

of Health and Human Services  
Public Health Services

PI: \_\_\_\_\_

Council: 05/2009

# Grant Application

NOV 5 2008

IPF \_\_\_\_\_

Dual: \_\_\_\_\_

IRG: \_\_\_\_\_

Received: 11/05/2008

Do not exceed character length restrictions indicated.

1. TITLE OF PROJECT (Do not exceed 81 characters, including spaces and punctuation.)  
\_\_\_\_\_

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION  NO  YES  
(If "Yes," state number and title)  
Number: \_\_\_\_\_ Title: \_\_\_\_\_

|                                                                                  |                                                                                                                  |                                                                                             |  |
|----------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|--|
| <b>3. PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR</b>                                |                                                                                                                  | <b>New Investigator</b> <input type="checkbox"/> No <input checked="" type="checkbox"/> Yes |  |
| 3a. NAME (Last, first, middle)<br>_____                                          | 3b. DEGREE(S)<br>MD                                                                                              | 3h. eRA Commons User Name<br>_____                                                          |  |
| 3c. POSITION TITLE<br>Fellow                                                     | 3d. MAILING ADDRESS (Street, city, state, zip code)<br>Department of Obstetrics and Gynecology<br>_____<br>_____ |                                                                                             |  |
| 3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT<br>Obstetrics and Gynecology  |                                                                                                                  |                                                                                             |  |
| 3f. MAJOR SUBDIVISION<br>Maternal Fetal Medicine                                 |                                                                                                                  |                                                                                             |  |
| 3g. TELEPHONE AND FAX (Area code, number and extension)<br>TEL: _____ FAX: _____ |                                                                                                                  | E-MAIL ADDRESS:<br>_____                                                                    |  |

|                                                                                                   |                                                                                            |                                                                                                                 |  |
|---------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|--|
| 4. HUMAN SUBJECTS RESEARCH<br><input type="checkbox"/> No <input checked="" type="checkbox"/> Yes | 4a. Research Exempt<br><input checked="" type="checkbox"/> No <input type="checkbox"/> Yes | If "Yes," Exemption No. _____                                                                                   |  |
| 4b. Federal-Wide Assurance No. _____                                                              | 4c. Clinical Trial<br><input checked="" type="checkbox"/> No <input type="checkbox"/> Yes  | 4d. NIH-defined Phase III Clinical Trial<br><input checked="" type="checkbox"/> No <input type="checkbox"/> Yes |  |

5. VERTEBRATE ANIMALS  No  Yes

5a. Animal Welfare Assurance No. \_\_\_\_\_

|                                                                                                                     |  |                                              |                                   |                                                   |                                   |
|---------------------------------------------------------------------------------------------------------------------|--|----------------------------------------------|-----------------------------------|---------------------------------------------------|-----------------------------------|
| 6. DATES OF PROPOSED PERIOD OF SUPPORT (month, day, year—MM/DD/YY)<br>From _____ Through _____<br>07/01/09 06/30/12 |  | 7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD |                                   | 8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT |                                   |
|                                                                                                                     |  | 7a. Direct Costs (\$)<br>\$127,000           | 7b. Total Costs (\$)<br>\$137,160 | 8a. Direct Costs (\$)<br>\$381,000                | 8b. Total Costs (\$)<br>\$411,480 |

9. APPLICANT ORGANIZATION  
Name \_\_\_\_\_  
Address Office of Sponsored Projects  
\_\_\_\_\_  
\_\_\_\_\_

10. TYPE OF ORGANIZATION  
Public: →  Federal  State  Local  
Private: →  Private Nonprofit  
For-profit: →  General  Small Business  
 Woman-owned  Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER  
DUNS NO. \_\_\_\_\_ Cong. District \_\_\_\_\_

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE  
Name \_\_\_\_\_  
Title Grants and Contracts Officer  
Address Office of Sponsored Projects  
\_\_\_\_\_  
\_\_\_\_\_  
Tel: \_\_\_\_\_ FAX: \_\_\_\_\_  
E-Mail: \_\_\_\_\_

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION  
Name \_\_\_\_\_  
Title Director, Office of Sponsored Projects  
Address Office of Sponsored Projects  
\_\_\_\_\_  
\_\_\_\_\_  
Tel: \_\_\_\_\_ FAX: \_\_\_\_\_  
E-Mail: \_\_\_\_\_

14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 13. \_\_\_\_\_  
(In ink. "Per" signature not acceptable.)  
DATE \_\_\_\_\_

PROJECT SUMMARY (See instructions):

This career development award is designed to support my transition into an independent clinician-scientist dedicated to the study of genetic susceptibility to adverse neurodevelopmental outcomes. I will be completing a Maternal Fetal Medicine Fellowship at the \_\_\_\_\_ in \_\_\_\_\_

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This proposal outlines \_\_\_\_\_  
My specific career development aims are to \_\_\_\_\_

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My specific research aims are to \_\_\_\_\_

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This proposal represents \_\_\_\_\_

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RELEVANCE (See instructions): Although various perinatal complications increase the risk of an individual developing cerebral palsy or other forms of neurodevelopmental delay, the mechanisms by which at-risk individuals develop these complications are incompletely understood. This work will contribute to an increased understanding of the genetic risk factors predisposing a premature fetus to brain injury, and will therefore identify possible prevention strategies and may provide a basis for future prevention/ intervention trials. It will also enhance the career development of a promising young clinician-scientist within an environment with proven career-development capabilities. Dr. \_\_\_\_\_ has the enthusiastic support of \_\_\_\_\_ Division, Department and Institution (including at least 75% protected time and appropriate institutional funding).

PROJECT/PERFORMANCE SITE(S) (if additional space is needed, use Project/Performance Site Format Page)

|                                                         |  |                 |  |                        |  |
|---------------------------------------------------------|--|-----------------|--|------------------------|--|
| <b>Project/Performance Site Primary Location</b>        |  |                 |  |                        |  |
| Organizational Name: _____                              |  |                 |  |                        |  |
| DUNS: _____                                             |  |                 |  |                        |  |
| Street 1: _____                                         |  | Street 2: _____ |  |                        |  |
| City: _____                                             |  | County: _____   |  | State: _____           |  |
| Province: _____                                         |  | Country: USA    |  | Zip/Postal Code: _____ |  |
| Project/Performance Site Congressional Districts: _____ |  |                 |  |                        |  |

Program Director/Principal Investigator (Last, First, Middle): \_\_\_\_\_

**SENIOR/KEY PERSONNEL.** See instructions. *Use continuation pages as needed* to provide the required information in the format shown below. Start with Program Director(s)/Principal Investigator(s). List all other senior/key personnel in alphabetical order, last name first.

| Name  | eRA Commons User Name | Organization | Role on Project |
|-------|-----------------------|--------------|-----------------|
| _____ | _____                 | _____        | Candidate       |
| _____ | _____                 | _____        | Mentor          |
| _____ | _____                 | _____        | Co-mentor       |
| _____ | _____                 | _____        | Co-Mentor       |

**OTHER SIGNIFICANT CONTRIBUTORS**

| Name  | Organization | Role on Project |
|-------|--------------|-----------------|
| _____ | _____        | Advisor         |

**Human Embryonic Stem Cells**  No  Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: <http://stemcells.nih.gov/research/registry/>. *Use continuation pages as needed.*

If a specific line cannot be referenced at this time, include a statement that one from the Registry will be used.

**RESEARCH CAREER DEVELOPMENT AWARD TABLE OF CONTENTS (Substitute Page)**

Page Numbers

**Letters of Reference\*** (attach unopened references to the Face Page)

**Basic Administrative Data**

|                                                                                                                                                                        |       |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| Face Page (Form Page 1) .....                                                                                                                                          | 1     |
| Description, Project/Performance Sites, Senior/Key Personnel, Other Significant Contributors, and Human Embryonic Stem Cells (Form Page 2) .....                       | 2-3   |
| Table of Contents (this CDA Substitute Form Page 3) .....                                                                                                              | 4     |
| Budget for Entire Proposed Period of Support (Form Page 5) .....                                                                                                       | 5-6   |
| Biographical Sketches (Candidate, Mentor[s], * Key Personnel and Other Significant Contributors*<br>—Biographical Sketch Format page) (Not to exceed four pages) ..... | 7-20  |
| Other Support Pages (for mentor(s) only) .....                                                                                                                         | N/A   |
| Resources (Resources Format page) .....                                                                                                                                | 21-28 |

**Career Development Plan**

**The Candidate**

|                                                                  |       |
|------------------------------------------------------------------|-------|
| Candidate's Background .....                                     | 29-30 |
| Career Goals and Objectives: Scientific Biography .....          | 30-32 |
| Career Development/Training Activities during Award Period ..... | 32-40 |
| Training in the Responsible Conduct of Research .....            | 38-39 |

(Items included in 25 page limit)

|                                                                                       |       |
|---------------------------------------------------------------------------------------|-------|
| <b>Statements by Mentor, Co-Mentor(s),* Consultant(s),* and Contributor(s)*</b> ..... | 41-59 |
|---------------------------------------------------------------------------------------|-------|

**Environment and Institutional Commitment to Candidate**

|                                                                           |       |
|---------------------------------------------------------------------------|-------|
| Description of Institutional Environment .....                            | 60-64 |
| Institutional Commitment to Candidate's Research Career Development ..... | 64-66 |

