

HYPOTH – Gene Environment Asthma Mar 27, 2002

The Role of Prenatal Maternal Stress, Genetics and Biotransformation in Childhood Asthma.

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I. Proposed Core Hypothesis – Primary outcome asthma/wheeze in children.

We propose to determine the role of prenatal, maternal stress exposure in predicting the risk of childhood asthma. We will also consider effect modifiers of the association between maternal psychological stress and the development of asthma by concurrent assessment of genetic and environmental factors thought to influence immune development and lung growth/airway inflammation in early life. We will also determine the comparative role of the maternal genetic background vs the child's genetic background in modifying the effect of prenatal environmental exposures in predicting asthma.

- 1 Children with mothers with high psychological stress levels during pregnancy will have increased risk of asthma/wheezing.** This effect of stress may be due to polarization of the infant to a T2 phenotype and/or due to increased susceptibility to *in utero* oxidative toxins.
- 2 Polymorphisms in *maternal* genes will interact with prenatal exposures to stress, smoking, air pollution in predicting subsequent asthma risk. This interaction effect will be greater than the interaction effect measured for the *child's* genotype for the same gene.** Because the mother's genetic make-up regulates the dose of an environmental toxin reaching the fetus, her genotype is the more relevant factor in a gene-environment interaction of an intra-uterine exposure. This concept that maternal genes mediated fetal exposure has been demonstrated in birth outcomes (cleft lip/palate, GST-T1 polymorphisms and maternal smoking for example) but to our knowledge has not been tested in health outcomes beyond the newborn period.

Specifically, we will test the following gene by environment interactions:

- 2a) 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 genes in the corticosteroid pathway (i.e. beta 11 hydroxylase, and the glucocorticoid receptor).** Children with stress exposure and specific genotypes will have a propensity to express the Th-2 phenotype in the preclinical state and will be more likely develop asthma.
- 2b) Children with mothers with high stress exposure during pregnancy will vary their increased risk of asthma/wheezing in part related to genetic variation in maternal toxin metabolizing genes (i.e. glutathione S transferase M1, T1, and P1 and Cytochrome P450 1A1).** Children with maternal prenatal smoking/air pollution exposure and specific genotypes will have increased airway inflammation via oxidative toxicity in a biologic pathway similar to that induced by stress.

Finally, we will test the following environment by environment interaction:

- 3. Prenatal stress exposure will modify the association between prenatal exposure to oxidative toxins (tobacco smoke and air pollution in particular) in predicting subsequent**

risk of asthma. Stress is a pro-oxidant biologic process with effects on biotransformation enzymes which can alter the response to oxidative exogenous toxins and increase their toxicity.

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IV. Public Health Importance. Asthma prevalence and the associated morbidity are increasing. These trends disproportionately affect poor minority children living in urban environments, and remain a paradox largely unexplained by accepted environmental risk factors and suggest the existence of as-yet-unidentified factors or unmeasured interactions (Wright RJ et al 1998). This paradox, in part, has led to reconsideration of the overlap between biological determinants and psychosocial factors (i.e., life stress) in understanding the rising asthma burden. Recently, we have identified parental stress as a risk factor for asthma (Wright RJ et al 2002). The factors which may explain these trends may be the interactions between established risk factors with genetic factors or may be due to interactions between highly prevalent environmental factors. These hypotheses propose a biologic pathway by which previously established environmental risk factors may interact with genetic risk factors and with each other. The interactions, if proven, will help to establish the mechanisms by which asthma occurs. By focusing on prenatal exposure, we restrict our specific aims to a critical window of exposure and by doing so, focus the question in a testable manner. The implications of these results are that disease prevention measures may be successfully implemented during pregnancy, a narrow window of opportunity in which even transient decreases in exposure (smoking, air pollution and stress) may produce substantial decreases in subsequent disease prevalence. By concurrently studying genetic and environmental risk, we will help to establish susceptible subpopulations and elucidate causative pathways which may lead to new insights in disease prevention.

V. Justification for a large prospective longitudinal study. Testing of gene environment interaction requires large sample sizes. Furthermore, our hypotheses are fundamentally a 3 way interaction (gene-environment-time) in that we propose that the critical period for environmental exposure and gene expression in predicting disease risk is prenatal. As such, only a large-scale longitudinal study with prospectively collected prenatal exposure data and DNA samples of mother-infant pairs can address these questions. The prospective nature of the NCS further enhances this research as the stress questionnaires will be administered prior to phenotype expression (childhood asthma) 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 that sufficient power exists for testing the gene by environment interaction hypotheses, which will require large sample sizes. *See appendix for sample size calculations.*

VI. Background/Scientific Merit. Some prenatal exposures, such as environmental tobacco smoke (ETS), have been demonstrated to increase the risk of childhood asthma and this risk is greatest among children with a family history of asthma, suggesting a gene-environment interaction (London et al 2001). Stress is a highly prevalent environmental exposure which has also been demonstrated

to increase asthma risk(Wright et al 2002). Unlike other risk factors like ETS or aeroallergens, the role of *prenatal* stress exposure in predicting subsequent asthma risk has not been studied to our knowledge. In addition, the mechanism by which stress increases risk of childhood asthma is not fully understood. We propose 2 potential pathways which may work in tandem. The first is that prenatal stress will increase polarization of the immune system to the T2 phenotype via dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis. Psychological stress results in activation of the HPA axis. Recent work suggests a complex response - disturbance of the HPA system varies based on the distinct psychological stress response in a given individual. This may best be understood within McEwen's concept of allostasis or the ability of the body to achieve stability through change, such that "the autonomic nervous system, the HPA axis, and cardiovascular, metabolic, and immune systems protect the body by responding to internal and external stress" (McEwen 1998). The potential cost of such accommodation is called 'allostatic load', which is the wear-and-tear from chronic over-activity or under-activity of the allostatic system (McEwen 1998). While in acute stress, increased cortisol and catecholamines promote allostasis by influencing cell trafficking and cytokines which fight infection (McEwen et al 1997; Brosschot et al 1994), in chronic stress over (or under) activity of these same mediators may result in allostatic load, and potential immunosuppressive effects (McEwen 1998). Imbalance, or the absence of appropriate levels of glucocorticoids and catecholamines, may allow other immune mediators to over-react and increase the risk of inflammatory disorders (Sternberg 1997). A substantial literature exists demonstrating how psychological stress can influence cell trafficking, T-cell function, and lymphocyte production of cytokines (Herbert & Cohen 1993). Stress can modulate immune responses by triggering the release of hormones and neuropeptides that interact with immune cells (Kiecolt-Glaser et al 1993, Cohen & Herbert 1996). This phenomenon includes stress-elicited changes of cytokine production (Dobbin et al 1991, Glaser et al 1990). Given this background, some speculate that stress triggers cellular signals that may modulate the direction of T cell differentiation and induce susceptibility to asthma (Herbert & Cohen 1993).

The concept that nongenetic factors act early in life to permanently organize or imprint physiological systems is known as perinatal 'programming'(Barker et al 1993). Adverse environmental influences (i.e., stress) may also produce permanent physiological changes, thus increasing the risk of later disease (Osmond and Barker 2000). Increasingly studies in animals and humans underscore the importance of the *in utero* environment in subsequent disease(Bertram & Hanson 2001). Thus, we propose that maternal stress *during pregnancy* may program the fetus towards an asthma phenotype. In addition, we propose that this programming is magnified by interactions with genetic polymorphisms of corticosteroid metabolism.

The second mechanism by which stress may enhance airway inflammation is through its role as a pro-oxidant. Two important environmental exposures which are associated with asthma morbidity (air pollution and ETS) have oxidative properties. Gergen (2001) has suggested that ETS exposure sensitizes susceptible individuals to triggers of asthma, and that this sensitizing effect would heighten the chances that an undiagnosed susceptible individual would experience more severe symptoms and become diagnosed (Gergen 2001). Stress activates various P450 enzyme systems which are involved in the biotransformation of environmental oxidative toxins, such as ETS. Several animal and human studies demonstrate that chronic stress will produced increased oxidative toxicity in the body. This may be due to a decreased ability to detoxify exogenous toxins. For example, in a birth cohort followed in mainland China, women with self-reported moderate to high levels of occupational stress had offspring with a modest reduction in birthweight(Chen et al 2000). Similar independent effects of occupational benzene exposure were also reported. Upon

further analysis, subjects with *both stress and benzene exposure* had an average reduction of 184 grams in the birthweight of their children. Subjects with only benzene exposure had a 15 gram reduction, and those with only maternal stress exposure had a 19 gram reduction. Benzene, a cyclic aromatic hydrocarbon, is an oxidative toxin which 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 of mother-infant pairs, genetic susceptibility to benzene and decreased gestational age was also described- (Wang et al 2001). Mothers with the P450 system CYP1A1 HincII polymorphism AA and occupational exposure to benzene had a statistically significant decreased gestational period(half a week). CYP1A1 is a phase I enzyme which activates polycyclic aromatic hydrocarbons. The association between the AA genotype and benzene exposure was postulated to be due to increased activation of benzene in the AA genotype mothers. The similar interaction between stress and benzene reported by Chen et al may be due to increased oxidation by stress mediated induction of P450 enzymes. While the A variant has no increased activity relative to the “a” variant, the A variant does have increased inducibility, and stress has been demonstrated to induce P450 enzyme systems in presence of a toxin. A study conducted in mice parallels these findings. Among mice exposed to chronic intermittent restraint stress, total P450 content was decreased; however, the presence of both stress and the toxic benzene derivative 1,4-bis(2-(3,5-dichloropyridyloxy)benzene (TCPOBOP) *increased* total P450 content (Konstandi et al 1998). Furthermore, there was a 7 fold decrease in glutathione content in the lungs of the TCPOBOP treated mice after stress, whereas glutathione levels in the unstressed TCPOBOP treated mice were unaffected.

Other human and animal studies support the concept that psychological stress has pro-oxidant properties and alters biotransformation(Capel 1983, Kosugi et al 1994, Tomei et al 1990, Irie et al 2000) These studies together with the study by Chen et al suggests that stress is a pro-oxidant exposure which modifies the host response to pro-inflammatory oxidative toxins and may mediate lung growth, increasing asthma susceptibility. Given this background, we propose that maternal stress will interact with *in utero* smoke exposure and air pollution exposure in predicting childhood asthma. Furthermore, via the same mechanism, we propose that polymorphisms in biotransformation enzyme system genes will interact with stress, and will also interact with oxidative environmental toxins such as tobacco smoke and air pollution in predicting asthma.

A second innovative approach of these hypotheses is to determine the role of maternal genetics on *in utero* environmental exposures. Given the physiology of pregnancy and the role of critical windows of exposure, we predict that maternal genetic polymorphisms are the most important genetic factor to measure in determining these interactions. While there are no studies of maternal genetic polymorphisms interacting with *in utero* exposures in predicting respiratory outcomes in children, there are some studies in birth defects which illustrate this concept. Van Rooij et al 2001, conducted a case control study of both maternal Glutathione-S-transferase (GST) T1 and child GST T1 genotypes and ETS exposure *in utero* in predicting cleft palate/cleft lip in children. Despite the small sample size (N=130), they found a 3.2 fold increase risk (95% CI 0.9-11.6) of cleft palate among mom's with GST T1 null genotype who smoked in pregnancy, but only a 1.9 fold effect (95% CI 0.5-6.6) if the baby had the GST- T1 null genotype and the mom smoked during pregnancy. For each of these odds ratios, the reference group consists of mothers or children without the GST T1 genotype and nonsmokers. Similar interaction effects may be found between maternal smoking or air pollution exposure during pregnancy and GST or CYP polymorphisms in predicting childhood asthma/wheeze. *Likewise, because stress is pro-oxidant, we hypothesize interactions between maternal stress during pregnancy and in utero exposure to TS and air*

pollution in predicting asthma/wheeze in children, similar to the study by Wang et al. Mechanistically, we propose that stress is biologically similar to alterations in detoxification produced by genetics, leading ultimately to the expression of disease by the same biologic pathway.

These hypotheses seek to determine how prenatal stress causes an increased risk of childhood asthma. The 2 pathways proposed in our hypotheses can be tested by collection of biomarkers (maternal and cord blood IgE and cortisol, maternal urinary cotinine levels) questionnaires (Perceived Stress Scale, The Parenting Stress Index, The Family Environment Scale, Qualitative and quantitative smoking history), environmental measures (Particulate matter less than 10 microns or PM10, and ozone levels), and DNA collected from both mother and infant.

VII. Potential for Innovative Research. Several aspects of these hypotheses are innovative. The role of prenatal stress exposure on subsequent childhood asthma, has not to our knowledge been 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 (the Barker hypothesis of decreased fetal nutrition and increased subsequent risk of hypertension and cardiovascular disease). The extension of this critical window concept to a psycho-social exposure, such as psychological stress, may be particularly fruitful in determining asthma causation. In addition, while the concept of critical windows of exposure is well established, the role of genetic susceptibility during these exposure windows has not been well studied. Reliance on purely 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 which may interact with *in utero* fetal exposures has not been studied outside the field of reproductive epidemiology and birth outcomes, but clearly deserves consideration for health outcomes in later life.

VIII. Feasibility. While studies of interaction require large sample sizes, we believe these hypotheses can easily be tested in the NCS(see appendix). Given the high prevalence of the outcome/phenotype (asthma), the high prevalence of the exposures (stress, smoking, air pollution) and the highly polymorphic nature of the glucocorticoid receptor, 11 beta hydroxylase enzyme, P450 and phase II biotransformation enzymes, these hypotheses will be testable within the framework of the NCS. The data for prenatal exposures and phenotype classification will likely be collected as part of the study protocol. All have well established, validated measurement instruments. The frequency of family contact will be within the framework of the overall study design (prenatal enrollment, collection of cord blood, follow-up until early childhood), thus, these hypotheses will not require additional visits and will not impose an additional burden on enrollees. The study could be performed on the cohort as a whole, however, for cost efficiency, a nested case control design should be employed with random control sampling (matching for age) after the cohort has reached age 3-5 and manifested early childhood wheezing. 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). All the important covariates (stress exposure, post-natal smoke exposure, allergen exposure, post-natal air pollution) are likely to be measured as part of the overall cohort goals, and will not require additional costs.

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Sample Size Appendix:

We present here sample size estimates for the gene-environment interaction hypotheses. Sample size estimates for the main effect of prenatal stress on subsequent risk of asthma are not presented, as a clinically relevant main effect is assumed to be adequately powered in a large cohort such as the NCS. Sample size estimates for interaction effects; however, may not be adequately powered even in a large cohort, and therefore require estimation.

Sample Size for Gene-Environment Interactions: Estimates of the frequencies of the environmental exposure and the genetic factor in the population are needed to estimate the sample size required to detect a gene-environment interaction with sufficient power (Hwang, et al. 1994). The software currently available to estimate sample size for interaction effects requires that both the environmental exposure and the outcome be treated as categorical variables (Lubin et al 1999). In the NCS some of these factors can be measured as continuous variables; thus, these calculations may overestimate the needed sample size if the environmental exposure can be measured on a continuous scale. Rather than calculate a separate sample size for each genetic variant and each exposure, there are some assumptions we can make to simplify these calculations. First, we believe that the prevalence of many of the functional genetic variants (or the variants in linkage disequilibrium with the functional variants) is approximately 10-25%. For some genetic polymorphisms, this may be high, but for others (GST M1 for example) this is too low. With respect to the environmental exposure, for purposes of this calculation, we will divide the data into quartiles and define the highest quartile of exposure as exposed, and the lowest 3 quartiles as unexposed. Keep in mind that for some exposures (air pollution for example) we will have data on a more continuous scale and that therefore these calculations using dichotomous measurements are conservative. Thus, the prevalence of both the genotype and the environmental exposure for the first of these calculations is estimated at 25% (Figure 1). We also ran the calculations using the prevalence of the genotype and environmental exposure as 10%. We assumed that this would represent the minimal prevalence for which 2 interacting factors would have important public health significance (1% of the general population affected).

Finally, we must estimate the independent and interaction effects of the environmental exposures and genetic variants. We are estimating a null effect for most genes and only a modest effect of the exposure in the absence of the genetic variants. Therefore, for our calculations, we will estimate the OR of the gene effect alone to be 1 and the OR of the exposure effect alone to be 1.5. We will estimate the OR for theta (or interaction effect) to be 2 (a conservative estimate). With respect to asthma prevalence, based on prevalence in other studies of other populations, we estimate the overall prevalence of wheezing/asthma at 10% of the cohort (10,000 subjects).

All power calculations were carried out using POWER version 2.2.4 (Lubin et al., 1999) which is tailored to perform calculations for studies of gene-environment interactions. POWER bases calculations on the binary response model:

$$P(D|E,G) = [OR(E,G)]^D / [1 + OR(E,G)],$$

where E = environmental exposure, G = gene, and the odds ratio (OR) for joint exposures under the alternative hypothesis (H_1) may be derived from a multiplicative relationship (i.e., a logistic model), such that

$$2^{[(E_1-E_0)(G_1-G_0)]} \text{OR}_{E_1-E_0, G_1-G_0} = (\text{OR}_{E=1, G=0})^{(E_1-E_0)} * (\text{OR}_{E=0, G=1})^{(G_1-G_0)} *$$

Where: D=1, indicates asthma present

D=0, indicates no asthma

E=1, indicates the highest quartile of environmental exposure (0.10-0.25) "exposed"

E=0, indicates the lowest three quartiles of environmental exposure (0.75-0.9) "unexposed"

G=1, indicates the presence of the gene SNP (0.10-0.25) "gene +"

G=0, indicates absence of the gene SNP (0.75-0.90) "gene -"

OR_{G=1, E=0} = OR for the gene SNP effect in the lowest quartile of environmental exposure (OR=1.0)

OR_{E=1, G=0} = OR for the stress effect (highest quartile of exposure) among those without gene (OR= 1.5) (Lubin et al 1999, Garcia-Clossas et al, 1999; and POWER 2.2.4 Readme document)

Figure 1: Sample Size needed if Genotype and Environmental Exposure Each Prevalent in 25% of NCS Cohort and Interaction Theta=2. (No Image)

As stated, we hypothesize a 1.5-fold increase in risk of asthma/wheeze among those children in the highest quartile of prenatal environmental stress exposure compared to those in the lowest 3 exposure categories (OR(ENV)=1.5). We further hypothesize that the presence of the gene potentiates the risk of the environmental exposure such that persons with both the gene and the environmental exposure will have at least a 2 fold increase in risk of asthma compared to those with only the prenatal environmental factor (OR(combined)=3.0). We believe the gene has no effect in the absence of environmental exposure (OR(gene)=1.0). Therefore, for the purposes of the power calculation, we are assuming the excess odds ratio for the interaction (theta)=2.0. Theta = Excess OR for interaction (2) = $\frac{\text{OR}_{E=1, G=1}}{\text{OR}_{E=1, G=0} * \text{OR}_{E=0, G=1}}$ for a multiplicative model and represents the proportional

Figure 2: Sample Size needed if Genotype and Environmental Exposure Each Prevalent in 10% of NCS Cohort and Interaction Theta=2. (No Image)

interaction parameter at unit increases in each exposure for our model.

Sample Size Required for Gene-Environment Interaction Hypotheses.

As shown in the figures, an estimate using $\theta = 2.0$, environment OR [environment]=1.5, OR[gene]=1.0, and OR[combined]=3.0 for a two-sided test of the null hypothesis of no effect, a total sample size of 1760 (880 cases and 880 controls) would be required to achieve 80% power. If the effect sizes are larger than predicted (i.e., $\theta = 3$ instead of 2), the number of subjects required to achieve similar power would decrease. Figure 1 is a power curve of the sample size under these assumptions and an exposure/genotype prevalence of 25% each. We also calculated the sample size when the genetic variant is only prevalent in 10% of the population with $\theta = 2.0$ (Figure 2). Using an environmental exposure prevalence estimate also set at 10%, the necessary sample size to achieve 80% power would be 7212 subjects (3606 cases and 3606 controls), an estimate well within the expected the number of subjects with childhood asthma in the NCS.