National Children's Study Assembly Meeting
Breakout Session: Exposures: Biological and Psychosocial
November 29, 2005
Omni Shoreham Hotel
Washington, DC

This meeting was held in conjunction with the National Children's Study, which is led by a consortium of federal agency partners: the U.S. Department of Health and Human Services (DHHS) (including the National Institute of Child Health and Human Development [NICHD] and the National Institute of Environmental Health Sciences [NIEHS], two parts of the National Institutes of Health, and the Centers for Disease Control and Prevention [CDC]), and the U.S. Environmental Protection Agency (EPA).

Co-Chair: Sarah S. Knox, Ph.D., Senior Scientist, National Children's Study Program Office, NICHD, NIH, DHHS

Co-Chair: Cynthia A. Moore, M.D., Ph.D., Medical Geneticist, Office of Genomics and Disease Prevention, CDC, DHHS

Invited Participant: George P. Daston, Ph.D., Miami Valley Laboratories, Proctor and Gamble; Member, Federal Advisory Committee

Dr. Knox explained that biological and psychosocial measures of exposure are important areas of the protocol of the National Children's Study (Study). Development of these measures for their incorporation into the Study's protocol has been proceeding for several years. Many groups and experts have provided valuable input into the protocol development, including the four lead government agencies (NICHD, NIEHS, CDC, and EPA), the ICC, and more than 3,000 individuals who have participated in numerous Study meetings and workshops. The protocol is still under development. Dr. Knox said that the purpose of this breakout session was to report on the status of protocol development with regard to measuring biological and psychosocial exposures.

Biological Measures of Exposure

Dr. Moore reviewed the use of biological measures of exposure in the Study. She focused her review on discussions of biological exposure assessment from two Study workshops and a smaller follow-up meeting:

- Collection and Use of Genetic Information in the National Children's Study, a workshop held September 8, 2004, in Washington, DC
- Measurement of Maternal and Fetal Infection and Inflammation, a workshop held May 20–21, 2004, in Linthicum, MD
- Assessment of Fetal Exposure to Infection and Inflammation, a meeting held August 22, 2005.

The workshop discussions included opportunities for use of biological measures of exposure, challenges and special considerations, and recommendations from the workshop participants. Dr. Moore commented that a unique opportunity exists in the Study to determine how environmental factors influence the health and development of children. Determining environmental influences requires consideration of potential uses of biological measures; collection and storage of biological materials in an appropriate manner; and awareness of the ethical, legal, and social implications of the use of this information.

Collection and Use of Genetic Information in the National Children's Study. This workshop brought together experts in the federal government (NIH, EPA, CDC, U.S. Food and Drug Administration) to explore opportunities and challenges and provide recommendations to the Study. The overall discussion focused on ensuring that biological samples are appropriately collected and stored to provide sufficient quality and quantity of genetic information to study health outcomes over time. Genetic information can be used to:

- Identify disease risk factors
  - Relation to disease susceptibility, severity, and prognosis; response to therapeutics; interaction with other risk factors
  - Focus of most genetic research to date
  - Requires germ line DNA
- Characterize disease outcomes
  - Using biomarkers to stratify study populations into more homogeneous groups
  - Current studies focused on various malignancies such as childhood leukemias
  - Assessed in DNA, RNA, and proteins
- Assess exposures to environmental agents
  - Changes in gene expression in response to, or changes in DNA structure as a result of exposure to an environmental agent
  - Limited research in infants or children
  - May be assessed in RNA, proteins, DNA.

Dr. Moore cited arsenic metabolism and polymorphisms in developmentally restricted arsenic (III) methyl-transferase as an example of DNA variation and relation to disease and health. She cited asthma phenotypes and beta2-adrenergic receptor polymorphisms as genetic studies to characterize outcomes. Dr. Moore cited (1) metallothionein gene expression and cadmium exposure and (2) carcinogen-DNA adducts in paired maternal and newborn blood samples as examples of genetic studies of exposure to environmental agents.

Recommendations from the workshop were:

- Specimens to be collected from the child
  - Cord blood collection (key)
  - Peripheral blood sampling in early and late childhood (key)
  - Blood spot in infancy (desirable)
- Family members to be sampled include mother, father, and, if possible, siblings enrolled in Study
- Flexibility desired to add other collections in certain situations such as acute, unpredicted exposures

- Essential to collect biological materials to study both genetic variation and gene expression
  - Collection, storage, and analytic approaches need to be reconsidered as studies are developed and new technologies become available
  - Planning should focus on collection of high quality biological specimens for genetic studies and storage of sample aliquots for genomic DNA, RNA, and protein; whole genome amplification; and cryopreservation.

Measurement of Maternal and Fetal Infection and Inflammation, and Assessment of Fetal Exposure to Infection and Inflammation. This workshop and follow-up meeting brought together experts to review the role of maternal and fetal infection and inflammation in perinatal and childhood outcomes such as cerebral palsy, autism, and preterm birth. The objectives of the workshops were to:

- Determine optimal and feasible measurements of maternal infection and inflammation
- Identify potential assessments of fetal infection and inflammation or fetal response to maternal infection and inflammation.

Three measurement strategies were identified:

- Before pregnancy—establish a baseline measure of a women's "inflammatory state" as well as capture evidence of past history of chronic infectious or inflammatory conditions
- During pregnancy—attempt to capture timing of maternal infection or inflammatory response
- Birth—identify maternal or fetal/infant infection at or shortly preceding birth, assess fetal exposure to variation in maternal temperature.

Selected measurements before pregnancy included:

- Questionnaire—assessment of prior, recurrent, or persistent infections (for example, urinary tract infections, sexually transmitted diseases)
- Vaginal swabs—gram stain for bacterial vaginosis, assessment of cytokines and "inflammatory profile"
- Urine—process and store for polymerase chain reaction (PCR) identification of specific organisms, proteomic analysis of additional markers
- Saliva—potential use for periodontal disease assessment
- Blood (serum or plasma)—immunoglobulins; "inflammatory profile" including general phase reactants
- Blood (cells)—T-cell subtypes for Th-type.

Selected measurements during pregnancy include:

- First trimester visit:
  - Questionnaire—history of infections, antibiotic use; assessment of elevations in body temperature
  - Vaginal swab, urine, blood, saliva—same collections as prepregnancy visit
- Second and third trimester visits:
  - Vaginal swab, urine, blood, saliva—same collections as pre-pregnancy visit
  - Physical exam—periodontal/dental assessment (once during pregnancy)
  - Selected measurements at birth.

Selected measurements at birth include:

- Chart review (mother, infant)—maternal temperature during labor, antibiotic therapy during pregnancy and labor and delivery, anesthesia received
- Maternal blood—storage (all inflammatory markers will be high due to labor, potential use for assessment of seroconversion)
- Cord blood (collected both in tubes and as blood spots to compare with infant samples)—"inflammatory profile," immunoglobulins, T-cell subtypes for Th-type, possible blood gas or pH
- Placenta—digital photo, block and hold for histology, possible PCR for infectious agents
- Infant blood spot—"inflammatory profile" and possible proteomics
- Colostrum—immunoglobulins and T-cells
- Other (if available)—amniotic fluid for "inflammatory profiles" and PCR, cerebrospinal fluid decidual material for inflammatory histology.

Recommendations from the Measurement of Maternal and Fetal Infection and Inflammation Workshop and the Assessment of Fetal Exposure to Infection and Inflammation meeting were:

- Important to use multiple data sources
- Imperative to make serial measurements
- Biological samples should be collected to maximize analytic flexibility as interest in specific infectious agents and inflammatory markers will likely evolve as planning progresses.

Challenges and Considerations for Biological Measures. Dr. Moore listed the following challenges and considerations for the collection of biological measures:

- Availability of biologic materials (quantity, stability, accessibility)
- Timing issues
  - Effects may be time limited (for example, gene expression in response to exposure; at different developmental stages)
  - Effects may not be time specific (for example, DNA adducts may persist over time)
  - Serial measurements often needed to capture timing and extent of exposure
  - Need to integrate collections for multiple purposes (for example, genetic studies, measurement of chemical blood levels)
- Some methods may need pilots (for example, use of filter paper for cytokine and inflammatory marker assessment)
- Technology to perform biological analyses and interest in specific research questions will likely evolve as planning progresses.

Psychosocial and Behavioral Measures of Exposure

Dr. Knox reviewed the role of behavioral and psychosocial factors in the Study. She noted that input to protocol development was from multiple sources, including 22 Working Groups, the Federal Consortium, pilot studies, Study Assembly meetings, Study workshops, literature reviews, and white papers.

Psychosocial and behavioral factors interact with each other and with other environmental, genetic, and behavioral factors to influence health and behavioral outcomes, from molecular to systemic levels. Dr. Knox said that psychosocial exposures could be conceptualized in three ways:

- Independent predictors
- Interactions with other environmental factors
- Gene-environment interactions.

The behavioral and psychosocial outcomes in the Study are complex and will be investigated from a perspective of multiple influences that vary in importance by developmental stages. Outcomes include:

- Psychiatric/mental health
- Cognitive
- Neurobehavioral
- Social/emotional health and development.

Dr. Knox provided an overview of the psychosocial and behavioral exposure domains, which include:

- Demographics
- Culture
- Family structure
- Family process
- Neighborhood factors
- Religion/spirituality
- Parental competencies
- Parenting practices
- Psychological stress
- Social support
- Public policies
- Child care
- School
- Diet
- Smoking, alcohol consumption, substance abuse
- Physical activity
- Cognitive stimulation

In assessing the psychosocial influences on health and development of children, the Study protocol will use the above factors in a multilevel interactive model that includes the individual, family, neighborhood, school, and community.

Independent predictors of health and development include neighborhood/community factors such as collective efficacy, media exposure, racism, family resources and process, and psychosocial stress, for which Dr. Knox gave the following definition:

> A feeling of distress experienced when demand exceeds an individual's ability to control what is happening in his or her life. Stress has consequences for physical health primarily when chronic, not acute.

Interactions with Other Environmental Factors. Dr. Knox noted that disparities in prevalence and severity of asthma by race and socioeconomic status are explained in part by psychosocial stress, effective management, and other health-related behaviors. These factors do not cause

asthma but contribute to increasing or decreasing its severity. Animal research indicates that maternal stress modulates the effects of maternal lead exposure in offspring.

Gene-Environment Interactions. As an example of the way in which psychosocial factors interact with genotype, Dr. Knox used the example of schizophrenia. She explained that, although a strong genetic etiology for schizophrenia is now generally accepted, adoption studies with children of schizophrenic mothers indicate that family environment modulates risk of manifest schizophrenia in offspring. Children at risk for schizophrenia are significantly less at risk for developing it if they are reared in a psychologically healthy home than if they are reared in a psychologically unhealthy home. Studies indicate that the risk of schizophrenia is also increased by famine and by prenatal infection.

The Children's Health Act of 2000 urged researchers to investigate basic mechanisms of developmental disorders and environmental factors, both risk and protective, that influence health and development processes. Dr. Knox explained the mechanisms through which substances such as cytokines, nutrients, and stress hormones can influence gene expression, demonstrating why there is a need to investigate psychosocial/behavioral factors as well as environmental toxicants in gene-environment interactions.

The proposed approach for measuring psychiatric/mental health outcomes includes:

- Periodic screening of all children starting in infancy for signs of psychological, psychiatric, and behavioral dysfunction.
- More definitive testing for those children with suspected problems
- Quantitative assessments rather than dichotomous (normal/abnormal) classifications
- Samples of behavior (for example, videotapes) may be "banked" for future analyses such as case control studies. It may be possible to code some observations directly so that simplified measures will be available on all children.

Neurobehavioral/cognitive measures will include:

- Omnibus IQ
- Executive function
- Language/verbal skills
- Academic skills
- Visuospatial abilities
- Learning/memory
- Attention
- Motor skills.

Social/emotional health and development measures will include:

- Social competence
- Attachment
- School readiness
- Emotional competence
- Temperament/personality
- Aggression.

Comments from Invited Participant

Dr. Daston—who has served on the Study's Federal Advisory Committee for several years—explained that his comments were from the perspective of the Federal Advisory Committee. He noted the following:

- The Study is the first large, longitudinal study to take advantage of a sufficiently large sample size (about 100,000) as well as the new information from the Human Genome Project. The Study will help to reveal information on the human genome, to examine the nature of individual genetic variability, and to systematically determine gene-environment interactions. Although the incidence of development outcomes such as birth defects is well established, little is known about the etiology of such outcomes. There are few single gene causes of abnormal developmental outcomes.
- The Study will set a precedent in terms of examining gene expression/RNA levels. This examination will pose particular challenges to the Study. Issues of sample collection, storage, and analysis will be very important to the success of examining gene expression. Animal models and the use of surrogate tissues will prove valuable in the aspect of the Study.
- One of the earlier goals of the Study will be to evaluate infections and their relationship to development outcomes. Early results on infections and developmental outcomes could have an impact by sensitizing the public and then Congress to the need for a long-term funding commitment to the Study. The Study needs big successes early on. These early successes will demonstrate the Study's potential.
- The behavioral and psychosocial measures will produce information that is very detailed and layered. These measures will provide insights on the underlying factors of environmental effects on disease outcomes. More important, behavioral and psychosocial factors will provide insights on why an individual fails to reach his or her full potential. The Study will help reveal which behavioral and psychosocial factors are positive and which are negative. Ultimately, the Study should provide answers on what individuals and society can do to help each child reach his or her full potential.

Discussion

The breakout session participants discussed the following topics and issues:

- Although the Study will have a standard protocol across all 105 Study sites, there will be opportunities for add-on or adjunct studies. The Study wants to keep subject burden to a minimum, but it does encourage adjunct studies.
- Measures of oral health, diet, nutrition, food security will be assessed, most likely, with questionnaires.
- Information on built environments, access to supermarkets, safety of playgrounds, crime statistics, and neighborhood observations/assessments can be included in the Study database without additional subject burden.
- Acceptability of biological measures is being assessed in the NC Herald Study.
- Information on family interactions, attachment measures such as mother-child bond, and didactic interactions will be gathered with validated questionnaires and other self-reporting tools.
- DNA, RNA, proteins, and blood volume measures will help researchers determine what is normal, or within normal variation, for different ages and developmental stages.
- Collecting, storing, and analyzing biological specimens will require prioritization. The cost and burden of analyses will need consideration because some analyses need to be performed immediately.

- Storage and retrieval of specimens in the biological repository require careful consideration.
- Samples/specimens will need to be stored for future hypotheses.
- Measures of cytokines and T-cell activation need to be refined.
- Dietary assessments will occur through a variety of approaches, including dietary recall, food frequency, and perhaps community food baskets.
- Children in foster care, juvenile justice system, or other nontraditional settings will be followed to the extent possible. The Study intends to follow all subjects from birth until 21 years of age.
- Parental smoking, alcohol consumption, and substance abuse will be assessed through a variety of approaches; newborn meconium will be collected and analyzed.
- Acute and chronic stress will be assessed. Cortisol levels in saliva are considered the best indicator of chronic stress, which is a good predictor of behavioral and psychosocial outcomes. Chronic stress is not well documented in children.
- Although the Study will not provide medical care to subjects, it may refer subjects for care.
- It is acknowledged that participation in this study, as in any other epidemiological study, may influence health and developmental outcomes.

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