**Research Plan**

|                                                                              |       |
|------------------------------------------------------------------------------|-------|
| 1. Introduction to Resubmission Application* (Not to exceed 3 pages) .....   | N/A   |
| 2. Specific Aims .....                                                       | 67    |
| 3. Background and Significance .....                                         | 68-74 |
| 4. Preliminary Studies/Progress Report .....                                 | 74-75 |
| 5. Research Design and Methods .....                                         | 75-79 |
| 6. Inclusion Enrollment Report (Renewal or Revision Applications only) ..... | N/A   |
| 7. Bibliography and References Cited/Progress Report Publication List .....  | 79-84 |
| 8. Protection of Human Subjects .....                                        | 84-85 |
| 9. Inclusion of Women and Minorities .....                                   | 85    |
| 10. Targeted/Planned Enrollment Table .....                                  | 86    |
| 11. Inclusion of Children .....                                              | 86    |
| 12. Vertebrate Animals .....                                                 | 86    |
| 13. Select Agents .....                                                      | 86    |
| 14. Multiple PD/PI Leadership Plan (Not applicable. Do not include.) .....   |       |
| 15. Consortium/Contractual Arrangements* .....                               | 86    |
| 16. Letters of Support/Consultants .....                                     |       |
| 17. Resource Sharing Plan(s) .....                                           | 86    |
| <b>Checklist</b> .....                                                       | 87    |

(Items 2-5 included in 25 page limit)

**Appendix** (Five identical CDs.)

Check if Appendix is included

Note: Font and margin requirements must conform to limits provided in the Specific Instructions.

\*Include these items only when applicable.

**CITIZENSHIP**

- U.S. citizen or non-citizen national       Permanent resident of U.S. (If a permanent resident of the U.S., a notarized statement must be provided by the time of award.)       Non-citizen with temporary visa (Applicable for only the K99 program)

**BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD  
DIRECT COSTS ONLY**

| BUDGET CATEGORY<br>TOTALS                                                  |            | INITIAL BUDGET<br>PERIOD<br><i>(from Form Page 4)</i> | ADDITIONAL YEARS OF SUPPORT REQUESTED |           |                   |     |
|----------------------------------------------------------------------------|------------|-------------------------------------------------------|---------------------------------------|-----------|-------------------|-----|
|                                                                            |            |                                                       | 2nd                                   | 3rd       | 4th               | 5th |
| PERSONNEL: <i>Salary and fringe benefits. Applicant organization only.</i> |            | 102,000                                               | 102,000                               | 102,000   |                   |     |
| CONSULTANT COSTS                                                           |            |                                                       |                                       |           |                   |     |
| EQUIPMENT                                                                  |            |                                                       |                                       |           |                   |     |
| SUPPLIES                                                                   |            |                                                       |                                       |           |                   |     |
| TRAVEL                                                                     |            | 2,000                                                 | 2,000                                 | 1,500     |                   |     |
| PATIENT CARE COSTS                                                         | INPATIENT  |                                                       |                                       |           |                   |     |
|                                                                            | OUTPATIENT |                                                       |                                       |           |                   |     |
| ALTERATIONS AND RENOVATIONS                                                |            |                                                       |                                       |           |                   |     |
| OTHER EXPENSES                                                             |            | 23,000                                                | 23,000                                | 23,500    |                   |     |
| CONSORTIUM/ CONTRACTUAL COSTS                                              | DIRECT     |                                                       |                                       |           |                   |     |
| <b>SUBTOTAL DIRECT COSTS</b><br><i>(Sum = Item 8a, Face Page)</i>          |            |                                                       |                                       |           |                   |     |
| CONSORTIUM/ CONTRACTUAL COSTS                                              | F&A        |                                                       |                                       |           |                   |     |
| <b>TOTAL DIRECT COSTS</b>                                                  |            | \$127,000                                             | \$127,000                             | \$127,000 |                   |     |
| <b>TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD</b>               |            |                                                       |                                       |           | <b>\$ 381,000</b> |     |

**BUDGET JUSTIFICATION**

\_\_\_\_\_, MD, Candidate, Maternal Fetal Medicine Fellow, Department of Obstetrics and Gynecology, \_\_\_\_\_, Dr. \_\_\_\_\_ will dedicate 75% (9 pers. mo.) of \_\_\_\_\_ time to research and training related to the Mentored Patient-Oriented Research Career Development Award for each of the 3 years. \$75,000 salary plus 36% benefits (\$102,000 subtotal) plus \$25,000 for training and research expenses have been budgeted for years 1-3. \_\_\_\_\_ will be primarily responsible for the execution of all aspects of the project including study design, data collection, management and analysis and preparation of all manuscripts resulting from this work. \_\_\_\_\_ will also maintain IRB approval and assure HIPAA compliance of the project. Although \_\_\_\_\_ Masters Degree in Clinical Investigation and \_\_\_\_\_ training and experience has given \_\_\_\_\_ valuable research experience and enhanced \_\_\_\_\_ professional contacts, there is still much to be learned and mastered before \_\_\_\_\_ can be truly considered an independent investigator. Dr. \_\_\_\_\_ will take didactic courses in study design, statistics, epidemiology, genetics, and ethics during the award period. \_\_\_\_\_ will enroll in, and complete, the \_\_\_\_\_ a multidisciplinary, multi-institutional DHHS-funded program that to date has never had any obstetric involvement. \_\_\_\_\_ will also actively participate in workshops, seminars, conferences, national meetings and research experiences with experts in the (cont.)

Program Director/Principal Investigator (Last, First, Middle): \_\_\_\_\_

**BUDGET JUSTIFICATION CONT.**

fields of genetics and neurodevelopmental disabilities. By the end of this proposed training award, \_\_\_\_\_ will be a competitive applicant for independent peer-reviewed research funding.

**Training and Research Expenses**

\$75,000 for training and research expenses is requested for years 1-3 (\$25,000/yr).

**Travel (\$5,500)**

- Annual

The candidate will attend 3 national meetings with support from the \_\_\_\_\_:

- Society of Maternal Fetal Medicine Meeting
- Society of Gynecologic Investigation Meeting
- Pediatric Academic Societies Meeting

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Didactic Training (\$12,092)**

- Years 1-3

\$2000 for books and course materials for didactic training

\$10,092 for tuition and fees (based on current tuition/fees at the \_\_\_\_\_ and 29 credit hours; 5 hours completed prior to grant award and will be covered by \_\_\_\_\_ Dept. of OBGYN)

**Laboratory Expenses (\$57,408)**

- Years 1-2

\$52,575 for 1,536 genotypes (GoldenGate Assay) on 701 subjects (\$75/subject), including development of assay, primers, reagents and other necessary lab equipment including plastic disposables, tips, polymer, and instrument maintenance. \_\_\_\_\_, MS, a Senior Laboratory Specialist with considerable experience in multiplex SNP analysis, will provide technical assistance. \_\_\_\_\_ duties will involve supervision of multiple steps, including quality control of DNA with determination of concentration using the Nanodrop Technologies instrument, extensive use of robotics for pipetting purposes, heat fragmentation, probe ligation, exonuclease clean up step, PCR reactions in 384 plates, and denaturation and hybridization steps. The final products will be run on a DNA sequencer and the multiplexed fluorescent signals converted to SNP results. All required instrumentation is located within the Department of Human Genetics. Any remaining funds will go towards salary for \_\_\_\_\_. Further salary support will be covered by funding within the Departments of Human Genetics and Obstetrics and Gynecology.

**As also noted in the accompanying letters of support, the \_\_\_\_\_ is committed to underwriting additional expenses as required both to 1) maintain Dr. \_\_\_\_\_'s salary consistent with the established salary structure of the \_\_\_\_\_ Department of Obstetrics and Gynecology for individuals of equivalent qualifications, rank and responsibilities, and, 2) pay for the balance of any research expenses not covered by this proposed award.**

**F&A Indirect Cost:** Per NICHD guidelines, F& A costs have been figured 8% of the total direct costs (\$127,000) at \$10,160 for years 1-3.

Program Director/Principal Investigator (Last, First, Middle): \_\_\_\_\_

### BIOGRAPHICAL SKETCH

|                       |                                          |
|-----------------------|------------------------------------------|
| NAME                  | POSITION TITLE                           |
| _____                 | Fellow, Maternal-Fetal Medicine Division |
| eRA COMMONS USER NAME | Department of Obstetrics and Gynecology  |
| _____                 | _____                                    |

EDUCATION/TRAINING

| INSTITUTION AND LOCATION | DEGREE<br><i>(if applicable)</i> | YEAR(s) | FIELD OF STUDY            |
|--------------------------|----------------------------------|---------|---------------------------|
| _____                    | BA                               | _____   | Biology                   |
| _____                    | MD                               | _____   | Medicine                  |
| _____                    | Residency                        | _____   | Obstetrics and Gynecology |
| _____                    | Fellowship                       | _____   | Maternal-Fetal Medicine   |
| _____                    | MS (anticipated)                 | _____   | Clinical Investigation    |

#### A. Positions and Honors

##### Positions

|       |       |       |
|-------|-------|-------|
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |

##### Scholastic Honors

|       |       |
|-------|-------|
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |

**B. Peer-Reviewed Publications**

Peer Review Publications

Invited Peer-Review Articles and Chapters

Published Abstracts

Abstract Accepted but not yet Published

Program Director/Principal Investigator (Last, First, Middle): \_\_\_\_\_

**C. Research Support**

Submitted and Funded

1. \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Submitted and Pending

1. \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Program Director/Principal Investigator (Last, First, Middle): \_\_\_\_\_

### BIOGRAPHICAL SKETCH

|                                |                                                                                                          |
|--------------------------------|----------------------------------------------------------------------------------------------------------|
| NAME<br>_____                  | POSITION TITLE<br>Professor, Maternal-Fetal Medicine Division<br>Department of Obstetrics and Gynecology |
| eRA COMMONS USER NAME<br>_____ | _____                                                                                                    |

#### EDUCATION/TRAINING

| INSTITUTION AND LOCATION | DEGREE<br><i>(if applicable)</i> | YEAR(s) | FIELD OF STUDY                                                   |
|--------------------------|----------------------------------|---------|------------------------------------------------------------------|
| _____                    | B.A.                             | _____   | Medicine<br>Obstetrics and Gynecology<br>Maternal-Fetal Medicine |
| _____                    | M.D.                             | _____   |                                                                  |
| _____                    | Residency                        | _____   |                                                                  |
| _____                    | Fellowship                       | _____   |                                                                  |

#### A. Positions and Honors

|       |       |
|-------|-------|
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| _____ | _____ |

#### B. Selected peer-reviewed publications (2005 – Present, from more than 200 entries)

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|-------|-------|
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |
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| _____ | _____ |





Program Director/Principal Investigator (Last, First, Middle): \_\_\_\_\_

II. Pending

|  |
|--|
|  |
|--|

III. Completed

|  |
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|  |
|  |

Program Director/Principal Investigator (Last, First, Middle): \_\_\_\_\_

## BIOGRAPHICAL SKETCH

|                                |                                                                        |
|--------------------------------|------------------------------------------------------------------------|
| NAME<br>_____                  | POSITION TITLE<br>Professor<br>Department of Obstetrics and Gynecology |
| eRA COMMONS USER NAME<br>_____ | _____                                                                  |

| EDUCATION/TRAINING       |            |          |                           |
|--------------------------|------------|----------|---------------------------|
| INSTITUTION AND LOCATION | DEGREE     | YEAR (s) | FIELD OF STUDY            |
| _____                    | BA         | _____    | Microbiology              |
| _____                    | MD         | _____    | Medicine                  |
| _____                    | Residency  | _____    | Obstetrics and Gynecology |
| _____                    | Fellowship | _____    | Maternal-Fetal Medicine   |

### A. Positions and Honors

#### Positions

|       |       |
|-------|-------|
| _____ | _____ |
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| _____ | _____ |
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#### Honors

|       |       |
|-------|-------|
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |

### B. Peer-Reviewed Publications

Select peer-reviewed publications (in chronological order)

|       |
|-------|
| _____ |
| _____ |
| _____ |













## RESOURCES

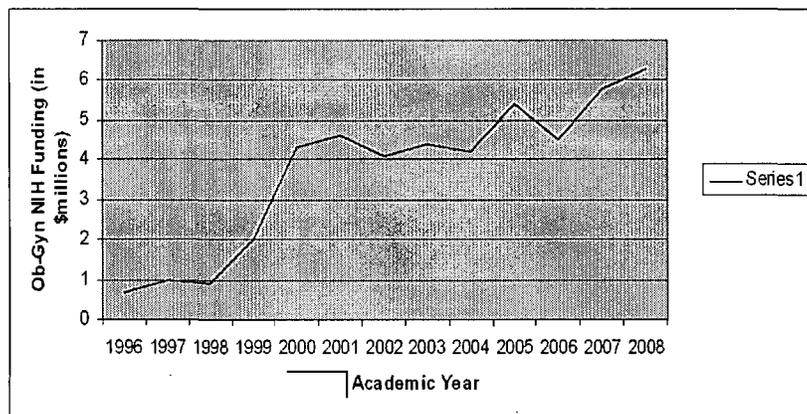
### Resources for Research, \_\_\_\_\_ Department of Obstetrics and Gynecology

#### **Department of Obstetrics and Gynecology**

The Department of Obstetrics and Gynecology at the \_\_\_\_\_ has become a leading academic force over the past 25 years. Numerous graduates and faculty have become national and, in some cases, world leaders in academic obstetrics and gynecology. The Department has had a sustained academic focus and now has nationally recognized academic programs in *perinatal medicine*, *reproductive genetics*, *urogynecology*, *reproductive immunology*, and *reproductive endocrinology*.

The Department of Obstetrics and Gynecology at the \_\_\_\_\_ | The Department remains committed to a strong academic environment that is founded on a successful scientific research program. The Department has funded and supported numerous young faculty in their efforts to become established clinical investigators and \_\_\_\_\_ faculty members currently enjoy extramural funding. The Department has been ranked between \_\_\_\_\_ in obstetrics and gynecology departments nationally in NIH funding for \_\_\_\_\_. Although these rankings are no longer published, the Department has continued its trend of increasing extramural peer-reviewed funding (Figure 1).

**Figure 1. \_\_\_\_\_ Ob-Gyn NIH Funding**



The five-year plan for the Department of Obstetrics and Gynecology at \_\_\_\_\_ calls for the expansion of a balanced research program incorporating basic, translational and clinical research. Significant financial support and physical space for the development of a clinical research core within the Department of Obstetrics and Gynecology has been obtained from the Senior Vice President

for Health Sciences in order to achieve this goal. The Department of Obstetrics and Gynecology has renovated its facilities to provide an expanded and dedicated home for the continually growing research efforts, including facilities to enhance and optimize research training.

#### **Division of Maternal Fetal Medicine**

The Maternal Fetal Medicine (MFM) Division at \_\_\_\_\_ was founded in \_\_\_\_\_ with the recruitment of \_\_\_\_\_ Dr. \_\_\_\_\_ served as the \_\_\_\_\_ director of the MFM Division until \_\_\_\_\_ at which point the Division consisted of \_\_\_\_\_ MFM subspecialists, all based at \_\_\_\_\_.

In \_\_\_\_\_ assumed the Directorship of the \_\_\_\_\_ MFM Division. Importantly, Dr. \_\_\_\_\_ integrated the MFM Division at the \_\_\_\_\_ with the evolving MFM services in the \_\_\_\_\_ | The \_\_\_\_\_ is the dominant health care provider in the \_\_\_\_\_, with more covered lives and hospital





### Department of Human Genetics

The Department of Human Genetics maintains many genomic resources that will be available to Dr. \_\_\_\_\_ for \_\_\_\_\_ proposed study. Within the Department, instrumentation for capillary ABI sequencing, Affymetrix gene expression studies, TaqMan SNP typing, SNPlex genotyping, and Affymetrix SNP chip genotyping, and GoldenGate genotyping is available to all researchers.

### K30 Training Program in Clinical Investigation (TPCI)

The TPCI is a two to three-year post-graduate training program in clinical investigation with an emphasis on the inherited basis of human disease. The TPCI is funded by an NIH Clinical Research Curriculum Award \_\_\_\_\_. The program consists of formal didactic coursework, a longitudinal seminar series, and a mentored clinical research project. Trainees who successfully complete the program are awarded a Masters Degree in Clinical Investigation from the \_\_\_\_\_. This course is designed to teach physician-scientists the essential concepts of clinical and/or laboratory-based research. The program's goals are to produce independent clinical investigators who are able to successfully apply for extramural grant support by the completion of their training.

The **specific aims** of the TPCI are:

- 1) To provide a rigorous didactic program consisting of six areas:
  - Epidemiology, biostatistics, and experimental design,
  - Bioethical issues in research on the inherited basis of human disease,
  - Human genetics for clinical investigators,
  - Molecular biology for clinical investigators,
  - Biochemistry for clinical investigators, and,
  - Utilization of animal models in the development of clinical research.
- 2) To create a program offering a specific, mentored, intensive research experience under the direct supervision of an accomplished clinical investigator.
- 3) To attract trainees most likely to succeed as independent clinical



\_\_\_\_\_ g several that have been conducted and published by Drs. \_\_\_\_\_ and their peers.

**Center for Clinical and Translational Sciences (CCTS)**

The \_\_\_\_\_ has been awarded a Clinical and Translational Science Award \_\_\_\_\_ ) to develop a Center for Clinical and Translational Sciences (CCTS). This Center builds upon the University's strengths in genetics and biomedical informatics and will incorporate the existing General Clinical Research Center (GCRC) and K30 Clinical Research Curriculum into the CCTS. The CCTS will be housed in \_\_\_\_\_ of space renovated specifically for the Center, comprising an entire floor of a building attached to the School of Medicine. Most CCTS members will be faculty in the School of Medicine, the College of Nursing, the College of Pharmacy, the College of Health, and the College of Engineering. Other CCTS members have primary appointments in other colleges, schools and departments located on the campus of the \_\_\_\_\_ and in our partner institutions of the Veterans Hospital, \_\_\_\_\_ and the \_\_\_\_\_ Department of Health. The goals of the \_\_\_\_\_ CCTS are to establish an academic home, located in a dedicated space, to support clinical and translational research by consolidating resources, promoting interdepartmental and interdisciplinary research, and facilitating increased interactions between basic scientists and clinical investigators. The Center will also promote the development of a new generation of clinical and translational investigators through a variety of educational and mentoring programs to provide support and recognition for these investigators.

**Pediatric Clinical and Translational (PCAT) Research Scholars Program**

Over the preceding decade the Department of Obstetrics and Gynecology, and particularly the MFM Division, has become increasingly collaborative and overlapping in its research efforts with the Department of Pediatrics (see letters from Drs. \_\_\_\_\_). The Department of Pediatrics' commitment to the success of clinical investigators, particularly during the early years of career development, is seen in the establishment of the Grants and Research Support Office for Clinical and Translational Research ( \_\_\_\_\_ ) and the development of the Pediatric Clinical and Translational (PCAT) Research Scholars Program. Both are directed by Dr. \_\_\_\_\_. The goal of the PCAT program is to support the career development of promising clinician scientists. The program provides an extensive research training curriculum as well as biostatistical support, research project coordination and administrative assistance, and scientific and career development mentorship from senior investigators in the Department of Pediatrics. As part of the increasingly collaborative and overlapping research interests of the Departments of Pediatrics and Obstetrics and Gynecology, PCAT resources are now available to promising clinician-scientists from both Departments. As per Dr. \_\_\_\_\_'s letter of support, these resources are all available for Dr. \_\_\_\_\_.

**The success of these programs confirms that the \_\_\_\_\_ has a well-established record of research career development activities and qualified research faculty to serve as mentors for Dr. \_\_\_\_\_.**

**Resources Outside the \_\_\_\_\_**

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_



Program Director/Principal Investigator (Last, First, Middle): \_\_\_\_\_

**Table 1.**

|            |            |            |
|------------|------------|------------|
| [REDACTED] | [REDACTED] | [REDACTED] |

|            |            |            |
|------------|------------|------------|
| [REDACTED] | [REDACTED] | [REDACTED] |

**Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)**

|            |            |            |
|------------|------------|------------|
| [REDACTED] | [REDACTED] | [REDACTED] |

Advisory Group) is further confirmed by the Letters of Support from Drs. \_\_\_\_\_

**Dr. \_\_\_\_\_ has access to a strong network of well-established research and career development resources and outstanding mentors outside the \_\_\_\_\_**









Program Director/Principal Investigator (Last, First, Middle): \_\_\_\_\_

**- Primary Mentor**

\_\_\_\_\_ has the research qualifications to serve as a mentor in the scientific area proposed by Dr. \_\_\_\_\_. \_\_\_\_\_ has an extended record of fostering the development of independent researchers and will actively provide guidance and training to Dr. \_\_\_\_\_. \_\_\_\_\_ also has sufficient independent research and administrative support to cover the costs of the proposed research project in excess of the allowable cost of the award. \_\_\_\_\_ will work closely with Dr. \_\_\_\_\_ on the planning, direction and execution of \_\_\_\_\_ proposed training and research programs.

**- Co-Mentor**

\_\_\_\_\_ has the research qualifications to serve as a co-mentor for Dr. \_\_\_\_\_'s proposal. \_\_\_\_\_ has a track record of fostering the development of independent researchers and will actively provide guidance and training to Dr. \_\_\_\_\_.

**- Co-Mentor**

The \_\_\_\_\_ is committed to the development of Dr. \_\_\_\_\_ into a productive, independent investigator. As referenced in the letters of support which follow, \_\_\_\_\_ Department and Institution are also committed to protecting at least 75% of \_\_\_\_\_ time in order to allow \_\_\_\_\_ full utilization of the opportunities provided by a K23 award.

**Advisory Committee**

I have identified additional advisors who will be responsible for guidance in specific areas of research. The contributions of these individuals are detailed within each training aim. I will meet with each advisor at least quarterly during the portions of the grant in which they are involved.

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**1c2. Training Plan**

**Training Aim 1: Expand existing skills in study design, analysis, and interpretation of results**

**Rationale** The study of perinatal risk factors associated with adverse neurodevelopmental outcomes demands advanced training in study design, analysis and interpretation of data that is absent from my previous training. The K30 Training Program in Clinical Investigation gave me a basic foundation in these subjects, however, more rigorous and in-depth training is necessary to achieve my career goals.

**Coursework**

- 1. FPMD 6100 Biostatistics I.** Course in the use of statistical methods in the analysis of outcome studies and quality improvement. Fall 2009 (3 credit hours).
- 2. FPMD 7100 Biostatistics II.** Course explores the use of statistical modeling of analysis of health and medical data, focusing on the analysis of complex data using a variety of regression and analysis of variance techniques. Spring 2010 (3 credit hours).
- 3. FPMD 6300 Epidemiology I.** Basic epidemiology, with emphasis on determining causation of chronic disease. Fundamentals of study design and data resources. Fall 2009 (3 credit hours).
- 4. FPMD 7300 Epidemiology II.** Intermediate and advanced principles in epidemiology, with emphasis on advanced designs (e.g. clinical trials, nested case-control, case-cohort, case-only, case-crossover), topics in statistical methods in epidemiology (e.g. survival analysis, categorical data analysis, multivariate models) and other topics. Spring 2010 (3 credit hours).
- 5. FPMD 6320 Perinatal and Women's Health Epidemiology.** Application of epidemiological methods to studies of perinatal, reproductive, and women's health. Students will learn to critically evaluate research articles, design epidemiologic studies, and apply health data to improve public health programs and policy. Spring 2011 (2 credit hours).
- 6. FPMD 7120 Linear and Logistic Regression Models.** Students will study multiple linear regression, logistic regression, ordinal and generalized least squares, multinomial and ordinal

logistic regression, hypothesis testing, prediction, measure of goodness-of-fit, regression diagnostics, collinearity, model selection, ANOVA. Spring 2011 (3 credit hours).

7. **FPM 7140 Applied Multivariate Data Analysis.** Students will study multivariate normal distribution, multivariate regression, MANOVA, principal components, classification, factor analysis cluster analysis. Focuses on applications in health science research. Fall 2010 (3 credit hours).

#### **Mentorship**

Drs. \_\_\_\_\_ will provide strong mentorship for Training Aim 1. Both Drs. \_\_\_\_\_ have extensive experience in longitudinal data collection, management, and analysis, including work focused on the challenges of missing data and multivariate analysis. Drs. \_\_\_\_\_ also bring expertise in construction and maintenance of long-term follow-up cohorts.

Dr. \_\_\_\_\_, Senior Research Statistician for the \_\_\_\_\_, will collaborate with me on data analysis and statistical concerns during every phase of the research projects described in this application. As described in \_\_\_\_\_ letter of support, \_\_\_\_\_ will also oversee the statistical training portion of this aim, continuing to assure that courses are suited for the statistical needs of my research. A week-long stay at Research Triangle Institute in Bethesda, Maryland, is planned for years 1 and 2 of the grant. This will function as an intense mentoring experience that will allow for discussion of the analysis plan, data analysis, and interpretation of results. Dr. \_\_\_\_\_ and I will discuss data analysis and study design issues pertinent to this project and potential future projects.

#### **Training Aim 2: Develop additional skills in the acquisition, management, and analysis of complex genetic data**

**Rationale** The complex nature of CP and neurodevelopmental delay, which likely involves interactions between environmental and genetic risk factors, makes it unlikely that any single mutation or risk factor will explain a substantial portion of the cases. Thus, improved methods of characterizing patients based on etiology, and methods that allow for the evaluation of multiple candidate genes simultaneously, are most likely to be successful in identifying genetic influences that increase the risk for neurodevelopmental delay and CP. As genome-wide association studies and copy-number variant technologies advance (and I have opportunities to participate in these investigations), additional training, both didactic and experiential, in the acquisition, management, and analysis of complex genetic data is clearly necessary.

#### **Coursework**

1. **MDCRC 6400 Medical Genetics for Clinical Investigators.** Broad overview of medical genetics with an emphasis on issues most relevant for clinical investigators. Cytogenetics and new molecular techniques, gene mapping through linkage analysis, positional cloning and candidate gene analysis will be covered. Summer 2009 (1 credit hour).
2. **MDCRC 6420 Genetics of Complex Diseases.** Addresses issues relevant to the identification of genes underlying susceptibility to complex disorders. Advantages and disadvantages of isolates versus large populations, utilization of affected sibling pairs, discordant sibling pairs and extended families, locus and allelic heterogeneity, phenotypic heterogeneity, gene-gene and gene-environment interactions and density of polymorphic markers will be discussed. Spring 2009 (2 credit hours).
3. **BMI 6040 Foundations of Genetic Epidemiology.** This course includes an introduction to fundamental concepts in bioinformatics and genetic epidemiology and will explore methods to determine if a trait is genetic, traditional and family-based association studies, concepts for haplotype blocks and tagging-SNPs, and an overview of both parametric and non-parametric linkage methods. Fall 2011 (1.5 credit hours).

#### **Mentorship**

\_\_\_\_\_, \_\_\_\_\_, and Co-Chair of the Department of Human Genetics, will be my primary mentor with regards to my training in genetic concepts, technique, and analysis. A significant component of genetics research training is gained by practical experience on research projects. