146-152.

Dillon, H.K., Miller, J.D., Sorenson, W.G., Douwes, J., & Jacobs, R.R. (1999). Review of methods applicable to the assessment of mold exposure to children. *Environmental Health Perspectives, 107*(Suppl. 3), 473-80.

Douwes, J., van Strien, R., Doekes, G., Smit, J., Kerkhof, M., Gerritsen, J., et al. (2006). Does early indoor microbial exposure reduce the risk of asthma? The Prevention and Incidence of Asthma and Mite Allergy birth cohort study. *Journal of Allergy and Clinical Immunology, 117*(5), 1067-1073.

Downs, S.H., Marks, G.B., Mitakakis, T.Z., Leuppi, J.D., Car, N.G., & Peat, J.K. (2001). Having lived on a farm and protection against allergic diseases in Australia. *Clinical Experimental Allergy, 31*(4), 570-575.

Eder, W. &. von Mutius, E. (2004). Hygiene hypothesis and endotoxin: What is the evidence? *Current Opinion in Allergy and Clinical Immunology, 4*(2), 113-117.

Eder, W., Klimecki, W. Yu, L., von Mutius, E., Riedler, J., Braun-Fahrlander, C., et al. (2005). Opposite effects of CD 14/-260 on serum IgE levels in children raised in different environments. *Journal of Allergy and Clinical Immunology, 116*(3), 601-607.

Eder, W., Klimecki, W. Yu, L., von Mutius, E., Riedler, J., Braun-Fahrlander, C., et al. (2004). Toll-like receptor 2 as a major gene for asthma in children of European farmers. *Journal of Allergy and Clinical Immunology, 113*(3), 482-488.

Eder, W., Klimecki, W. Yu, L., von Mutius, E., Riedler, J., Braun-Fahrlander, C., et al. (2006). Association between exposure to farming, allergies and genetic variation in CARD4/NOD1. *Allergy, 61*(9), 1117-1124.

Ege, M.J., Bieli, C., Frei, R., van Strien, R.T., Riedler, J., Ublagger, E., et al. (2006). Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. *Journal of Allergy and Clinical Immunology, 117*(4), 817-823.

Eisenbarth, S.C., Piggott, D.A., Huleatt, J.W., Visintin, I., Herrick, C.A., & Bottomly, K. (2002). Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *Journal of Experimental Medicine, 196*(12), 1645-1651.

Enriquez, R., Addington, W., Davis, F., Freels, S., Park, C.L., Hershow, R.C., et al. (2005). The relationship between vaccine refusal and self-report of atopic disease in children. *Journal of Allergy and Clinical Immunology, 115*(4), 737-744.

Gehring, U., Bolte, G., Borte, M., Bischof, W., Fahlbusch, B., Wichmann, H.E., et al. (2001). Exposure to endotoxin decreases the risk of atopic eczema in infancy: A cohort study. *Journal of Allergy and Clinical Immunology, 108*(5), 847-854.

Hoffjan, S., Nicolae, D., & Ober, C.. (2003). Association studies for asthma and atopic diseases: A comprehensive review of the literature. Respiratory Research, 4(4), 14. Holt, P.G. & Macaubas, C. (1997). Development of long-term tolerance versus sensitisation to environmental allergens during the perinatal period. *Current Opinion in Immunology, 9*(6), 782-787.

Hysi, P., Kabesch, M., Moffatt, M.F., Schedel, M., Carr, D., Zhang, Y., et al. (2005). NOD1 variation, immunoglobulin E and asthma. *Human Molecular Genetics, 14*(7), 935-941.

Jabara, H.H. & Vercelli, D. (1994). Engagement of CD14 on monocytes inhibits the synthesis of human Igs, including IgE. *Journal of Immunology, 153*(3), 972-978.

Jones, C.A., Holloway, J.A., Popplewell, E.J., Diaper, N.D., Holloway, J.W., Vance, G.H., et al. (2002). Reduced soluble CD14 levels in amniotic fluid and breast milk are associated with the subsequent development of atopy, eczema, or both. *Journal of Allergy and Clinical Immunology, 109*(5), 858-866.

Kabesch, M. (2006). A glitch in the switch? Of endotoxin, CD14, and allergy. *American Journal of Respiratory and Critical Care Medicine, 174*(4), 365-366.

Kalliomaki, M., Salminen, S., Arvilommi, H., Kero, P., Koskinen, P., & Isolauri, E. (2001). Probiotics in primary prevention of atopic disease: A randomised placebo-controlled trial. *Lancet, 357*(9262), 1076-1079.

Lau, S., Illi, S., Platts-Mills, T.A., Riposo, D., Nickel, R., Gruber, C., et al. (2005). Longitudinal study on the relationship between cat allergen and endotoxin exposure, sensitization, cat-specific IgG and development of asthma in childhood—Report of the German Multicentre Allergy Study (MAS 90). Allergy, 60(6), 766-773. Lauener, R.P., Birchler, T., Adamski, J., Braun-Fahrlander, C., Bufe, A., Herz, U., et al. (2002). Expression of CD14 and Toll-like receptor 2 in farmers' and non-farmers' children. *Lancet, 360*(9331), 465-466.

Leynaert, B., Guilloud-Bataille, M., Soussan, D., Benessiano, J., Guenegou, A., Pin, I., et al. (2006). Association between farm exposure and atopy, according to the CD14 C-159T polymorphism. *Journal of Allergy and Clinical Immunology, 118*(3), 658-665.

Liu, A.H. & Leung, D.Y., (2006). Renaissance of the hygiene hypothesis. *Journal of Allergy and Clinical Immunology, 117*(5), 1063-1066.

Liu, A.H. (2004). Something old, something new: Indoor endotoxin, allergens and asthma. *Paediatric Respiratory Reviews, 5*(Suppl. A), S65-S71.

Malone, D.C., Lawson, K.A., & Smith, D.H. (2000). Asthma: An analysis of high-cost patients. *Pharmacy Practice Management Quarterly, 20*(1), 12-20.

Martinez, F.D. & Holt, P.G. (1999). Role of microbial burden in aetiology of allergy and asthma. *Lancet, 354*(Suppl. 2), SII12-5.

National Academy of Sciences Institute of Medicine, Division of Health Promotion and Disease Prevention. (2000). *Clearing the air: asthma and indoor air exposures*. Washington, DC: National Academy Press.

Newacheck, P.W., & Halfon, N. (2000). Prevalence, impact, and trends in childhood disability due to asthma. *Archives of Pediatric and Adolescent Medicine, 154*(3), 287-293.

Ng, N., Lam, D., Paulus, P., Batzer, G., & Horner, A.A.. (2006). House dust extracts have both TH2 adjuvant and tolerogenic activities. *Journal of Allergy and Clinical Immunology, 117*(5), 1074-1081.

Ober, C., & Hoffjan, S. (2006). Asthma genetics 2006: The long and winding road to gene discovery. *Genes and Immunity, 7*(2), 95-100.

Panigrahi, A., Kiron, V., Satoh, S., Hirono, I., Kobayashi, T., Sugita, H., et al. (2007). Immune modulation and expression of cytokine genes in rainbow trout Oncorhynchus mykiss upon probiotic feeding. *Developmental and Comparative Immunology, 31*(4), 372-382.

Perzanowski, M.S., Miller, R.L., Thorne, P.S., Barr, R.G., Divjan, A., Sheares, B.J., et al. (2006). Endotoxin in inner-city homes: Associations with wheeze and eczema in early childhood. *Journal of Allergy and Clinical Immunology, 117*(5), 1082-1089.

Platts-Mills, T.A., Erwin, E., Heymann, P., & Woodfolk, J. (2005). Is the hygiene hypothesis still a viable explanation for the increased prevalence of asthma? *Allergy, 60*(Suppl. 79), 25-31.

Pyo, Y.-H., & T.-C. Lee. (2005). Effect of lactic acid fermentation on enrichment of antioxidant properties and bioactive isoflavones in soybean. *Journal of Food Science, 70(*3), S215-S220.

Racila, D.M. & Kline, J.N. (2005). Perspectives in asthma: Molecular use of microbial products in asthma prevention and treatment. *Journal of Allergy and Clinical Immunology, 116*(6), 1202-1205.

Riedler, J., Braun-Fahrlander, C., Eder, W., Schreuer, M., Waser, M., Maisch, S., et al. (2001). Exposure to farming in early life and development of asthma and allergy: A cross-sectional survey. Lancet, 358(9288): 1129-1133.

Sasaki, T., Fukami, S., & Namioka, S. (1994). Enhancement of cytotoxic activity of lymphocytes in mice by oral administration of peptidoglycan (PG) derived from Bifidobacterium thermophilum. *Journal of Veterinary Medical Science, 56*(6), 1129-1133.

Schaub, B., Lauener, R. & von Mutius, E. (2006). The many faces of the hygiene hypothesis. *Journal of Allergy and Clinical Immunology, 117*(5), 969-977; quiz 978.

Sepp, E., Julge, K., Vasar, M., Naaber, P., Bjorksten, B., & Mikelsaar M. (1997). Intestinal microflora of Estonian and Swedish infants. *Acta Paediatria, 86*(9), 956-661.

Shida, K., Makino, K., Morishita, A., Takamizawa, K., Hachimura, S., Ametani, A., et al. (1998). Lactobacillus casei inhibits antigen-induced IgE secretion through regulation of cytokine production in murine splenocyte cultures. *International Archives of Allergy and Immunology, 115*(4), 278-287.

Simpson, A., John, S.L., Jury, F., Niven, R., Woodcock, A., Ollier, W.E., et al. (2006). Endotoxin exposure, CD14, and allergic disease: An interaction between genes and the environment. *American Journal of Respiratory and Critical Care Medicine, 174*(4), 386-392.

Stafford, R.S., Ma, J., Finkelstein, S.N., Haver, K., & Cockburn, I. (2003). National trends in asthma visits and asthma pharmacotherapy, 1978-2002. *Journal of Allergy and Clinical Immunology, 111*(4): 729-735.

Strachan, D.P. & Cook, D.G. (1998). Health effects of passive smoking. 6. Parental smoking and childhood asthma: Longitudinal and case-control studies. *Thorax, 53*(3), 204-212.

Strachan, D.P. (1989). Hay fever, hygiene, and household size. *British Medical Journal, 299*(6710), 1259-1260.

Suissa, S., Ernst, P. Benayoun, S., Baltzan, M., & Cai, B. (2000). Low-dose inhaled corticosteroids and the prevention of death from asthma. *New England Journal of Medicine, 343*(5), 332-336.

Suissa, S., Ernst, P., & Kezouh, A. (2002). Regular use of inhaled corticosteroids and the long term prevention of hospitalization for asthma. *Thorax, 57*(10), 880-884.

Sung, M.H., Park, C., Kim, C.J., Poo, H., Soda, K., & Ashiuchi, M.. (2005). Natural and edible biopolymer poly-gamma-glutamic acid: Synthesis, production, and applications. *Chemical Record, 5*(6), 352-366.

Taussig, L.M., Wright, A.L., Holberg, C.J., Halonen, M., Morgan, W.J., & Martinez, F.D. (2003). Tucson Children's Respiratory Study: 1980 to present. *Journal of Allergy and Clinical Immunology, 111*(4), 661-675; quiz 676.

Vercelli, D. (2003). Learning from discrepancies: CD14 polymorphisms, atopy and the endotoxin switch. *Clinical and Experimental Allergy, 33*(2), 153-155.

Vercelli, D. (2006). Mechanisms of the hygiene hypothesis–molecular and otherwise. *Current Opinion in Immunology, 18*(6), 733-737.

Von Ehrenstein, O.S., von Mutius, E., Illi, S., Baumann, L., Bohm. O., & von Kries, R. (2000). Reduced risk of hay fever and asthma among children of farmers. *Clinical Experimental Allergy, 30*(2), 187-193.

Warner, J.A., Jones, C.A., Jones, A.C., & Warner, J.O. (2000). Prenatal origins of allergic disease. *Journal of Allergy and Clinical Immunology, 105*(2 Pt 2), S493-S498.

Woodfolk, J.A. (2006). Cytokines as a therapeutic target for allergic diseases: A complex picture. *Current Pharmaceutical Design, 12*(19), 2349-2363.

Yurong, Y., Ruiping, S., Shimin, Z., & Yibao, J. (2005). Effect of probiotics on intestinal mucosal immunity and ultrastructure of cecal tonsils of chickens. *Archives of Animal Nutrition, 59*(4), 237-246.

ENVIRONMENTAL EXPOSURES INTERACT WITH GENES TO INCREASE THE RISK OF ASTHMA AND WHEEZING IN CHILDREN

1. Meta Hypothesis

There will be a significant association with gene-environment, gene-gene, and genotype-phenotype relationships that contribute to wheezing and asthma in children.

General approaches have been proposed for evaluating gene-environment interactions (Weiss, 2001; Martinez, 2007), but groundbreaking gene-environment asthma studies in the literature also have many limitations. Some of these limitations can be addressed in the National Children's Study. Three specific hypotheses have been formulated to demonstrate how the National Children's Study can address critical questions still left unanswered.

2. Specific Hypotheses

In addition to this hypothesis on gene-environment interaction, the outcome domain of asthma has three specific hypotheses provided based on potential exposure related to air pollution, environmental tobacco smoke, viral and microbial agents, and more general "environmental" exposures such as stress and diet.

1. Environmental exposures to such agents as air pollution and environmental tobacco smoke (ETS) will increase risk for wheezing and asthma, and this effect will be moderated by certain oxidative stress genetic phenotypes such as GSTM1, GSTP1, NAT1 and NAT2.

2. Exposure to viral agents or microbial products will be significantly associated with wheezing and asthma outcomes and this effect will be dependent on certain innate immunity genes such as CD14, TLR2, TLR4, iNOS and/or, adaptive immunity genes such as IL4, IL13, IL4RA.

3. Other prenatal or postnatal environmental exposures such as stress and diet will significantly increase the risk for wheezing and/or asthma, and this effect will be moderated by specific genotype-phenotype relationships.

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

Among children, asthma is the most common chronic illness (National Academy of Sciences, 2000). Data on asthma prevalence from the United States from the National Health Interview Survey (NHIS), CDC's National Center for Health Statistics show the prevalence of current asthma increased 85 percent from 1982 through 1996 to an estimated 14.6 million persons (55.2 per 1,000 persons), according to reports by the American Lung Association (2006). This increase was 76 percent in children younger than 18 years to 4.43 million children in 1996 (62.0 per 1,000 persons). This trend paralleled increasing asthma hospitalization and death rates in children (American Lung Association, 2006; Akinbami, 2006). Similar or greater increases in the prevalence of asthma during the second half of the 20th century were reported from other countries (Eder, Ege, & von Mutius, 2006) and increases may still be occurring (e.g., see Lodrup Carlsen et al., 2006, for recent information from a birth cohort study in Oslo, Denmark, where now every fifth child is affected by asthma).

Between 1997 and 2000, the asthma questions in NHIS used a different measure of current asthma prevalence, which suggested a decrease. However, this difference made it impossible to appropriately compare the data during this period to past trends. In 2001, another change was made (a question was restored to restate the prevalence of current asthma regardless of attack occurrence in the past 12 months) making comparisons possible again. It then became apparent that current asthma prevalence had continued to increase until 2001, later reaching a plateau in most groups except black children and adolescents, who have continued to experience increased prevalence annually (American Lung Association, 2006).

Hospitalization and death rates in children have also reached a plateau or decreased (Akinbami, 2006). Despite a plateau in prevalence in 2001, nonambulatory visits for asthma to physician offices by children have continued to increase (Akinbami, 2006), possibly due to increased public and health provider awareness, which improved treatment of asthma. For example, there was a dramatic increase in the use of preventive (controller) medications, chief among them being inhaled corticosteroids (Stafford et al., 2003; Suissa et al., 2000; Suissa, Ernst, & Kezouh, 2002), which may have mitigated increases in hospitalization and mortality.

In 2004, 30.2 million Americans (104.7 per 1,000 persons) at some time during their lifetime had been diagnosed with asthma by a health professional. This estimate included 6.5 million children younger than 18 years. Almost four million children younger than 18 years were estimated to have experienced an asthma attack in 2004. Prevalence data in the United States from both the 12-month prevalence (before 1997) and 12-month attack prevalence of asthma (since 1997) were highest among children aged 5-14 years, blacks compared with whites, and females (American Lung Association, 2006; Akinbami, 2006). In the U.S. population, approximately 38 percent of the asthma hospital discharges in 2004 were children younger than 15 years, even though only 21 percent of the U.S. population was younger than 15 years.

Economic and social burden

In 2004, the direct health care cost of asthma was estimated to be $11.5 billion. Indirect cost (lost productivity) was estimated to be $4.6 billion. Total cost (direct plus indirect) was estimated to be $16.1 billion (American Lung Association, 2006). The more severe forms of asthma account for a disproportionate amount of the total direct costs. Malone, Lawson, & Smith (2000) estimated that less than 20 percent of asthmatics account for more than 80 percent of the direct costs. Asthma also poses a substantial and increasing public health burden from school absence and restriction of usual physical and social activities (Newacheck & Halfon, 2000).

3.2 Justification for a Large Prospective Longitudinal Study

Opinions differ on the types of studies to use to address complex common disorders. In a recent debate in the literature, Willett et al. (2007) supported the combination and use of available databases to study common disorders such as asthma, while Collins and Manolio (2007) supported a new large cohort study mainly of adults (Manolio, Bailey-Wilson, & Collins, 2006). This debate covered the strengths and weaknesses of both of these approaches, but both Willet et al. and Collins and Manolio praised the strengths of a design based on a large birth cohort but questioned the feasibility of a large prospective cohort study design that incorporates in utero and early postnatal data as well as data from stages of development from infancy through childhood and into adulthood. However, the National Children's Study will provide valuable data that could be used to accurately assess the timing of exposures, particularly during critical windows of vulnerability (e.g., specific trimester of pregnancy, and

early vs. later postnatal periods, and later in childhood) on the onset of asthma in childhood and persistence into adulthood.

Accurate exposure and phenotypic data are needed to assess the importance of many asthma and allergy genotypes interacting with many exposures in relation to complex and variable asthma phenotypes. Interactions of maternal and fetal genotypes contribute to the requirement for a large sample. A large sample will be required to evaluate the susceptibility to the adverse effects of certain exposures and their sources in specific genotype-phenotype clusters.

Also, data are needed from children of different ethnicities living across the United States under varied housing, socioeconomic, and geographic conditions. These factors may account for some of the ethnic disparities related to asthma, which may be correlated with variations in exposures. A large sample size will be necessary to allow adjustments to account for this. Landrigan et al. (2006) discuss in more detail the rationale and strengths for a large prospective study, which is expected to direct the development of prevention strategies for both diseases of childhood and chronic diseases of adulthood whose developmental origins likely begin in childhood.

3.3 Scientific Merit

A recent review by Ober and Hoffjan (2006) identified a group of 10 genes that have been associated with asthma in 10 or more studies; 15 genes that have been associated with an asthma or atopy phenotype in six to 10 studies; and 54 genes that have been associated in two to five populations. The genes associated with asthma in more than 10 studies are IL4, IL13, ADRB2, TNF, HLA-DRB1, FCER1B, IL4RA, CD14, HLA-DQB1 and ADAM33. On the basis of their review, Ober and Hoffjan suggest the total number of genes associated with asthma will likely exceed 100, and the individual effect of any one of these genes on disease risk will be small. However, this review did not discuss gene-environment interaction, which was addressed in a separate article (Ober & Thompson, 2005). Both articles to which Ober contributed state there is no single "asthma gene" in all populations, and it is therefore critical to consider environmental context or gene-environment interactions to understand genetic susceptibility to asthma. They emphasized that asthma is a complex common disease with genetic susceptibility that is context-dependent due to gene-environment interactions resulting in effects apparent only in specific environments. To address these factors, studies of asthma must take into account environmental context (e.g., gender, parental asthma status, parent-of-origin effects) and use large samples, careful ascertainment of asthma phenotypes, comprehensive genetic surveys of targeted genes or genome-wide variation, and rigorous statistical approaches that account for multiple comparisons. Thus, the National Children's Study will provide data to test gene-environment effects hypothesized to be important now as well as associations that may be proposed in the future.

Examples of gene-environment interactions

One straightforward example of a gene-environment hypothesis is based on the CD14 gene and exposure to endotoxin (see Vercelli, 2004, and Vercelli & Martinez, 2006, for a review and discussion). CD14 is one of the groups of genes with evidence of association with asthma in more than 10 studies (Ober & Hoffjan, 2006). The literature suggested that a functional promoter polymorphism (CD14/-260C>T) in this gene influences IgE and asthma in opposite ways depending on level of exposure to endotoxin. Recent examples are based on studies of children exposed to small animals or large animals (Eder et al., 2006) and to low or high levels of house dust (Zambelli-Weiner et al., 2005; Simpson et al., 2006). Another example of a gene-environment hypothesis is based on the effect of ETS on asthma (Cook & Strachan, 1997). In the Children's Health Study of 6,000 children in the Los Angeles region (Peters et al., 1999), in utero ETS increased asthma and wheezing in children and current ETS increased wheezing. Pathway analysis of xenobiotic metabolism and antioxidant defenses suggested candidate genes in the

GST superfamily (GSTP1, GSTM1, and GSTP1), which are expressed in the respiratory tract and function as an enzymatic antioxidants and hydroperoxidases. Cross-sectional studies have supported the hypothesis of gene-environment interaction between GST genes and ETS. In a subset of the Children's Health Study, Gilliland et al. (2002) showed a common genotype that results in the complete lack of the GSTM1 enzyme (GSTM1 null) interacts with in utero exposure to ETS to increase the prevalence of asthma and wheezing in children.

Crossover interactions

One type of gene-environment interaction will mask main effects of genes. The crossover interaction (see Ober and Thompson, 2005, Figure 1a) has been documented for several genes (CD14, HLA-G, TLR4, and IR4RA genes). Two of these (TLR4 and HLA-G) are not in the elite group of genes with more than 10 replications but fall in the middle class with two to five replications (Ober & Hoffjan, 2006). The underlying concept of the crossover gene-environment interaction has been discussed by Vercelli and Martinez (2006). They point out that, in the nuances of human subjects, variation in regulatory processes may create variation in gene expression that is difficult to understand without consideration of appropriate exposures. The studies by Eder et al. (2005) and Zambelli-Weiner et al. (2005) were cited as examples of the same genetic polymorphism (CD14-260C>T) being associated with risk or protection depending on environmental exposures (in these two instances, to farm animals or house dust). Thus, including individuals with different environmental exposures in the same analysis may dilute a genetic main effect to the point of erasing it. Vercelli and Martinez point out that, contrary to the suggestions of some (Hall & Blakely, 2005), Nevertheless, the NCS provides the best opportunity to compensate for heterogeneity of environmental exposures do date.

Therefore, the search for gene-environment interactions in the elite group of genes with multiple replications of association with asthma (10 genes in more than 10 studies and 15 genes in six to 10 studies) may not be the best strategy, at least not for the class of crossover gene-environment interactions that dilute the main effect of genes. NCS researchers intend to evaluate gene-environment interactions for multiple exposures across all of the genes identified by Ober and Hoffjan (2006) with particular emphasis on those with few replications (i.e., the set of 54 genes with two to five replications and the set of 39 genes with a single study documenting association).

3.4 Potential for Innovative Research

Vulnerability to particular risk factors is determined not only by the genome acquired at conception but by the nature of the environment influences during critical periods of development, so a longitudinal assessment of the environment from before conception through pregnancy, fetal life, birth, and infancy could provide an opportunity to understand time-dependent susceptibility to asthma. For example, the review by Saglani and Bush (2007) emphasized the importance of the first 3 years of life and concluded that fetal and maternal genotype interact with environmental exposures to establish a risk phenotype, which interacts with antenatal exposures to set by age 3 the lifelong course of chronic asthma and lung impairment.

Also, the interplay between genes and environment is characterized by dynamic modifications to the genome, such as epigenetic modifications to nuclear and mitochondrial DNA that have been hypothesized as likely mechanisms for gene-environment interactions. For example, Valle (2004) reviews the contributions of epigenetic mechanism to genetic individuality, compares these stable alterations in gene expression that arise during development to variation in expression due to promoter allelic variation and alternative splicing, and points out that environmental experiences can have profound effects on epigenetics and individual variation in gene expression. Sanders (2006) described epigenetic regulation of Th1 and Th2 cell development that is important in asthma. Hanson and Gluckman (2005)

propose the primary mechanism for fetal adaptations and development of "thrifty phenotypes" is by epigenetic modification. An investigation of a cohort over time and under various environmental conditions provides an opportunity to collect repeated specimens for genetic analysis (see Callinan and Feinberg, 2006, for a description of this process), which could be used to assess some likely candidate epigenetic mechanisms involved in gene-environment interactions and susceptibility to asthma.

3.5 Feasibility

The National Children's Study protocol specifies the collection of biological samples that could be the source of DNA for traditional genetic analysis of candidate genes and for gene discovery based on genome-wide association scans. The anticipation of chip-based genotyping of all participants based on current technology or complete sequencing of all individuals at some point in the future will provide extraordinary detail about genetic variation of nuclear DNA. Along with the planned definition of cases based on asthma phenotypes, this provides an opportunity to use the efficient nested-case control design for subsets of the sample or the proportional hazard design for the entire sample to evaluate effects of genes, environments, and their interaction.

In addition, the Study protocol specifies collection of biological samples at multiple points over time, which provides the opportunity to evaluate epigenetic changes proposed to be the molecular mechanisms of some gene-environment effects (Hanson & Gluckman, 2005). The epigenetic assays for environmental effects of methylation and chromatin status are rapidly evolving, and current methods are surely to improve rapidly as a result of extensive current work (Callinan & Feinberg, 2006) and anticipated future work (NIH Epigenetics Workshop, 2007).

Callinan and Feinberg (2006) describe the two major modifications of DNA or chromatin (DNA methylation, the covalent modification of cytosine; post-translational modification of histones including methylation, acetylation, phosphorylation, and sumoylation) that act to regulate gene expression and review the current status of high-throughput microarray technologies for assessing DNA methylation and chromatin modification and the status of the Human Epigenome Project. Bjornsson, Fallin, and Feinberg (2004) describe an integrated framework to evaluate, in addition to direct or independent effects of DNA genotype (g_{ind}), the epigenotype (epg) and how it influences disease phenotype.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/child

- Genetics: Family history of allergy, asthma, and respiratory illness; family immune history
- Environmental exposures (home, school and daycare):
  - Environmental samples (e.g., mold, endotoxins, allergens, environmental tobacco smoke)
  - Other exposure information (housing characteristics, product usage in home, parental occupational/hobby data, food/diet questionnaire, child time activity patterns [questionnaire, diary, or GPS])

4.2 Methods

Primary/maternal

- Interview/questionnaire
- Medical record review
- Blood for genotype
- Physical sampling (air, dust) of home, school, and daycare

Primary/child

- Medical record review
- Blood for genotype
- Physical sampling (air, dust) of home, school, and daycare

4.3 Life Stage

- Periodic, birth through year 20

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Asthma, wheezing, pulmonary function/dysfunction
  - Allergic sensitization
  - Airway reactivity
  - Immune system function (e.g., lymphocytes, cytokines, IgE, interleukins)

5.2 Methods

- Examination and interview by medical professional
- Medical record review
- Blood samples
- Pulmonary function: Pulmonary function tests and spirometry

5.3 Life Stage

- Periodic, birth through year 20

6. Important Confounders, Mediators, and Effect Modifiers

- **Health care access:** Poor health care access in inner cities, especially among African Americans and Hispanics, exacerbates the risk of asthma attacks and deaths (Lara et al., 2003; Sin, Bell, & Man, 2004; Wallace, Scott, Klinnert, & Anderson, 2004). This and other disparity factors could be behind the greater asthma prevalence, especially among African Americans. These issues are more thoroughly discussed in hypothesis 4 and in the review by Gold and Wright (2005).

- **Diet, physical activity, bioaerosols, stress, and infections:** These exposures will be the topic of other hypotheses and could interact with or confound associations of asthma onset with air pollutants.

- **Polymorphisms in genes:** For the analysis of gene-environment interactions, it will be important to include polymorphisms in genes involved in oxidative stress responses to air pollutant exposures and candidate genes.

- **Socioeconomic status:** Lower socioeconomic status and some minority groups, such as Blacks and Hispanics, are likely to live in areas with increased air pollution, such as near freeways or industrial sites. Careful assessments of these characteristics will be important to properly control for their influence on outcomes.

- **Environmental exposures:** Diet, physical activity, bioaerosols, stress, and infections, which will be the topic of other hypotheses, could interact with or confound associations of asthma onset with air pollutants (specific hypothesis 1). Though the following list is not exhaustive, some important factors associated with atopy and asthma include demographic group (age, race, income, or education), familial characteristics (number of siblings, parent and sibling phenotypes for asthma), daycare attendance, pet ownership, living on a farm, body mass index, immunizations, several dietary factors, respiratory infections, and diabetes.

7. Power and Sample Size

Several methods are available for the evaluation of gene-environment hypotheses stated above. A standard approach is to use the nested case-control design, with affected cases defined by asthma phenotype and matching to select control subjects. When samples are stored and measures of exposure are expensive, the nested-case control design is preferred since it is efficient and minimizes the number and cost of measures. This approach described in Gauderman (2002) and Gauderman and Morrison (2007) provides the computer program QUANTO to estimate power and sample size.

Schwartz (2006) emphasizes the difference between the use of additive and multiplicative scales of measurement. The multiplicative scale of measurement is typically adopted, which dictates the use of ratio measures such as relative risk or odds ratio to assess disease risk based on the assumption that risk is multiplied in individuals with a risk factor compared to those without. When the additive scale is adopted, the risk difference is used based on the assumption that disease risk is added in individuals with the risk compared to those without.

Interactions in the additive model will be easier to detect than in the multiplicative model. Although the traditional approaches are used in the examples below, the use of an additive model (which would increase statistical power with all other factors held constant) could be considered.

Nested case-control example

The power calculations presented below were performed by James Gauderman using the QUANTO 1.2 computer program. The following assumptions were made:

- Initial cohort size: N
- Three percent per year loss to follow-up
- Incidence rates of 0.01, 0.05, 0.10, 0.20, 0.50, and 3.00 percent per year
- Study period of 5 years

In addition, an incidence rate of 2.5 percent (which is the approximate average annual incidence rate observed in the Children's Health Study) is assumed. Specifically, as reported in McConnell et al. (2006), 14 percent of children aged 5-7 reported physician-diagnosed asthma and 13 percent reported prevalent asthma at that age. An annual incidence rate of 2.5 percent would yield a prevalence by age 6 of 13.1 percent. From the above incidence rates, the expected number (n) of cases that will accrue during the 5-year period for each incidence rate can be derived. Based on the initial cohort size of N = 100,000 projected for the National Children's Study, these are as follows:

Disease incidence (%)	Cases expected (n)
0.01	47
0.05	235
0.10	470
0.20	938
0.50	2,331
2.50	11,191
3.00	13,304

From these, the following minimum detectable odds ratio for a genetic main effect relative odds ratio for a gene-environment effect is calculated:

- Gene with 10 percent allele frequency and dominant inheritance model
- Environmental exposure with 10 percent prevalence
- Marginal genetic and environmental effects of 1.5 in gene-environment calculations
- Eighty percent power
- 0.0001 Type I error rate, two-sided alternative hypothesis

With disease incidence rates less than 0.1 percent per year and the above assumed prevalences of genetic susceptibility and environmental prevalence, gene-environment interactions will be nearly impossible to detect. For asthma (with assumed annual incidence rate of 2.5 percent), interactions of modest size will be detectable.

The above calculations assumed environmental factor prevalence of 10 percent. An increased prevalence may be present, and this would increase the ability to detect gene-environment

interactions. The following table shows the change in minimum detectable relative odds ration for gene-environment interaction effect with increased environmental factor prevalence (20 percent and 30 percent), for a few values of incidence/year and 1.5 controls per case.

	Environmental factor prevalence		
Disease incidence (%)	10%	20%	30%
0.01	>>10.00	>>10.00	>>10.00
0.05	>10.00	9.00	7.80
0.10	7.10	4.70	4.20
0.20	4.00	3.00	2.70
0.50	2.50	2.00	1.90
2.50	1.52	1.35	1.34
3.00	1.47	1.39	1.31

The prevalence of the environmental factor has a large impact on the minimum detectable interaction effect size when the incidence rate is small (e.g., for 0.20, a decrease from 4.0 to 2.7, about 33 percent). The improvement is less when the incidence rate is high (e.g., for 2.50, a decrease from 1.52 to 1.34, about 12 percent). The same patterns would be observed as a function of the prevalence of genetic susceptibility.

8. Other Design Issues

There may be ethical considerations associated with genetic tests, but such considerations are relevant to the NCS as a whole and would not be unique to this specific hypothesis. For nongenetic factors, the study will need to have a formal strategy and process for effective communication of clinically relevant and actionable physiological and biochemical results to the child's parents. The study also will need to have a formal strategy and process for effective communication of clinically relevant and actionable results of environmental monitoring to the child's parents along with appropriate and feasible recommendations regarding the correction of any unhealthful environmental findings. Daycare and school cooperation would be required for some of the intended environmental exposure measures. In addition, a mechanism needs to be identified to ensure that children found to have asthma through this study can receive adequate medical care for their illness. Repeated assessments can be burdensome, and the total respondent burden must be evaluated and be reasonable.

9. References

Akinbami, L. (2006). The state of childhood asthma, United States, 1980-2005 (pp. 1-24). *Advanced Data from vital and health statistics, no. 381*. Hyattsville, MD: National Center for Health Statistics.

American Lung Association Epidemiology and Statistics Unit, Research and Program Services. (2006) *Trends in asthma morbidity and mortality*. New York: American Lung Association. Retrieved from http://www.lungusa.org

Bjornsson, H.T., Fallin, M.D., & Feinberg, A.P. (2004). An integrated epigenetic and genetic approach to common human disease. *Trends in Genetics, 20*(8): 350-358.

Callinan, P.A., & Feinberg, A.P. (2006). The emerging science of epigenomics. *Human Molecular Genetics, 15*, R95-R101.

Collins, F.S., & Manolio, T.A. (2007). Merging and Emerging Cohorts: Necessary but not sufficient. *Nature, 445*(7125), 259.

Cook, D.G., & Strachan, D.P. (1997). Health effects of passive smoking. 3. Parental smoking and prevalence of respiratory symptoms and asthma in school age children. *Thorax, 52*(12), 1081-1094.

Eder, W., Ege, M.J., & von Mutius, E. (2006). The asthma epidemic. *New England Journal of Medicine, 355*(21), 2226-2235.

Eder, W., Klimecki, W. Yu, L., von Mutius, E., Riedler, J., Braun-Fahrlander, C., et al. (2005). Opposite effects of CD 14/-260 on serum IgE levels in children raised in different environments. *Journal of Allergy and Clinical Immunology, 116*(3), 601-607.

Eder, W., Klimecki, W., Yu, L., von Mutius, E., Riedler, J., Braun-Fahrlander, C., & et al. (2006). Association between exposure to farming allergies and genetic variation in CARD4/NOD1. *Allergy*, 61, 1117-1124.

Gauderman, W.J. (2002). Sample size requirements for matched case-control studies of gene-environment interaction. *Statistics in Medicine, 21*, 35-50.

Gauderman, W.J., & Morrison, R. (2007). QUANTO 1.2: A computer program for power and sample size calculations for genetic-epidemiologic studies [Computer software]. Retrieved from http://hydra.usc.edu/gxe

Gilliland, F.D., Li, Y.F., Dubeau, L., Berhane, K., Avol, E., McConnell, R., et al. (2002). Effects of glutathione S-transferase M1, maternal smoking during pregnancy, and environmental tobacco smoke on asthma and wheezing in children. *American Journal of Respiratory & Critical Care Medicine, 166*(4), 457-463.

Gold, D.R., & Wright, R.J. (2005). Population disparities in asthma. *Annual Review of Public Health, 26*, 1-25.

Hall, I.P., & Blakely, J.D. (2005). Genetic association studies in Thorax. *Thorax, 60*(5), 357-359.

Hanson, M. & Gluckman, P. (2005). Developmental processes and the induction of cardiovascular function: conceptual aspects. *The Journal of Physiology, 565,* 27-34.

Hoffjan, S., Nicolae, D., Ostrovnaya, I., Roberg, K., Evans, M., Mirel, D.B., et al. (2005). Gene-environment interaction effects on the development of immune responses in the 1st year of life. *American Journal of Human Genetics, 76*(4), 696-704.

Illi, S., von Mutius, E., Lau, S., Niggemann, B., Gruber, C., Wahn, U., et al. (2006). Perennial allergen sensitisation early in life and chronic asthma in children: A birth cohort study. *Lancet, 368*(9537), 763-770.

Landrigan, P.J., Trasande, L., Thorpe, L.E., Gwynn, C., Lioy, P.J., D'Alton, M.E., et al. (2006). The National Children's Study: a 21-year prospective study of 100,000 American children. *Pediatrics, 118*(5), 2173-2186.

Lara, M., Duan, N., Sherbourne, C., Halfon, N., Leibowitz, A., & Brook. R.H. (2003). Children's use of emergency departments for asthma: persistent barriers or acute need? *Journal of Asthma, 40*(3), 289-299.

Lodrup Carlsen , K.C., Haland, G., Devulapalli, C.S., Munthe-Kaas, M., Pettersen, M., Granum, B. (2006). Asthma in every fifth child in Oslo, Norway: A 10 year followup of a birth cohort study. *Allergy, 61*(4), 454-460.

Lowe, L.A., Simpson, A., Woodcock, A., Morris, J., Murray, C.S., Custovic, A., et al. (2005). Wheeze phenotypes and lung function in preschool children. *American Journal of Respiratory and Critical Care Medicine, 171*(3), 231-237.

Malone, D.C., Lawson, K.A., & Smith, D.H. (2000). Asthma: An analysis of high-cost patients. *Pharmacy Practice Management Quarterly, 20*(1), 12-20.

Manolio, T.A., Bailey-Wilson, J.E., & Collins, F.S. (2006). Opinion: Genes, environment and the value of prospective cohort studies. *Nature Reviews Genetics, 7*(10), 812-820.

Martinez, F. (2007). Genes, environments, development and asthma: A reappraisal. *The European Respiratory Journal, 29*(1), 179-184.

McConnell, R., Berhane, K., Yao, L., Jerrett, M., Lurmann, F., Gilliland, F., et al. (2006). Traffic, susceptibility, and childhood asthma. *Environmental Health Perspectives, 114*(5), 766-772.

Morgan, W.J., Stern, D.A., Sherrill, D.L., Guerra, S., Holberg, C.J., Guilbert, T.W., et al. (2005). Outcome of asthma and wheezing in the first 6 years of life: Followup through adolescence. *American Journal of Respiratory and Critical Care Medicine, 172*(10), 1253-1258.

National Academy of Sciences Institute of Medicine, Division of Health Promotion and Disease Prevention. (2000). *Clearing the Air: Asthma and Indoor Air Exposures*. Washington, DC: National Academies Press.

Newacheck, P.W., & Halfon, N. (2000). Prevalence, impact, and trends in childhood disability due to asthma. *Archives of Pediatric and Adolescent Medicine, 154*(3), 287-293

NIH Epigenetics Workshop. (2007). The National Children's Study Genetic Measures and Epigenetics Workshop, March 23, 2007, National Institute of Child Health and Human Development, National Institutes of Health, Washington, DC.

Ober, C., & Hoffjan, S. (2006). Asthma genetics 2006: the long and winding road to gene discovery. *Genes and Immunity, 7*(2), 95-100.

Ober, C., & Thompson, E.E. (2005). Rethinking genetic models of asthma: The role of environmental modifiers. *Current Opinion in Immunology, 17*(6), 670-678.

Peters, J.M., Avol, E., Gauderman, W.J., Linn, W.S., Navidi, W., London, S.J., et al. (1999). A study of 12 Southern California communities with different levels and types of air pollution. II. Effects on pulmonary function. *American Journal of Respiratory and Critical*

Saglani, S., & Bush, A. (2007). The early-life origins of asthma. *Current Opinion in Allergy & Clinical Immunology, 7*(1), 83-90.

Sanders, V.M. (2006). Epigenetic regulation of Th1 and Th2 cell development. *Brain, Behavior, and Immunity, 20*(4), 317-324.

Schmoor, C., Sauerbrei, W., & Schumacher, M. (2000). Sample size considerations for the evaluation of prognostic factors in survival analysis. *Statistics in Medicine, 19*(4), 441-452.

Schwartz, S. (2006). Modern epidemiological approaches to interaction: Applications to the study of genetic interactions. In L.M. Hernandez & D.G. Blazer, (Eds.). *Genes, Behavior, and the Social Environment: Moving Beyond the Nature/Nurture Debate* (Appendix E, pp. 310-337). Washington, DC: National Academies Press.

Sherriff, A., Farrow, A., Golding, J., & Henderson, J. (2005). Frequent use of chemical household products is associated with persistent wheezing in preschool age children. *Thorax, 60*(1), 45-49.

Simpson, A., John, S.L., Jury, F., Niven, R., Woodcock, A., Ollier, W.E., et al. (2006). Endotoxin exposure, CD14, and allergic disease: an interaction between genes and the environment. *American Journal of Respiratory and Critical Care Medicine, 174*(4), 386-392.

Sin, D.D., Bell, N.R., & Man, S.F. (2004). Effects of increase primary care access on process of care and health outcome among patients with asthma who frequent emergency departments. *American Journal of Medicine, 117*(7), 479-483.

Stafford, R.S., Ma, J., Finkelstein, S.N., Haver, K., & Cockburn, I. (2003). National trends in asthma visits and asthma pharmacotherapy, 1978-2002. *Journal of Allergy and Clinical Immunology, 111*(4): 729-735.

Stein, R.T., & Martinez, F.D. (2004). Asthma phenotypes in childhood: Lessons from an epidemiological approach. *Paediatric Respiratory Review, 5*(2), 155-161.

Suissa, S., Ernst, P. Benayoun, S., Baltzan, M., & Cai, B. (2000). Low-dose inhaled corticosteroids and the prevention of death from asthma. *New England Journal of Medicine 343*(5), 332-336.

Suissa, S., Ernst, P., & Kezouh, A. (2002). Regular use of inhaled corticosteroids and the long term prevention of hospitalisation for asthma. *Thorax 57*(10), 880-884.

Valle, D. (2004). Genetics, individuality, and medicine in the 21st century. *American Journal of Human Genetics, 74*(3), 374-381.

Vercelli, D. (2004). Genetics, epigenetics, and the environment: switching, buffering, releasing *Journal of Allergy & Clinical Immunology, 113*(3), 381-386, quiz 387.

Vercelli, D., & Martinez, F. (2006). The Faustian bargain of genetic association studies: Bigger might not be better, or at least it might not be good enough. *Journal of Allergy & Clinical Immunology, 117*(6), 1303-1305.

Wallace, A., Scott, J., Klinnert, M., & Anderson, M.E. (2004). Impoverished children with asthma: a pilot study of urban healthcare access. *Journal for Specialists in Pediatric Nursing, 9*(2), 50-58.

Weiss, K.B. (2001). The health economics of asthma and rhinitis. I. Assessing the economic impact. *Journal of Allergy & Clinical Immunology 107*(1), 3-8.

Willett, W.C., Blot, W.J., Colditz, G.A., Folsom, A.R., Henderson, B.E., & Stampfer J. (2007). Not worth the wait. *Nature, 445*(7125), 257-258.

Zambelli-Weiner, A., Ehrlich, E., Stockton, M.L., Grant, A.V., Zhang, S., Levett, P.N., & et al. (2005). Evaluation of the CD14/-260 polymorphism and house dust endotoxin exposure in the Barbados Asthma Genetics Study. *Journal of Allergy and Clinical Immunology, 115*, 1203-1209.