NONPERSISTENT PESTICIDES AND POOR NEUROBEHAVIORAL AND COGNITIVE SKILLS

1. Broad Meta Hypothesis

Repeated, low-level exposure to nonpersistent pesticides, including carbamates, organophosphates, and pyrethroids, in utero or postnatally increases risk of poor performance on neurobehavioral and cognitive examinations during infancy and childhood.

2. Specific Hypotheses

1. After adjusting for potential confounders, exposure to nonpersistent insecticides will be associated with decrements in standardized tests of development, intelligence, language, motor ability, and visual-motor integration.

2. The risk of poor performance on neurobehavioral and cognitive examinations due to exposure to nonpersistent pesticide exposure will be more pronounced among children with genetically decreased paraoxonase activity.

3. Children with certain polymorphisms affecting paraoxonase activity (i.e., $PON1_{Q129}$) will be vulnerable to pesticide exposures at all ages while children without this polymorphism will be more susceptible to pesticide exposures during the first 6 months of life than at later ages (gene-by-exposure-by-age interaction).

3. Background and Justification

3.1 Public Health Importance

National survey data show that the general adult population has widespread exposure to pesticides as reflected by levels of urinary metabolites (Hill et al., 1995). In many settings, children have a greater opportunity for exposure because they have greater dermal contact with surfaces near the floor or ground and because of their greater hand-to-mouth activity (Moya, Bearer, & Etzel, 2004). Within existing literature, investigations of children's exposures to pesticides as reflected by the levels of urinary metabolites reveal surprisingly frequent exposure even in populations without agricultural exposure. Because children may be more sensitive than adults to low levels of neurotoxic substances and because pesticide exposure is generally widespread, concern about the potential health effects is warranted. Although recent regulations have decreased the use of organophosphate pesticides (e.g., chlorpyrifos) in homes, other pesticides with similar mechanisms are still used. Organophosphate pesticides are also used extensively in agriculture, and children's exposure from food sources is substantial (Curle, Fenske, & Elgethun, 2003). Members of the pyrethroid and organophosphate classes of synthetic insecticides have been identified as toxic to developing nervous systems (Olson, Blank, & Menton, 1998; Roy, Andrews, Seidler, & Slotkin, 1998; Weiss, 2000). Data from animal models and epidemiological studies suggest that even low levels of exposure during critical periods of development could cause subtle neurological effects in humans (Dam, Garcia, Seidler, & Slotkin, 1999; Auman, Seidler, & Slotkin, 2000; Dam et al., 2000; Rice & Barone, 2000; Shafer, Meyer, & Crofton, 2005; Kofman, Berger, Massarwa, Friedman & Jaffar, 2006). Experiments on animals show that low levels of organophosphate pesticides in utero or postnatally have subtle, detrimental, and permanent effects on behavior (Eskanazi, Bradman, & Castorina, 1999). Their toxicity owes to inhibition of cholinesterase (Brimijoin & Koenigsberger, 1999), an action shared by carbamate pesticides. The lowest level of organophosphate exposure used in these experiments

(Muto, Lobelle, Bidanset, & Wurpel, 1992) resulted in exposures within an order of magnitude of what humans experience in buildings where pesticides are used (Currie, McDonald, Chung, & Higgs, 1990).

Prevalence/incidence

It is assumed that the frequency of detectable exposure may vary with the specific pesticide being measured. A recent report detected chlorpyrifos, diazinon, and propoxur in 100 percent of personal air samples of pregnant urban women correlated significantly with maternal and cord plasma concentrations (Whyatt et al., 2003). The 1999 National Health and Nutrition Examination Survey (NHANES) indicated that more than 90 percent of a sample of the U.S. population had detectible levels of at least three metabolites of organophosphate insecticides in their blood or urine. The metabolites detected in the highest number of samples, diethylphosphate and diethylthiophosphate, are nonspecific metabolites of approximately 10 regularly used organophosphate insecticides.

Estimating the prevalence of poor cognitive development in children is a more difficult task. The definition of poor cognitive development can include cases of severe mental retardation to mild cases of attention deficit in the absence of hyperactivity. While mental retardation can be easily assessed using IQ testing, the diagnosis of more subtle developmental disabilities, such as hyperactivity and some autism spectrum disorders, makes a true national estimate of developmental disabilities in the United States difficult. In addition, the standards used to identify and classify developmental disabilities continue to change as more is understood regarding childhood cognitive development. Developmental disabilities affect approximately 17 percent of children younger than 18 years old in the United States and have resulted in substantial financial and social costs for affected families and educational and health care systems (Centers for Disease Control and Prevention [CDC], 2006).

Economic and/or social burden

Applying the methodology in Grosse, Matte, Schwartz, and Jackson (2002), a one IQ point loss in 90 percent of births results in $49 billion in annual cost in 2003 dollars, and lifetime savings from an IQ point gain of 0.5 percent of at-risk live births (2003) is about $294 million.

While no studies have precisely calculated costs associated with autism, a U.K. report estimates the lifetime custodial costs of autism spectrum disorders from $3 million to $4 million per child, with societal costs likely to be triple the individual estimate (Jarbrink & Knapp, 2001). The lifetime cost of mental retardation for persons born in 2000 was estimated at $51.2 billion (in 2003 dollars) (CDC, 2006). These costs are likely to underestimate the true cost of mental retardation since they are based on prevalence estimates obtained through only one CDC monitoring program.

3.2 Justification for a Large Prospective Longitudinal Study

Well-designed prospective, longitudinal studies of the impact of prenatal and postnatal insecticide exposure on child outcomes are critical in determining actual effects. It is likely that there are interactions between genotypes at multiple loci and that gene-by-environment and gene-by-environment-by-age interactions also exist. For example, the paraoxonase-1 (PON1) enzyme plays a role in detoxification of organophosphates but its levels are lower in neonates than in older children and adults (Cole et al., 2003), suggesting neonates have a reduced ability to detoxify organophosphates. In addition, individuals with a particular allele related to PON1 (i.e., $PON1_{Q129}$) may be more vulnerable to organophosphates at all ages. While all individuals without this allele are vulnerable to organophosphates during the neonatal period, they do not demonstrate this vulnerability later in life. Detecting these interactions will require larger sample sizes than studies to date. In addition, prospective studies will

allow for measurement of both prenatal and postnatal exposures prior to the development of adverse outcomes.

3.3 Scientific Merit

This study will help discover the effects of exposure to pesticides at various points in development from prenatal to early adulthood and possibly identify critical periods at which exposure is most damaging. The large sample size will allow for detection of subtle effects of exposure and gene-by-environment-by-age-at-exposure interactions. The nationally representative sample will help describe types of exposures by setting or geographic region and further elucidate and perhaps reveal exposure effects most damaging to development.

3.4 Potential for Innovative Research

This large, longitudinal study could lead to discovering the best markers of exposure, peak periods of exposure, and various aspects of neurodevelopment and behavior affected by exposure to pesticides with the possibility of determining very specific effects (e.g., contribution to learning disabilities). The size of the study also will enable investigators to study gene-by-environment and gene-by-environment-by-age interactions, allowing for the identification of subpopulations or age groups at increased risk from pesticide exposure.

3.5 Feasibility

This hypothesis fits within the general scope of the National Children's Study (NCS). Careful consideration of information gained by repeated environmental and biological measurements will be weighed against costs and participant burden. Recent investigations have demonstrated little within-home variability in a two-week average of indoor air pesticide concentrations, indicating that reducing the frequency of obtaining such samples can reduce the burden to NCS participants without sacrificing access to valuable data (Whyatt et al., 2007). The number of molecular techniques currently available to the study, combined with the number of environmental exposures to be measured, lends to the feasibility of investigating multiple types and sources of exposure to nonpersistent pesticides and outcomes related to poor cognitive development in children. Utilizing readily accessible molecular and genetic techniques to investigate gene-by-environment interactions is not only feasible, it can be beneficial and cost efficient. It is important to stress the need to utilize multiple approaches to assess pesticide exposure and not to rely on either biomarkers or physical indoor/outdoor air samples (Bradman & Whyatt, 2005).

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Pesticide concentration: Pesticide metabolites
- Genetic markers (e.g., paraoxonase gene)

Secondary/maternal

- Predictors of maternal pesticide exposure: Pesticide usage log, occupational data, food frequency questionnaire during pregnancy, food preparation patterns (washing fruits/vegetables or special cooking techniques)

Primary/paternal

- Some paternal measures (genetic markers such as paraoxonase activity [inheritable to child], pesticide usage log, occupational data)

Primary/child

- Child pesticide concentration: Pesticide metabolites in body fluid or concentrations in environmental media
- Child paraoxonase activity: Genetic markers of susceptibility

Secondary/child

- Predictors of child pesticide exposure: Food frequency diary, food preparation patterns (washing fruits/vegetables or special cooking techniques)

4.2 Methods

Primary/maternal

- Biological specimens: Blood, urine, breast milk
- Environmental samples: Air, dust, soil, water, food (residential/occupational)

Secondary/maternal

- Predictors of maternal pesticide exposure: Pesticide usage log, occupational data, food frequency questionnaire during pregnancy, food preparation patterns (washing fruits/vegetables or special cooking techniques)

Primary/child

- Biological specimens: Blood, urine
- Environmental samples: Air, dust, soil, water, food (microenvironments)

Secondary/child

- Interview/questionnaires with parent

4.3 Life Stage

Primary/maternal

- Repeated measures from preconception through birth
- Repeated measures from birth to age 1 (for milk collection)

Secondary/maternal

- Repeated measures from preconception through birth
- Repeated measures from birth to age 1 (for milk collection)

Primary/child

- Repeated measures from birth through age 7

Secondary/child

- Repeated measures from birth through age 21

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

Primary

- Poor neurobehavioral and cognitive performance

Secondary

- Poor academic performance (under cognitive performance)

5.2 Methods

Primary

- Examination by a trained professional (neurological and psychological testing): assessments selected will include those that evaluate broad areas of abilities (e.g., neurodevelopment, intelligence, behavior) as well as more specific skills (e.g., executive function, memory, reading, language, motor skills, and attention). At birth, general neurobehavioral functions will be assessed using the Neonatal Intensive Care Unit Network Neurobehavioral Scale, which is considered to be sensitive to toxic exposures. In early childhood, poor performance on the cognitive, language, and motor subscales of the Bayley III at 12 months will be used to identify delays in general cognitive development. The MacArthur-Bates CDI will be used at 12 months to assess delayed language development from parental report. Social competence and behavioral problems will be assessed at 12 months with the Brief Infant-Toddler Social and Emotional Assessment short form. The Modified Checklist for Autism in

Toddlers will be used for autism screening. For those instruments not yet specified, the location of the study visits will be considered when selecting the instrument to be used at each visit.

Secondary

- School record reviews will be used to demonstrate poor academic performances in children. Achievement testing, possibly age-appropriate sections of the Woodcock-Johnson test, may also be administered at multiple points in time to participating children to identify poor academic performance.

5.3 Life Stage

Primary

- Repeated from birth through 21 years with age-appropriate instruments

Secondary

- Repeated throughout childhood and adolescence with age-appropriate instruments

6. Important Confounders, Mediators, and Effect Modifiers

- **Genetically decreased paraoxonase activity and stored DNA:** Genetic polymorphisms of paraoxonase may decrease paraoxonase activity and increase risk for neurotoxic effects (Berkowitz et al., 2004). It is likely that additional, yet currently nonidentified, genes influence the metabolism of nonpersistent pesticides. DNA will be stored so gene-by-environment and gene-by-environment-by-age interactions can be investigated in the future.

- **Exposure to other neurotoxins such as lead, mercury, and persistent pesticides:** Increased exposure to other neurotoxins would increase adverse neurodevelopment and behavior (Steenland et al., 1994; Calver et al., 1998).

- **Residential and daycare environment:** Home environment is one of the most prominent factors in children's intellectual and behavioral development. Rural living and size of farm is associated with decreased performance on neurobehavioral test batteries (e.g., digit span, Benton visual retention, simple reaction time) (Van Wijngaarden, 2003; Misra, Prasad, & Pandey, 1994). Although pesticide exposures could cause these associations, it is possible other factors analogous with rural lifestyle may be causing these differences.

- **Prenatal and postnatal nutrition:** Folate and vitamin D deficiency have been implicated in adverse neurodevelopmental outcomes. Caffeine and tobacco consumption during pregnancy may be associated with neuropsychological functions and behavioral problems (e.g., ADHD).

- **Socioeconomic status:** Low socioeconomic status, including low levels of parental education, is the strongest and most consistently identified risk factor associated with poor performance across all cognitive domains.

- **Prenatal and recent infection and cytokine response:** Prenatal infection and cytokine concentrations are associated with neurodevelopmental disabilities and will need to be controlled for the analysis (also see Prenatal Infection and Schizophrenia and Prenatal Infection and Neurobehavioral Disabilities hypotheses).

7. Power and Sample Size

The example given above for the relationship between the presence of the $PON1_{Q129}$ allele (occurs at a rate of 0.3 to 0.75 among the general population) (Furlong, Richter, Seidel, & Mutulsky, 1988; Costa et al., 2003) organophosphate (OP) exposure and age may be hypothesized to affect IQ in the following direction:

Table 1. Mean IQ Scores by Presence of $PON1_{Q129}$ allele, Organophosphate Exposure, and Age at Exposure

$PON1_{Q129}$	Organophosphate	Neonate	Older	Totals
Present	Exposed	↓↓↓	↓↓↓	↓↓↓
	Non-exposed	100	100	100
Absent	Exposed	↓↓↓	↓	↓↓
	Non-exposed	100	100	100

To simplify the sample size calculation, we used the presence of the $PON1_{Q129}$ gene (present/absent) and a four-level variable exposure: 1) as a neonate, 2) as an older child, 3) as a neonate and an older child, or 4) not exposed. Loss to follow-up was assumed to be 2 percent per year and assessment age 18 was used. It is estimated that approximately 70,000 children will still be in the Study at 18. The minimum difference that can be reliably detected is 0.53 IQ units with a 30 percent exposure rate. The minimum detectable difference will be smallest if 50 percent of children are exposed (0.49) and bigger if the percentage is close to 0 percent or 100 percent (minimum effect size is 0.82 if 10 percent are exposed).

For a two-way analysis of variance model with an interaction, the minimum interaction that can be reliably detected is about twice that for a main effect. The minimum interaction that could be reliably detected would be 1.11 IQ units assuming that 30 percent are exposed as neonates, 20 percent are first exposed when older, and 50 percent are not exposed.

These results indicate that if the proportion exposed is not too close to 0 percent or 100 percent, the minimum detectable difference will be between 0.5 and 1.0 IQ units. For the interaction of the allele and exposure, if the proportion of the population with the allele is not too close to 0 percent or 100 percent, an interaction of between 1.0 and 2.0 IQ units can be reliably detected.

8. Other Design Issues

- **Ethical/burden considerations:** The study will have a formal process for effectively communicating results of physiological and biochemical measures to the child's parents and a responsible health care provider; and 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:** The study will have a protocol for reporting neurodevelopmental and behavioral test results to primary care providers when they suggest significant developmental delay or behavioral disorder and may indicate need for more detailed assessment or intervention services.

- **Cost/complexity of data collection:** Potentially time consuming and costly instruments to assess exposures and outcomes in later stages of the study will be selected on the basis of providing most accurate data while maximizing retention rate through minimization of subject burden.

- **Cost of sample analysis:** Some tests are costly and repeated assessments can add to the overall cost. The use of nested case-control design for some outcomes will reduce expenses.

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

- **Training on proper administration of neurobehavioral assessments:** Rigorous training will guarantee all individuals are tested properly according to the Study protocol. Provisions will need to be made for training, certification, and annual recertification of examiners. Given the magnitude of the Study, training efforts may be costly and time consuming but are imperative to ensure quality data.

- Other potential measurement issues that could be investigated:

  - Body burdens or excretion rates of nonpersistent insecticides or their metabolites in children are directly related to insecticide concentrations in environmental exposure media, and these relationships are modified by human behaviors.

  - Longitudinal insecticide exposure for individual children can be ascertained from repeated short-term sampling of noninvasive biological markers of exposure (e.g., insecticide metabolites in urine).

  - Longitudinal insecticide exposure for individual children can be estimated from repeated (e.g., annual) residential exposure media (e.g., settled dust indoors) and longitudinal questionnaire/diary information on residential pest pressure, residential insecticide use, microactivity patterns, and food consumption.

- Long-term (e.g., annual) exposure to nonpersistent insecticides varies among groups of children defined by age and other demographic parameters.

- Long-term exposure to nonpersistent insecticides for populations of children can be estimated from personal exposure information obtained from representative samples of individual children.

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PRENATAL INFECTION AND NEURODEVELOPMENTAL DISABILITIES

1. Meta Hypotheses

Prenatal infection and mediators of inflammation are risk factors for neurodevelopmental disabilities such as cerebral palsy and autism.

2. Specific Hypotheses

1. The risk of neurodevelopmental disabilities will vary depending on the type of infection (i.e., chorioamnionitis, etc.) and timing of infection during gestation.

2. Maternal changes in cytokines in response to infection will predict the risk of neurodevelopmental disabilities.

3. The risk of neurodevelopmental disabilities due to exposure to infection will be influenced by obstetric complications and genetic risk factors.

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

Cerebral palsy affects as many as 0.3 percent (Bhasin, Brocksen, Avchen, & Van Naarden, 2006; Kuban & Leviton, 1994) and autism affects about 0.7 percent of children (Centers for Disease Control and Prevention [CDC], 2007; Yeargin-Allsopp et al., 2003). Whether the frequency of autism is increasing is controversial. Recent estimates of higher prevalence may be due to inclusion of less severe cases, better identification, or other disorders that may now be classified as autism whereas previously they were not (e.g., intellectual disabilities).

Seizures, reduced intellectual and communication skills, and increased injuries can co-occur with cerebral palsy. Fractures are prevalent in children with cerebral palsy, and repeated fractures are common. They diminish the quality of life and add to the care requirements for these children (Henderson et al., 2002). Increased mortality from seizures, injuries, and respiratory diseases is observed among persons with autism, and, although mortality is especially increased among persons with accompanying severe intellectual disabilities, life expectancy is also reduced for persons with mild intellectual disabilities (Shavelle, Strauss, & Pickett, 2001).

Literature findings suggest prenatal exposure to infection is a risk factor for cerebral palsy, autism, and other neuropsychiatric disorders (Meyer et al., 2006; Nelson & Willoughby, 2002). Chorioamnionitis is thought to play a role in the development of cerebral palsy, and approximately 1-2 percent of term pregnancies are affected by chorioamnionitis. Pregnancies ending in preterm births have an even higher prevalence of such infection (Wu et al., 2003).

Fetal inflammatory response to chorioamnionitis (intrauterine infection) includes increased levels of fetal cytokines, which can be neurotoxic (Yoon et al., 1998; Dammann & Leviton, 1998). While few studies of chorioamnionitis and cerebral palsy among children born at term have been done (Grether & Nelson, 1997; Nelson & Ellenberg, 1985), it has been estimated that about 28 percent of cerebral palsy

in preterm infants and 12 percent of cerebral palsy in term infants may be due to chorioamnionitis (Wu & Colford, 2000). A study by Wu et al. (2003) observed a four-fold increased risk (adjusted) of cerebral palsy in subjects with diagnosed chorioamnionitis. Periodontal disease is also a source of infection during pregnancy and may be associated with adverse pregnancy outcomes due to metastatic spread of infection, metastatic injury from circulating microbial toxins, or metastatic inflammation induced by microorganisms (Li, Kolltveit, Tronstad, & Olsen, 2000).

Another recent study, which found an association between fetal herpes group B exposure and cerebral palsy, found a prevalence of neurotropic viral nucleic acids from any herpes virus (group A and B) to be 38.3 percent (95 percent confidence interval: 34.8 to 41.9) in stored neonatal blood spots from a control population (Gibson et al., 2006a). The odds ratio for the association between herpes exposure and any type of cerebral palsy was 1.52 (1.09 to 2.13).

Economic and/or social burden

Cerebral palsy and autism are serious developmental disabilities that have a dramatic effect on the lives of the affected persons and their families. The lifetime economic costs of cerebral palsy have been estimated at $11.5 billion per annual cohort (CDC, 2004). A U.K. report estimates the lifetime custodial costs of autism spectrum disorders in the range of $3 million to $4 million per child (Bradstreet, 2002; Jarbrink & Knapp, 2001). The estimated annual monetary cost of autism in the United States is $26 billion (Maltby, 2000) with lifetime incremental societal costs per capita estimated at $3.2 million. Lost productivity and adult care are the largest components of these costs (Ganz, 2007). Estimates of dollars currently spent on special education annually are from $30.9 billion to about $34.8 billion (National Center for Education Statistics, 1998). Research indicates early identification of and interventions for children with autism using behavioral modification programs would lead to significant cost savings (Jacobson, Mulick, & Green, 1998).

3.2 Justification for a Large Prospective Longitudinal Study

Investigation of the influence of prenatal infection or mediators of inflammation on the origins of neurodevelopmental disabilities such as cerebral palsy and autism requires a large prospective cohort that has been followed from early gestation through childhood.

A prospective study is required to obtain prenatal exposure data. For the exposures of interest (e.g., prenatal infection), biological specimens are essential to obtain data during early and late gestation. Although case-control studies have shown an association between cerebral palsy and either recall of prenatal viral infection or presence of viral nucleic acids at birth, there are no prospective studies that have investigated the influence of the timing of the infection, whether the infection is primary or recurrent, the trajectory of the immune response to the infection, or genetic factors that may influence the risk of adverse outcomes of the trajectory in the immune system. To conserve specimens and reduce cost, a nested case-control design will be employed when assessing viral nucleic acids.

The longitudinal design is required because it is important to trace the development of autism through childhood. Identifying and validating early predictors of autism may lead to significant improvement in outcomes.

A large cohort is required to study infrequent outcomes such as autism and the possible gene-by-environment interactions. Using a nested case-control approach, the National Children's Study (NCS) can be used to identify genes associated with autism that are conditional upon infection or inflammatory responses to infection.

3.3 Scientific Merit

Herpes viruses and enteroviruses can cross the placenta and infect the fetus. These neurotropic viruses can either contribute directly to the causation of cerebral palsy or indirectly by increasing proinflammatory cytokines that may adversely affect the developing brain (Cai, Lin, Pang, & Rhodes, 2004). An association between perinatal viral exposure, as determined by detection of specific viral nucleic acids in newborn blood samples, and the risk of developing cerebral palsy has recently been reported (Gibson et al., 2006a). Children with specific cytokine polymorphisms (i.e., tumor necrosis factor-alpha [TNF-∀] and mannose-binding lectin) are at a greater risk of developing cerebral palsy than children without the polymorphisms (Gibson et al., 2006b). These polymorphisms are associated with a greater immune response to infection.

While there are some data on the relation of viral infections in pregnancy to occurrence of autism, few causal agents have been established. One specific infection, rubella, has been commonly associated with autism spectrum disorders (Rodier & Hyman, 1998). The general relationship of prenatal infection, such as chorioamnionitis, and mediators of inflammation to risk of autism has not been well studied. Such studies are overdue because of the role immune abnormalities may play in autism and the increased knowledge of the neurotoxicity of inflammatory cytokines (Meyer et al., 2006).

The genetic basis for autism is covered in greater detail in a separate hypothesis (see "Gene-Environment Interactions"). The focused research efforts and an intense search for genes associated with autism have not identified a gene with a large effect, but linkage scans have identified some overlap of regions harboring many genes with some related to the central nervous system (Muhle, Trencoste, & Rapin, 2004). The heterogeneity of findings across genetic studies of autism may be due to unidentified gene-by-environment interactions that may be masking some of these effects.

The risk of neurodevelopmental disabilities resulting from infection is determined, in part, by the specific type of infection (i.e., viral, bacterial, etc.), the gestational age of the fetus at the time of infection, whether the maternal infection is primary or recurrent, and possibly an inherited predisposition that makes the fetus more susceptible to infection or inflammation. Prospective studies are needed to determine timing of the specific maternal infection, the gestational age at the time of infection, whether the infection is primary or recurrent, and genetic predisposition. In addition, there is increasing evidence neurodevelopmental disorders such as cerebral palsy can result from subclinical maternal infection that cannot be identified retrospectively (Schendel, 2001).

3.4 Potential for Innovative Research

The technologies used in the assessment of relevant prenatal exposures will include serologic and viral nucleic acid analyses, including measurement of inflammatory cytokines, and are common to other research hypotheses within the NCS. Each assessment during pregnancy provides a window on fetal exposure and holds the potential to improve our knowledge and understanding of the identity, timing, and mechanisms of exposures adversely influencing neurodevelopment and increasing risk for cerebral palsy and autism.

In addition to basic serologic analyses for specific infections (e.g., influenza, HSV2), high throughput methods will be used to detect and quantitate viral nucleic acids, antiviral antibodies, and cytokine response. This will permit the simultaneous measurement of multiple analytes using small amounts of sera. The use of nested case-control design will allow this to be done in a cost-effective manner. No human studies have been reported that investigated longitudinal changes or timing of prenatal infection and immune response and how these changes or timing influence the risk of neurological outcomes of the infant.

3.5 Feasibility

Due to the size and longitudinal nature of the Study, these hypotheses can be addressed. Collection of biological samples during the first, second, and third trimesters will allow investigation of longitudinal changes in infection and immune response and determine whether the gestational age at the time of exposure to infection or cytokines has a significant impact on these neurobehavioral outcomes. Obtaining family history and genetic material also will allow for the determination of genetic susceptibility to infection and inflammatory response and whether this genetic susceptibility modifies the risk of neurodevelopmental disabilities resulting from exposure to infection. The burden of sample collection at multiple time points during pregnancy is feasible, and the use of a nested case-control design is cost-effective.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Infection serology (lymphocytes, antibodies, cytokines/interleukins, inflammatory markers)
- Blood for viral nucleic acids. The specific type of viral infection is thought to modify the risk of neurological disorders (Gibson et al., 2006a)
- Medical history of fever and infection (medicine usage)
- Dental exams

Primary/child

- Infection serology (lymphocytes, antibodies, cytokines/interleukins, inflammatory markers)
- Umbilical cord/placental (antibodies, cytokines, viral nucleic acids)

Secondary/maternal

- Retrospective medical records reviews

4.2 Methods

Primary/maternal

- Blood samples
- Vaginal/cervical cultures
- Examinations by a medical professional
- Interviews

Secondary/maternal

- Medical and obstetrical history
- Family history

Primary/child

- Umbilical cord blood culture/pathology

4.3 Life Stage

Primary/maternal

- Repeated measures at early and late gestation

Secondary/maternal

- Repeated measures at early and late gestation

Primary/child

- Birth

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

Primary

- Neurological development

Secondary

- Parental assessment and screening for child's autistic traits (e.g., modified-checklist for autism in toddlers)

5.2 Methods

Primary

- Direct observation by medical professional: Fetal ultrasound, neurological exam, autism screening and diagnostic tests (e.g., autism diagnostic observation schedule)

Secondary

- Parental and perhaps teacher screening and assessment for autistic traits in child (e.g., modified checklist for autism in toddlers, autism diagnostic interview-revised)

5.3 Life Stage

Primary

- Prenatal through year 7

Secondary

- Follow-up in year 7 and older

6. Important Confounders, Mediators, and Effect Modifiers

- **Family history of psychiatric and neurodevelopmental disorders:** Children of parents with a history of psychiatric disorders (including schizophrenia-like psychosis or affective disorders) may be at increased risk of autism (Larsson et al., 2005). Twin and family studies have suggested a genetic component to neurodevelopmental disorders, which may be determined by assessing family histories (Muhle et al., 2004).

- **Maternal and infant genetic polymorphisms:** Genetic polymorphisms have been identified that influence the concentration of circulating cytokines in response to a viral infection (Gibson et al., 2006b). Therefore, it is expected the presence of specific polymorphisms or genes may modify the risk of exposure to infectious agents.

- **Gestational age:** Studies have shown the relationship between viral type and neurological disorders varies depending upon whether the infant was born preterm or term and small for gestational age (Larsson et al., 2005).

- **Mother's medical and obstetrical histories:** Various obstetric complications are often cited as possible causes for fetal neurodevelopmental disruption and consequent disorders (Nelson & Willoughby, 2002). Increased risk for autism has been associated with specific breech presentation and low Apgar score at 5 minutes (Larsson et al., 2005).

- **Environmental exposures and drug use during pregnancy:** Maternal smoking (Hultman, Sparen, & Cnattingius, 2002) and maternal drug usage (Rodier & Hyman, 1998; Williams et al., 2001) have been implicated as risk factors for autism. Prenatal use of thalidomide early in gestation was correlated with increased risk for autism, implicating xenobiotics as a possible factor in the pathway to autism (Newschaffer, Fallin, & Lee, 2002). Prenatal use of valproic acid and other anticonvulsants has been associated with increased risk for autism in animal models (Rodier & Hyman, 1998) and reported in case studies (Palmer, Blanchard, Stein, Mandell & Miller, 2006). Although heavy metals such as mercury have been implicated as risk factors for autism, the evidence for a relationship between low-dose exposures to methylmercury are conflicting (Palmer et al., 2006).

- **Parental age:** Maternal and paternal age may be risk factors for autism. For every 10-year increase in parental age, the relative risk for autism spectrum disorders increases by 28 percent (paternal) to 31 percent (maternal) (Croen, Najjar, Fireman, & Grether, 2007). Children born to men 40 years or older were 5.75 times more likely to have an

autism spectrum disorder compared to children with fathers younger than 30 (Reichenberg et al., 2006).

7. Power and Sample Size

Assuming 100,000 infants are born into the Study, with an exposure prevalence of 2 percent, the smallest detectable relative risk would be 2.8 for cerebral palsy and 2.4 for autism.

8. Other Design Issues

Retention of index children at least to or beyond the average age of diagnosis for these disorders (e.g., age 6 or 7 for autism) will be important to address this hypothesis with sufficient power. It is important to collect maternal serum and umbilical cord blood samples or blood samples taken early in infancy to measure cytokine concentrations. This will require coordination with medical professionals to ensure the collection of these samples.

Because the contacts with these patients will essentially fall within the scope of standard of care during the pregnancy, additional cost will be relatively minor and entail maintaining the pregnancy and infant/childhood database and the tissue sample repositories.

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GENE-ENVIRONMENT INTERACTIONS AND BEHAVIOR

1. Meta Hypothesis

Exposures to adverse psychosocial, chemical, and physical environments and other stressors during vulnerable periods of pregnancy and early childhood can interact with genotype to cause or modulate behavioral problems in childhood.

2. Specific Hypotheses

Based on the literature, specific hypotheses about gene-environment interactions and behavior can be formulated around the neurotransmitter systems and modulation by hormonal and other factors often used to account for etiology and pharmacological treatments of psychiatric disorders, including depression and serotonin, ADHD (and schizophrenia) and dopamine, and conduct (and anxiety) disorders and norepinephrine.

1. The neurotransmitter serotonin has been associated with depression in animal studies with primates and in clinical studies of pharmacologic treatment with specific serotonin reuptake inhibitors.

    1.1 Children with one or two copies of the short allele of the 5-HTT promoter polymorphism will be more likely to exhibit depressive symptoms when exposed to stressful life events than those with the same genotype, but without stressful life events.

    1.2 Children homozygous for the long variation in the 5-HTTLPR serotonin transporter gene will be at a greater risk for developing alcoholism in adolescence if they are also exposed to early life stress than those who have this same allelic variant coupled with low early life stress.

    1.3 Children with the l/s form of the 5-HTT gene will exhibit abnormal impulsivity and aggression when their early rearing environment is stressful compared with children who have the same genotype but are reared in a low-stress environment.

2. The neurotransmitter norepinephrine has been associated with antisocial and anxious behavior in animal studies and pharmacologic treatment of conduct and anxiety disorders. Based on this literature, an example of this hypothesis is that children with low monoamine oxidase A (MAOA) activity who are maltreated will be more likely to exhibit antisocial behavior than children with low MAOA who are not maltreated or maltreated children with high MAOA activity.

3. The neurotransmitter dopamine has been associated with ADHD in animal and clinical studies. Psychostimulants are known to affect dopaminergic systems and thus dopamine-related genes make plausible candidates for studies investigating gene-environment effects on ADHD. Several environmental toxicants affect dopamine as well. Example hypotheses include:

    3.1 Children exposed to prenatal tobacco smoke who are homozygous for the dopamine transporter (DAT) gene 480-bp allele are at greater risk for hyperactive-impulsive behaviors than children who have either smoke exposure or the DAT genotype alone.

3.2 Children exposed to maternal insensitivity show increased oppositional and aggressive behavior, but only if they possess at least one DRD4 allele 7-repeat allele.

4. For other psychiatric disorders without a standard pharmacologic intervention, such as autism, this candidate gene or candidate pathway approach has not been as useful. However, the serotonin transporter gene (SLC6A4) and the promoter polymorphism (5-HTTLPR) have been implicated. Differences in phenotype within and across autism genetic studies have contributed to heterogeneity of findings, with over transmission of the short allele in some studies and the long allele in others. Also, the variability of effects may depend on specific environmental conditions that interact to mask association or linkage of autism and SLC6A4. Using a nested case-control approach, the Study can be used to identify genes associated with autism conditional upon environmental factors (i.e., chemical and biological exposures) that emerge in this and other studies currently underway.

3. Background and Justification

Psychiatric disorders are responsible for significant burden related to disabilities of children and adults in the United States. The prevalence of psychosocial dysfunction in school-aged and preschool-aged children presenting for primary pediatric care is estimated to be 12 percent and 14 percent, respectively (Jellinek et al., 1999).

The effects of early exposures on adult outcomes have been addressed by Developmental Origins of Health and Disease (Gillman, 2005). This organization evolved from focused studies that followed an insightful observation of an association of birth weight and cardiovascular disease (Barker & Osmond, 1986), and its direction and purpose have been described by Gluckman, Hanson, & Pinal (2005) with a focus on obesity, insulin resistance, and other physical conditions. Similar observations have been made about psychiatric disorders, with reports of an association between low birth weight and/or premature birth and a variety of disorders, including ADHD (Lou, 1996; Linnet et al., 2006). A leading account of early origins is based on the concept of adaptive fetal or infant responses that produce "thrifty phenotypes." These adaptive responses are assumed to have an epigenetic basis (Gluckman et al, 2005). This assumption can be evaluated with the use of emerging epigenetic measures such as genome-wide methylation scans (Callinan & Feinberg, 2006) that allow for the evaluation of imprinting of genes related to some psychiatric disorders, (Luedi, Hartemink, & Jirtle, 2005), including autism (Lamb et al., 2005), schizophrenia (Francks et al., 2003; DeLisi et al., 2002), and Tourette's Disorder (Simonic et al., 2001).

Maternal genotype also has been evaluated in terms of enzymes that affect fetal exposure to toxic substances. For example, the maternal genes in the detoxification pathways related to tobacco smoke (Van Rooij et al, 2001; Shi et al., 2007) and alcohol (Jacobson et al., 2006) affect exposure of the fetus to the teratogenic effects of these substances. Both maternal and fetal genotypes may play a significant role in moderating the effects of environmental conditions that have a relatively high prevalence, such as maternal smoking (about 20 percent) and alcohol consumption (20 percent).

Research in animal models has demonstrated the influence of mothering on gene expression (Rutter, 2007). This illustrates that knowing the genotype is not sufficient for predicting phenotypic expression without additional knowledge of the psychosocial environment (Meyer, Palchaudhuri, Scheinin, & Flugge, 2000). Intracellular and extracellular environments play an important role not only in the magnitude of expression but also in the direction of expression (i.e., whether it is up- or down-regulated). The intracellular environment is a function of its genetic components and influences from extracellular factors, such as hormones, neurotransmitters, cytokines, and nutrients (Meaney, 2001b).

Variation in these constituents can determine how and when a gene is functionally expressed. Psychosocial factors are involved in these interactions through their influence on hormones, neurotransmitters, and nutrient intakes.

Several recent studies suggest genetic predisposition and psychosocial stressors interact to shape neurobehavioral outcomes. Seminal studies based on the Dunedin birth cohort of 1,000 children, (Caspi et al., 2003) found a serotonin transporter polymorphism moderated the influence of stressful life events on depressive symptoms, depression, and suicide. They also found a functional polymorphism in the MAOA gene moderated the effect of child maltreatment on subsequent antisocial behaviors and criminality (Caspi et al., 2002). Results from initial replication studies have been mixed, though study design and exposure assessments have varied (Dick et al., 2007; Haberstick et al., 2005; Young et al., 2006). This is not unexpected, because the size of the critical subgroups (defined by extreme placement in the genotype-exposure combinations) was small (i.e., approximately 20 to 30). These early mixed findings highlight the need for a definitive, large, prospective study to address these research questions. The NCS will provide a birth cohort that may produce critical subgroups of 200-300.

In the first published molecular genetic studies of ADHD, the candidate gene approach was used and statistical association was documented for the dopamine transporter (DAT) gene (Cook et al., 1995) and the dopamine receptor type 4 (DRD4) gene (LaHoste et al., 1996). These candidate genes were chosen based on dopamine theories of ADHD and the sites of action of drugs used to treat ADHD (Swanson et al., 2000). Cook et al. (1995) investigated parent-to-child transmission rates of the DAT alleles and reported an increased prevalence (0.85) and transmission (0.60) of the most prevalent 10R-repeat allele in a sample of 119 ADHD children. LaHoste et al. observed a higher than expected frequency of the DRD4 7R allele (0.28) in a group of ADHD cases, and Swanson et al. (1998) replicated this finding and extended it by showing linkage-disequilibrium in proband-parent triads. A recent meta-analysis confirmed the association of ADHD with alleles of the DRD4 gene and suggested association of ADHD with another dopamine gene, DRD5 (Li, Sham, Owen, & Lin, 2006). This meta-analysis did not confirm an association of ADHD with the allele of the 40 bp variable number tandem repeat (VNTR) of the DAT gene, suggesting this allele may not be associated with ADHD, may be in linkage disequilibrium, or interact with another polymorphism nearby. Another possibility is that the conflicting results arise from a gene-environment interaction such that the gene creates susceptibility and the environmental risk factor promotes expression. Other meta-analyses have reviewed the limited evidence of association of ADHD with non-dopamine genes (Faraone, Doyle, Mick, & Biederman, 2001). The failure to detect a strong signal does not discount the existence of genes with high risk alleles, of multiple genes that combine to confer ADHD risk, or of genes with effects dependent on interactions with environmental factors. Signals for genes in several different loci have been reported (Ogdie et al., 2003; Arcos-Burgos et al., 2004). Brookes et al. (2006) described a combination of candidate gene and genome scan approaches and confirmed association of ADHD with the DRD4 and DAT genes and also provided suggestive evidence of association of ADHD with 16 other genes. A large, longitudinal birth cohort study, such as the NCS, with multiple environmental exposures will provide a much needed database to facilitate resolution of these issues.

The recognition of autism has increased during the past few decades from a prevalence of less than one per 1,000 to more than six per 1,000 children (Centers for Disease Control and Prevention [CDC], 2007). Focused research efforts and an intense search for genes associated with autism have not identified a gene with a large effect, but linkage scans have identified some overlap of regions harboring many genes with some related to the central nervous system (Muhle, Trentcoste, & Rapin, 2004). Proposals (Herbert et al., 2006) and studies (Hertz-Picciotto et al., 2006; Hu, Frank, Heine, & Lee, 2006) have recently incorporated the evaluation of environmental influences, which has redirected efforts to address environmentally sensitive genes (Nickerson et al., 2005). The Hu et al. study of gene expression was based on only five monozygotic twins discordant for autism. This approach of using highly

informative cases can be extended using the systems biology approach to track perturbations, as described by Hood, Heath, Phelps, & Lin, (2004), related to regression of autism. Longitudinal samples obtained within the Study will allow investigators to evaluate cases using RNA from serial samples taken on the same child during normal development and after the regression of autism occurs. The CHARGE study described by Hertz-Picciotto et al. is a large, ongoing case-control study of 500 autism cases focusing on chemical and biological exposures. Using a nested case-control approach, the NCS can be used to identify genes associated with autism conditional upon environmental factors (i.e., chemical and biological exposures) that emerge in the CHARGE study and other current studies.

3.1 Public Health Importance

Prevalence/incidence

Major depressive disorder affects as many as 2.5 percent of children and 8.3 percent of adolescents, an age group in which the female-to-male ratio of depression is 2:1 (Birmaher et al., 1996). On a commentary on the mental illness epidemic among teens, Friedman noted that in 2005, the CDC estimated 17 percent of U.S. high school students seriously considered suicide and 8 percent attempted suicide at least once during the preceding year (Friedman, 2006). Physical violence also is a serious problem in adolescents. In 2003, 17 percent of American high school students carried a weapon (gun, knife, or club) and 33 percent were involved in a physical fight (CDC, 2004). The Study provides an opportunity to examine etiological factors and gene-environment interactions in the development of psychosocial problems, such as depression and violent behavior.

Childhood onset disorders such as autism and ADHD also offer opportunities for the investigation of gene-environment interactions. The prevalence of autism is low, but its recognition and perhaps even prevalence has been increasing. The 2002 prevalence estimate from the Autism and Developmental Disabilities Monitoring Network indicates autism now affects about 1 out of every 150 children in the United States (CDC, 2007). The prevalence of ADHD is much higher, and its recognition and treatment have been increasing for decades (Swanson, Lerner, & Williams, 1995; Swanson et al., 2007). An estimated 8.7 percent (7.3-10.1 percent) of U.S. children aged 8 to 15 years meet DSM-IV criteria for ADHD, and almost 15 percent of 10-year-old boys and 5 percent of 10-year-old girls carry this label (CDC, 2005).

Economic and/or social burden

According to the Global Burden of Disease Study, depression is the fourth most important cause of death and disability (Murray & Lopez, 1997; Holden, 2000). The worst outcome of depression is suicide, which accounts for 6.8 percent of all deaths in 10- to 14-year-olds and 11.9 percent of deaths in 15- to 19-year-olds (Arias, MacDorman, Strobino, & Guyer, 2003). The annual cost of depression in the United States ranged between $43.7 billion and $52.9 billion in 1990. Adjusted for inflation, this estimate would be close to $70 billion today (Greenberg, Leong, Birnbaum, & Robinson, 2003). Due to its high prevalence, the total cost attributed to ADHD is high. A recent analysis estimated the societal cost for ADHD in childhood and adolescence is between $36 billion and $52 billion annually. Autism, which has a low prevalence, also has significant economic impact and social burden. This has been recognized by special NIH programs that focus on the causes of autism and legislation that gives priority for investigations in this area.

3.2 Justification for a Large Prospective Longitudinal Study

A prospective study is required because the exposures in question precede the outcomes and because these exposures influence gene expression at different time points. The prospective design also provides more accurate and timely exposure measurements relative to subclinical and clinical outcomes.

A longitudinal design is required because it is important to trace the development of the cohort through childhood and adolescence to measure the neurobehavioral outcomes of relevance. Several reports from smaller cohorts have indicated childhood antecedents (or perhaps risk factors) can predate symptom development by long periods. In addition, the interactions under investigation require data on prenatal and early childhood exposures.

Gene expression is subject to multiple environmental influences: social connections (Meany, 2001), nutrition (Waterland & Jirtle, 2003), and toxic exposures (Jacobson, 2006; Braun, Kahn, Froelich, Auinger, & Lamphear, 2006). A large cohort is needed to provide enough power for the candidate gene approach to studying these neurobehavioral outcomes while accounting for multiple environmental influences and interactions. The use of a large cohort enables the examination of gene-environment and gene-gene interactions.

3.3 Scientific Merit

Animal research on genes associated with behavior and on the modification of gene expression by psychosocial factors has been accumulating for a number of years. This research covers a fairly broad range of factors from physiological risk to behavioral traits and has emphasized the extent to which the functional importance of a gene is determined by factors which influence expression. Intracellular and extracellular environments play an important role not only in the magnitude of expression but in the direction of expression (i.e., whether it is up- or down-regulated) (Meaney, 2001). The intracellular environment is a function not only of its genetic components but also of influences from the extracellular factors such as hormones, neurotransmitters, cytokines, and nutrients. Variation in these constituents can determine how and when a gene is functionally expressed. Psychosocial factors are involved in these interactions through their influence on hormones and neurotransmitters. This can be illustrated by the influence of stress on transcription factors. Glucocorticoids such as cortisol are part of the hormonal response to stress.

The transcription factors c-jun and c-fos are sequence-specific DNA-binding proteins that bind to DNA in a multiprotein complex that controls cell proliferation and growth. These transcription factors are extremely responsive to extracellular stimuli and have been shown to influence glucocorticoid receptor-induced transcription of proliferin, causing it to increase or decrease according to their presence together or alone in the surrounding environment (Diamond, Miner, Yoshinaga, & Yamamoto, 1990). Research in rats has demonstrated immobilization stress (physically restraining the animal) upregulates both of these transcription factors in endothelial, myocardial, and smooth muscle cells of coronary vessels (Ueyama, Yoshida, & Senba, 1999). Similarly, it has been demonstrated that immobilization stress influences gene expression in the hippocampus in rats, causing increased expression of corticotropin-releasing hormone mRNA and a decrease in 5-HT1_a mRNA levels in the dentate gyrus (Givalois, Arancibia, & Tapia-Arancibia, 2000; Lopez, Lierzon, Vazquez, Young, & Watson, 1999). These latter data complement other research that has demonstrated serotonin polymorphisms differentially influence heart rate and blood pressure responsivity to stress (Barr et al., 2003) by showing stress also influences the functional expression of serotonin polymorphisms. Similar interactions have been demonstrated for the alpha$_2$-adrenoceptor variants, which influence the magnitude of stress responsivity. Animal research shows the functional expression of this gene is itself influenced by stress (Meyer et al., 2000). In addition, stress-induced expression of corticosteroid receptors has been demonstrated to vary by gender

(Karandrea, Kittas, & Kitraki, 2000). Together, these data illustrate the complexity and ongoing nature of gene-environment interactions, emphasizing that "genes or environment" is not a meaningful question. The presence of a linked allele at a locus is an indication of risk, but it is not enough to predict functional expression. Understanding function requires knowledge of the factors that influence when and in what direction it is expressed.

The Study's core hypothesis addressing the impact of maternal stress during pregnancy and risk of asthma states that excessive maternal psychosocial stress during pregnancy, in conjunction with maternal and fetal genetic susceptibilities, is reflected in specific measures of biologic function and results in an altered trajectory of fetal growth and development. During a Study-related workshop centered on this hypothesis, one of the mechanisms postulated for this influence was gene-environment interactions associated with gene expression (Gluckman et al., 2005; Gillman et al., 2006). Research on nonhuman primates has demonstrated the influence of mothering on gene expression. Research in Rhesus monkeys has demonstrated a short form of the serotonin transporter (rh5-HTTLPR) gene is associated with drinking alcohol to excess in monkeys reared in an environment with same-age peers and no mother, indicating a genetic link to alcohol abuse (Barr et al., 2003). However, monkeys with this same genotype reared together with their mothers actually consume less alcohol, indicating this same polymorphism under different circumstances confers a protective effect. These data further illustrate that knowing the genotype is not sufficient for predicting phenotypic expression without additional knowledge of the psychosocial environment.

Many diseases of interest to the NCS have complex etiologies and pathogenesis involving multiple genes and environmental factors. The disease prevalence varies from very common (e.g., 8.7 percent for ADHD) to relatively rare (e.g., 0.6 percent for autism). The risk alleles also vary from common (e.g., 0.7 for the DAT 10 repeat allele) to relatively rare. Thus, the common variant-common disorder model as well as the rare variant-common disorder model must be considered. Linkage and association studies are the primary tools used to study the role of genes and environment on disease occurrence. The main difference, in a general sense, between association and linkage studies is related to the sampling method. Association studies use the candidate gene approach, where association can be tested in populations of unrelated individuals. Linkage studies differ from association studies in that they require the recruitment of related individuals in a family. Both approaches have advantages and disadvantages, but most experts agree that linkage studies are good for identifying new genes while association methods are good for testing known ones. The opportunity to collect exposure and genetic information on parents of the children, and perhaps even on some grandparents, offers an opportunity for the Study to use linkage methods as well as association methods in studying gene-environment interactions on behavior.

The Study provides a unique opportunity to study candidate genes for disorders such as depression and antisocial behaviors, ADHD and autism, and psychosocial and environmental influences that interact with these genetic factors. As noted by Moffitt, Caspi, and Rutter (2005), incorporating these psychosocial and environmental influences may help researchers identify candidate genes for these disorders. In several studies, genotype was unrelated to disease phenotype when examined in the full cohort; however, significant genetic associations were revealed when the sample was stratified by exposure to an environmental factor. Such gene-environment interactions also provide an explanation for the inability to replicate genetic associations across studies. If an environmental exposure is a key trigger of a genetic effect, then variations in environmental exposure rates from one sample to the next could lead to inconsistent findings. The Study's detailed exposure assessments throughout the prenatal period and early childhood will be critical for illuminating environmentally contingent genetic effects and for clarifying the inconsistent results previously reported.

The Environmental Genome Project (EGP) (Goehl, 2005) has focused on genes sensitive to environmental factors that also meet other acknowledged criteria, such as variation in a coding region or evolutionary origin by conservation or selection (Nickerson et al., 2005). In the investigation of autism, the overlap in chromosome regions implicated by linkage studies and from the EGP have been identified (Herbert et al., 2006). Some of these genes are related to gastrointestinal and immune abnormalities that have systemic impact and are environmentally responsive. These genes and others related to the central nervous system (e.g., the serotonin transporter gene) will be evaluated in the Study. The polymorphism in the DRD4 gene has two characteristics used by the EGP to target genes of special interest: functional variation and signs of positive selection (Nickerson et al., 2005). The functional significance of the DRD4 risk-related polymorphism has been investigated by multiple groups. The initial unexpected results found the "risk" allele of the DRD4 polymorphism apparently has protective effects instead of conferring risk, and the authors suggested the subgroup of ADHD children with a 7R allele present had a partial syndrome characterized by behavioral excesses without cognitive deficits while the 7R-absent subgroup have the full syndrome characterized by both behavioral excesses and cognitive deficits (Swanson et al., 2000). Several independent groups have reported similar results (Manor et al., 2002; Langley et al., 2004; Bellgrove et al., 2005), which support functional variation with theoretical positive selection in the DRD4 polymorphism.

3.4 Potential for Innovative Research

This hypothesis has high potential for innovative research. Complex behaviors such as aggression, substance abuse, depression, schizophrenia, ADHD, and autism have defied attempts at simple genetic or environmental explanations. For example, the recent report on merged cohorts to evaluate autism made this point with the discovery of a new chromosome region of interest in the large sample not implicated in the multiple smaller studies (Autism Genome Project Consortium, 2007). In fact, even in areas where genetic and environmental main effects have been documented (e.g., ADHD), the emphasis now is on gene-environment effects that may account for inconsistencies or discrepancies in results across studies based on single sites or small samples.

Although there are a number of candidate genes and a number of environmental factors associated with these outcomes, none of them alone explain enough variance to suffice as targets for intervention. Complexity introduced by gene-environment and gene-gene interactions may require an approach to predict outcomes or responses in subgroups rather than to address single causes of disorders (Clark, Boerwinkle, Hixson, & Sing, 2005). The Study enables the measurement and timing of early exposures and the tracking of neurobehavioral outcomes throughout childhood and adolescence, thus providing an unprecedented opportunity to investigate gene-environment interactions.

3.5 Feasibility

This study is feasible. The size of the study provides the power for genome-wide scans of association for many childhood onset psychiatric diagnoses, including ADHD (about 5,000 cases are expected to be identified by the age of 7 years) and autism (in the Study sample of 100,000, about 700 cases are expected to be identified by the age of 3 to 5 years). These cases can be matched with nonaffected (2:1) or clinical controls (e.g., with nonautistic developmentally disabled cases) for prospective nested case-control studies. The prospective nature of the Study avoids many of the problems of retrospective case-control studies (recall bias, etc.) (Manolio, Bailey-Wilson, & Collins, 2006). In addition, if disorders are partially dependent on early developmental origins related to adverse exposures of psychological, chemical, biological, and physical factors during fetal and early childhood stages of development, then the birth cohort design of the Study is superior (Willett et al., 2007). In addition to association studies based on the prospective, nested case-control design made possible by the Study birth cohort, the opportunity to collect information on family members offers opportunities for linkage studies.

Because of the currently available molecular techniques and the number of environmental exposures to be measured in this study, investigation of gene-environment interactions is not only feasible, it can be done at reasonable cost with high benefit to the Study.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Exposure to psychosocial stressors during pregnancy:
  - Parental depression, stress, life events
  - Social support
  - Financial strain
- Exposure to smoking and alcohol during pregnancy
  - Maternal genotype and detoxification: GSTT1, NAT2, ADH2
- Genotype: Examples include 5-HTTLPR, DAT VNTR, and COMT
- Parental antisocial behaviors, psychopathology

Primary/child

- Genotype: Examples include 5-HTTLPR, MAOA, DAT, and COMT
- Psychosocial environment
  - Salivary cortisol at 6 months, 1 year, 2 years, 3 years
  - Parenting style: Nurturance, discipline techniques
  - History of child neglect and abuse: Records from child protective services
  - Quantitative and qualitative home environment assessments (e.g., the HOME inventory)

4.2 Methods

Primary/maternal

- Questionnaire
- Clinical diagnosis
- Blood for genotyping (appropriately collected and stored)

Primary/child

- Blood for genotyping (appropriately collected and stored)
- Salivary cortisol at 6 months, 1 year, 2 years, 3 years
- Parenting style: Nurturance, discipline techniques
- Measure of depression in parents
- Measure(s) of child abuse: Parental discipline style, records from child protective services

4.3 Life Stage

Primary/maternal

- Pregnancy

Primary/child

- First 3 years and beyond

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- ADHD
- Autism
- Depression
- Schizophrenia (adolescents)
- Antisocial behavior
- Other psychosocial and neurobehavioral outcomes

5.2 Methods

- DSM-IV based assessments for:
  - Depression
  - Antisocial personality disorder

- Conduct disorder
- Oppositional defiant disorder

- ADHD
- Autism
- Anxiety disorder
- Convictions for violent crimes
- Medical records review provided from parents
- School record review
- Observations of family/friends

5.3 Life Stage

- 3-5 years (disruptive behaviors), 6-12 (ADHD, oppositional defiant disorder, depression), preadolescence and adolescence (depression, conduct disorder)

6. Important Confounders, Mediators, and Effect Modifiers

- **Smoking:** Nicotine induced c-fos mRNA expression was found in several brain areas associated with cognitive function in rats. This may be associated with development of schizophrenia in adults.
- **Postnatal parental depression:** Parental depression may influence child depression and other behavior.
- **Prenatal infection:** Prenatal infection may be related to neurodevelopmental disorders (see other hypotheses) and will need to be controlled for the analysis.

7. Power and Sample Size

For any condition or disorder with a given prevalence at a given assessment point (or age of the cohort), the power to detect associated genes depends on the risk allele proportion. This is especially important for the investigation of hypotheses about common variants in common disorders. The high prevalence of disorders such as depression, anxiety, and ADHD ensure high statistical power, especially for evaluation of risk alleles that also have high expected frequencies in the population (i.e., common variants). The power to detect gene-environment and gene-gene interactions can be calculated (Gauderman, 2002a; Gauderman & Morrison, 2006), and for the combination of prevalence, allele frequency, and cases expected for the high-frequency conditions, the power provided by the Study is adequate to address multiple gene-environment and gene-gene interactions. Examples will be provided for depression, antisocial behavior, ADHD, and autism.

For depression with an expected population prevalence of 2.5 percent of children and 8.3 percent of adolescents, a hypothesized gene (serotonin transporter gene) and environment (stress) interaction was evaluated for three variants of the risk allele, three levels of stress, 0.05 probability and 80 percent power. The frequency of the "l" form of the serotonin transporter gene is about 60 percent (50-60 percent in Caucasians and close to 70 percent in African Americans). Therefore the gene frequency is 0.36 for the "ll" variant, 0.48 for the "ls" variant and 0.16 for the "ss" variant. To achieve 80 percent power, the total number of subjects required is 47,920.

For antisocial behavior (aggression), the population prevalence among adolescents ranges from 17-33 percent depending on the specific measure. A hypothesized gene (MAOA) and environment (child abuse) interaction was evaluated by considering the two most common VNTR polymorphisms, the three-repeat variant with low activity and the four-repeat variant with high activity. These alleles have a population prevalence of about 33 percent and 62 percent, respectively (Caspi et al., 2003). Child abuse was cited as 8 percent severe, 28 percent probable abuse, and 64 percent with no abuse. Power analyses based on a prevalence of 5 percent in the unexposed population (conservative according to Caspi, et al., 2003) result in a required sample size of 31,650.

For ADHD with a population prevalence of 5 percent by age 7 years, the gene (DAT) and environment (maternal smoking) interaction was evaluated by considering the homozygote genotype for the 10 repeat allele of the 40 bp VNTR, which has a population prevalence of about 50 percent. Maternal smoking was estimated to be about 20 percent. Given the prevalence of exposure and outcome discussed above, the minimum detectable odds ratio, based on 80 percent power and a 5 percent type I error is 1.27.

8. Other Design Issues

- **Ethical/burden considerations:** Human subjects issues associated with observation of certain psychosocial stressors (e.g., abuse) must be carefully addressed in the study protocol, with any planned remedial actions approved by appropriate institutional review boards.

- **Cost/complexity of data collection:** The longitudinal design is required because it is important to trace the development of the cohort through childhood and adolescence in order to measure not only the exposures but also the neurobehavioral outcomes of relevance (e.g., depression, substance abuse, antisocial behavior). Several reports from smaller cohorts have indicated childhood antecedents (or perhaps risk factors) predate symptom development by long periods.

9. References

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PRENATAL AND PERINATAL INFECTION AND SCHIZOPHRENIA

1. Meta Hypothesis

Prenatal infection and mediators of inflammation during pregnancy and the perinatal period are associated with increased risk of schizophrenia.

2. Specific Hypotheses

1. A child born to a mother experiencing an infection of toxoplasa, rubella, or other known pathogens during the first 22 weeks of gestation has an increased risk for developing schizophrenia in adulthood.

    1.1 Mothers of low socioeconomic status (SES) vs. mothers of a higher SES who experience a prenatal or perinatal infection during pregnancy will have children with an increased risk for schizophrenia as adults.

    1.2 Consuming a diet high in vitamins and minerals (i.e, folate, vitamin A, vitamin D) that are known to influence immune function and fetal development during pregnancy will reduce the affect of a mother experiencing a prenatal or perinatal infection(s) and having a child that will be at an increased risk for developing schizophrenia in adulthood.

2. The presence of specific genetic factors, such as candidate receptor genes 5-HT2a, D3, and NMDA, will increase the likelihood that a child born to a mother experiencing a prenatal or perinatal infection will develop schizophrenia in adulthood.

3. Background and Justification

Schizophrenia is a severe psychiatric disorder typically appearing in late adolescence or early adulthood. It is associated with significant long-term morbidity, occupational disability, social disadvantage, and high mortality from suicide and other causes. The burden of the disease extends to the family for whom there are major economic and social implications. Converging evidence suggests that many cases of schizophrenia are neurodevelopmental in origin, that both genes and environment play a role in the etiology of these cases, and that exposures in early gestation, in particular, infection and nutritional deficiency, may be linked to schizophrenia, (Cannon, Jones, Susser, van Os, & Murray, 2002; Susser, Brown, & Gorman, 1999).

While infectious agents have been suspected of increasing the risk of schizophrenia, their role has not been well established (Bromet & Fennig 1999; Buka, Tsuang, Torrey, Klebanoff, Bernstein, et al., 2001; Buka, Tsuang, Torrey, Klebanoff, Wagner, et al., 2001). Herpes simplex viruses (HSV) are known to cause encephalitis in infants (Corey, Whitley, Stone, & Mohan, 1988), thus latent effects of less severe infection are biologically plausible. In recent data from long-term follow-up of participants in the U.S. Collaborative Perinatal Project (a longitudinal study conducted in the 1960s), maternal serum immunoglobulins (IgG and IgM) obtained during pregnancy were associated with increased risk of psychosis in offspring (Buka, Tsuang, Torrey, Klebanoff, Bernstein, et al., 2001). However, others have not found an increased risk of schizophrenia spectrum disorders associated with IgG antibody to herpes simplex virus type 2 (Brown, Schaefer, Quesenberry, Shen, & Susser, 2006). Conflicting results among studies may be due to differences in outcome (psychosis vs. schizophrenia spectrum disorders), varying effects of exposure depending upon nutritional status of the mother, timing of infection during gestation,

or chance findings due to small numbers of cases. For example, risk for schizophrenia was increased seven-fold for influenza exposure during the first trimester, but no increased risk was observed if influenza occurred during the second or third trimester (Brown et al., 2004). HSV is just one of many infectious agents, including respiratory infections (Brown et al., 2004; Brown et al., 2001), rubella, and perhaps exposure to toxoplasmosis (Brown et al., 2006), that have been linked with risk for schizophrenia in offspring. Not only is prenatal infection important, it is possible that exposure to infections during conception is also relevant. Exposure to genital/reproductive infections during the periconception period, which has been cited to occur in 4.2 percent of pregnancies, has been associated with a five-fold increase in the risk of schizophrenia in adults (Babulas, Factor-Litvak, Goetz, Schaefer, & Brown, 2005).

Prenatal infection can either contribute directly to adverse effects on the developing brain or indirectly by increasing pro-inflammatory cytokines (Cai, Lin, Pang, & Rhoades, 2004). Cytokine polymorphisms have been identified (i.e., tumor necrosis factor-a [TNF-a] and mannose-binding lectin [MBL]) and are associated with a greater immune response to infection (Gibson et al., 2006). Whether the presence of these polymorphisms increases risk of schizophrenia with exposure to prenatal infection has not been studied in humans, although animal studies support this hypothesis (Ashdown et al., 2006). This is an example of a type of gene-by-environment interaction that may be tested within the context of the National Children's Study (NCS), and it is expected that during the next several decades specific candidate genes will be more clearly implicated in the risk to schizophrenia or with risk factors associated with schizophrenia.

3.1 Public Health Importance

Prevalence/incidence

Infections of many different types are common in the U.S. population. More importantly, some types of infections are more prone to occur during pregnancy. For example, one out of five adolescents nationwide, and similarly in adults ages 21 and older, have had a genital HSV infection (Centers for Disease Control and Prevention [CDC], 2004). Antibodies to HSV-2 have been detected in approximately 20 percent of pregnant women, although only 5 percent report a history of symptomatic infection (Brown, 1998). Physical changes during pregnancy can also incur vulnerability to respiratory disorders. Pregnant women in their second and third trimesters of pregnancy have a greater risk of influenza-related morbidity compared to nonpregnant and postpartum women (CDC, 2004). Additionally, about 1-2 percent of term pregnancies are affected by chorioamnionitis (intrauterine infection). In pregnancies ending in preterm births, the prevalence of such infection is higher (Wu & Colford, 2000). Periodontal disease is also a source of infection during pregnancy and may be associated with adverse pregnancy outcomes due to metastatic spread of infection, metastatic injury from circulating microbial toxins, or metastatic inflammation induced by microorganisms (Li, Kolltveit, Tronstad, & Olsen, 2000).

Schizophrenia is a chronic, severe, and disabling disorder that affects approximately 1 percent of people worldwide. The schizophrenia incidence rates per 10,000 person-years for males ages 15-19 is 9.4, for 20-24 is 5.6, and for 25-29 is 3.3. The incidence rates for females in the same age groups are 1.5, 1.3, and 4.1, respectively (Bresnahan et al., 2000). The cumulative risk for schizophrenia by age 38 was 0.93 percent for men and 0.35 percent for women.

Economic and/or social burden

Annual costs of schizophrenia in the United States were recently estimated at $62.7 billion (Wu et al., 2005), with a substantial portion due to disease in young adults (Genduso & Haley, 1997). Pharmacological treatment for schizophrenia can relieve many of the disorder's symptoms, but most individuals with schizophrenia live with residual symptoms for many years after diagnosis. About two

thirds of those who develop schizophrenia continue to be affected throughout adulthood (Bromet & Fennig 1999).

Individuals with schizophrenia and other mental illnesses are at increased risk for cardiovascular disease due to high rates of smoking, obesity, diabetes, and hypertriglycerdemia. Substance abuse and high-risk sexual behavior (which may partially explain the observed higher rates of HIV and infectious hepatitis in individuals with schizophrenia) also are increased in schizophrenia (Lambert, Velakoulis, & Pantelis, 2003; Goff et al., 2005).

3.2 Justification for a Large Prospective Longitudinal Study

Investigation of the prenatal origins of schizophrenia requires a large prospective cohort followed from early gestation through adulthood.

A prospective study is required to obtain precise prenatal exposure data. For the exposures of interest (e.g., prenatal infection), biological specimens are essential for precise measurement and timing. Serum and placental specimens can be collected prospectively but stored for later analysis. To conserve specimens and reduce cost, a nested case-control design will be employed.

The longitudinal design is required because it is important to trace the development of the cohort through childhood and adolescence, before the onset of schizophrenia. Several reports from smaller cohorts have indicated that risk factors occurring during childhood can predate a formal diagnosis by decades (Cannon et al., 2002). In addition, while prenatal exposures may play an important role in schizophrenia, this by no means precludes an important role for postnatal experience. Indeed, some reports have suggested that adverse childhood experiences contribute to increased risk of schizophrenia.

A large cohort is required to study this relatively infrequent outcome. As noted above, earlier studies with good prenatal data have suggested relationships of prenatal exposures to the outcome of schizophrenia, but their interpretation is limited by small numbers.

A large cohort is virtually a precondition for extending this field to investigate several types of hypothesized interactions, including gene-environment and nutrition-by-infection interactions.

There are many potential confounders, mediators, and effect modifiers, such as the presence of other developmental disabilities (ADHD, autism); race; SES; postnatal infection and trauma; and low birth weight. A longitudinal study design can consider these potential multiple interactions, inform temporality, and support causality.

3.3 Scientific Merit

The "neurodevelopmental theory" proposes that the neural basis for schizophrenia originates in prenatal brain development that begins long before any clinical symptoms are apparent, and is caused by the combination of genetic and environmental effects (Cardno et al., 1999; Singh, McDonald, Murphy, & O'Reilly, 2004). Findings in schizophrenic patients suggest that early harbingers of schizophrenia include minor physical anomalies (Waddington, 1990; Rosso et al., 2000) and motor (Walker et al., 1994), cognitive (Comblatt et al., 1999; Cannon et al., 2002, Davidson et al., 1999, Kremen, 1998), and social (Jones, Rodgers, Murray & Marmot, 1994; Done, Crow, Johnston, & Sacker, 1994; Olin & Mednick, 1996) disturbances in childhood. By onset, schizophrenia is associated with ventricular enlargement; decreased total brain, gray, and white matter volumes (Lawrie et al., 1998; Wright et al., 2000); and decreased hippocampal (Nelson, Saykin, Flashman, & Riordan, 1998), thalamus (Konick & Friedman 2001), and frontal lobe (Davidson & Heinrichs, 2003) volumes. Definitive evidence for an

environmental prenatal exposure that increases risk of schizophrenia is necessary. By providing such evidence or refuting the hypothesis, the Study will contribute in a unique and fundamental way to our understanding of the etiology of schizophrenia.

3.4 Potential for Innovative Research

The technologies used in the direct and indirect assessment of relevant prenatal exposures will include serologic analyses and placental pathology. These methods, which are common to many of the research hypotheses addressed in the study, are exceptionally useful in the context of the present hypothesis. Each provides a window on gestational experience, and holds the potential to improve the knowledge of the timing and mechanisms of exposures adversely influencing neurodevelopment and elevating risk of schizophrenia.

- **Serum samples:** In addition to basic serologic analyses for specific candidate infections (e.g., influenza, HSV-2), high throughput methods will be used to detect viral nucleic acids, antiviral antibodies, and host factors. This will permit the simultaneous measurement of multiple analytes using small amounts of sera.

- **Placenta:** Placental data can be used to indicate nutritional, infectious, toxic, and other exposures. Novel technologies of digital image capture for gross assessment of placenta and image analysis will standardize gross measures and extend what can be measured in the placenta. These advances will permit us to assess potential mechanisms for the influence of adverse exposures.

Taken together, these measures will provide unprecedented access to early gestational life and address the core of the neurodevelopmental hypothesis.

3.5 Feasibility

Due to the longitudinal nature of the study, these hypotheses can be addressed. A minimum of three visits during pregnancy will be required to investigate longitudinal changes, or trajectories, in infection and subsequent immune responses. Direct assessment of infection during pregnancy is optimal. Clinical visits and medical record abstraction will also yield valuable information. The large sample size of the study provides adequate power to detect any associations between infectious agents during pregnancy and later manifestation of schizophrenia symptoms in offspring. Using the widely cited rate of 1 percent in the population, approximately 1,000 cases of schizophrenia can be potentially identified throughout the course of the study. Prospective, nested case-control studies can be used to compare cases to controls for which the study provides a richly diverse sample to ensure adequate matching.

4. Exposure Measures

The central interest of this hypothesis is in the periconception environment and prenatal development. Early gestation is a time of unique vulnerability to the consequences of environmental insult. There is evidence that both infectious and nutritional exposures during this period profoundly affect brain development. From the perspective of exposure measurement, early gestation is therefore of critical interest in the work proposed here.

4.1 Individuals Targeted for Measurement

Primary/maternal

Maternal infection/inflammation:

- Infection serology (lymphocytes, antibodies, cytokines/interleukins, inflammatory markers)
- Blood for viral nucleic acids. The specific type of viral infection may modify the risk of neurological disorders.
- Cultures/vaginal swabs
- Medical histories of fevers and infections (medicine usage)
- Assessment of dental health via interviews for potential periodontal disease before and after pregnancy

Secondary/maternal

- Retrospective medical record review

Primary/child

Prenatal infection/inflammation:

- Infection serology (lymphocytes, antibodies, cytokines/interleukins, inflammatory markers)
- Umbilical cord (antibodies, cytokines, viral nucleic acids)
- Placental pathology

4.2 Methods

Primary/maternal

- Blood samples
- Vaginal/cervical cultures
- Examinations by a medical professional
- Interviews

Secondary/maternal

- Medical and obstetrical history
- Family history

Primary/child

- Umbilical cord blood culture/pathology
- Amniotic fluid analysis, if available

4.3 Life Stage

Primary/maternal

- Repeated measures from first through third trimesters and birth

Secondary/maternal

- Enrollment, all trimesters, follow-up after birth at predetermined intervals (for family history)

Primary/child

- Perinatal period and throughout the course of the study

5. Outcome Measures

From the perspective of outcome, we are interested in childhood markers of, and risk factors for, schizophrenia. Therefore, specific postnatal assessments will be periodically conducted during childhood and adolescence. Our interest lies in the trajectory of cognitive, language, motor, sensory, and psychological and social development, as well as the appearance of specific psychiatric symptoms over time. Although the median age of onset is in the early to mid-20s for men and late 20s for women (American Psychiatric Association, 1994), screening for schizophrenia should begin in early adolescence to capture any early onset cases and detection of subclinical schizophrenic traits.

5.1 Outcomes Targeted for Measurement in Child

- Schizophrenia screening and diagnosis: Standardized screening and diagnostic instruments, e.g., Schizotypal Personality Questionnaire (screening) and KID-SCID or SCID for diagnosis at ages 18-21 or the NIMH Diagnostic Interview Schedule for Children, will be administered in a face-to-face diagnostic interview. These instruments have been extensively tested. However, one must consider the possibility that during the next several decades, the state-of-the-art diagnostic interview may require use of an instrument's version that is not currently well-validated, but will be in the future, or an instrument that may not yet be developed.

5.2 Methods

- Examination by a medical professional
- Screening and diagnostic instruments for schizophrenia spectrum disorders, social function, neurological, and psychological testing

5.3 Life Stage

Primary/child

- Screening and diagnostic instruments throughout the course of the study at predetermined intervals when the child is at an appropriate age for specified instruments

Secondary/parental

- Screening and diagnostic instruments with parental respondent throughout the course of the study (when instruments are not suitable for direct assessment of younger children)

6. Important Confounders, Mediators, and Effect Modifiers

- **Age:** Schizophrenia typically manifests itself in late adolescence or early adulthood. Men tend to have earlier onset (early to mid 20s) than women (late 20s). Early childhood onset may have unique risks (Nurnberger et al, 1994).
- **Socioeconomic status, race, ethnicity status:** Individuals with schizophrenia tend to be of a lower socioeconomic class. However, like many mental illnesses, this may be that the symptoms of the disease themselves cause a drift into a lower class.
- **Paternal and maternal age:** Paternal age at the time of birth may be a risk factor for schizophrenia (Malaspina et al., 2001).
- **Stress:** Stress may trigger disease development. Maternal unwantedness of pregnancy has been found to be a risk factor for the development of adult schizophrenia (Herman et al., 2006). Indicators of maternal stress (cortisol) will be assessed during pregnancy.
- **Residential environment:** Residential environment is associated with risk of schizophrenia. Most studies consistently report a positive association with urban birth (Lewis, David, Andreasson, & Allenbeck, 1992; Marcelis, Navarro-Mateu, Murray, Selten & van Os, 1998; Mortensen et al., 1999) and rural living younger than age 16 is negatively correlated with the incidence of schizophrenia (Kendler, Gallagher, Abelson, & Kessler, 1996). However, more research is needed to determine the underlying factors that may account for this association (toxic exposures, stress, diet, selective migration) (McGrath & Scott, 2006; Pedersen & Mortensen, 2006).

- **Smoking status:** Rates of smoking among schizophrenics are about 66-88 percent (Herman et al., 2006), two to three times that of the general population. Smoking usually starts around adolescence prior to the average age of diagnosis (Hughes, Hatsukami, Mitchell, & Dahlgren, 1986).

- **Systematic infections:** There is evidence that children with postnatal systematic infections, particularly central nervous system infections, are 4.8 more times as likely to develop schizophrenia (Rentakallio, Jones, Moring, & Von Wendt, 1997).

- **Vaccinations:** Maternal vaccinations may be a plausible option for prevention of schizophrenia by reducing the rate of prenatal infection (McGrath, 2000).

- **Nutrition and diet:** A Finnish study hypothesized that poor nutrition, but not lower caloric intake, in utero and in early childhood is an increased risk factor for schizophrenia (Wahlbeck, Forsen, Osmond, Barker, & Eriksson, 2001). Associations have been reported between schizophrenia and maternal homocysteine concentrations suggesting that folate status during pregnancy may be important (Brown et al., 2007). Other investigators have suggested that low vitamin D status also may be a risk factor (McGrath, 2001).

- **Gestational age, weight, head circumference:** Schizophrenics are more likely to be premature and have a low birth weight (Jones, Rantakallio, Hartiainen, Isohanni, & Sipila, 1998). Certain prenatal infections also are associated with preterm birth.

- **Mother's medical and obstetrical histories:** Various obstetric complications are often cited as possible causes for fetal neurodevelopmental disruption and consequent disorders (Jones et al., 1998).

- **Maternal medication and drug use:** Illicit drugs taken during pregnancy, including marijuana, PCP, and methamphetamines, increase the risk of schizophrenia among the offspring (Kelly & McCreadie, 1999).

- **Environmental exposures:** Exposure to solvents (Perrin et al., 2006) and heavy metals such as lead (Opler et al., 2004) have been found to be associated with schizophrenia.

- **Family history of mental health:** Schizophrenia has been shown through family, sibling, and twin studies to have a strong genetic component (Cannon et al., 2002).

- **Maternal and infant DNA:** Genetic polymorphisms have been identified that influence the concentration of circulating cytokines in response to a viral infection (Gibson et al., 2006). Elevated cytokine concentrations can be neurotoxic to the fetus, therefore, the presence of specific cytokine polymorphisms or genes not yet identified may modify the risk of schizophrenia given exposure to infectious agents.

- **Social, language, motor, and cognitive development:** Deficits in social and cognitive development may increase risk for psychosis (Jones et al., 1994). Cognitive and achievement test scores are lower in pre-schizophrenic children compared to healthy children (Done, Crow, Johnston, & Sacker, 1994; Davidson et al., 1999). The severity of speech and language deficits may also be associated with the age of schizophrenia onset (Hollis, 1995). Declines in general intellectual function between

ages 4 to 7 may be specific to schizophrenia and associated spectrum disorders (Kremen et al., 1998).

- **Genetic risk factors as effect modifiers:** A number of candidate genes have been extensively studied as risk factors for schizophrenia. These include COMT, DTNBP, and NRG1. These susceptibility genes may modify the risk for schizophrenia incurred by perinatal and prenatal infection.

- **Premorbid psychopathology:** Some authors have suggested that schizophrenia is part of a psychopathic progression that begins during early childhood (Rutter, Kim-Cohen, & Maughan, 2006). The design and the data collected in the NCS will enable investigators to determine whether this continuum exists.

- **Migrant and minority populations:** A higher rate of schizophrenia has been observed in migrants compared to native-born populations (McGrath et al., 2004).

7. Power and Sample Size

Although nested case-control studies are planned within the study, for simplicity power is presented within this document for the entire cohort. The use of placental data and serum from early gestation (preferably first trimester) will reduce the effective sample size, thus we assume 80,000 participants with complete measures. We assume that the lifetime risk of the outcome is 0.01 (this risk will vary according to narrow or broad definitions of schizophrenia and schizophrenia spectrum disorders). For the present purposes, we have assessed the smallest detectable risk, varying the disease probability in the unexposed (appropriate to ascertainment at varying ages), and prevalence of exposure (dichotomized).

Table 1 The smallest detectable risks for a sample size of 80,000 and power of 80 percent and alpha = 0.05:

Smallest Detectable Relative Risk			
Prob (disease in unexposed)	Prevalence exposure = .05	Prevalence of exposure = .10	Prevalence exposure = .20
.003 (age 21)	RR = 1.9	RR = 1.6	RR = 1.4
.005 (age 30)	RR = 1.7	RR = 1.5	RR = 1.3
.009 (age 40)	RR = 1.5	RR = 1.3	RR = 1.2

For more common exposures such as influenza, the power of this study would be significantly higher. A recent study suggested, based on population attributable risk estimates, that up to 14 percent of schizophrenic cases would not occur if influenza exposure during early to mid-pregnancy had been prevented (Brown et al., 2004).

8. Other Design Issues

- While a simple examination of maternal antibody titers would advance science in this area, consideration of the timing of the infection in relation to birth would be useful. Multiple maternal blood samples during pregnancy will help determine the trimester that infection occurred and the immune response.

- Vaginal swabs for viral culture would enable an evaluation of the timing and rate of viral shedding in relation to subsequent schizophrenia.

- Ascertainment of schizophrenia among children born into the study will rely on reports of clinical diagnosis. However, for a more sensitive assessment of outcome, the study will employ screening questionnaires that specifically assess participants on a continuum of dysfunction. This will enable detection of early subclinical syndromes and a spectrum of related psychotic disorders (schizophreniform, etc.).

- Because schizophrenia is generally not diagnosed until late adolescence and through the early 20s, response and retention rates of the study participants will be important for assessing this hypothesis.

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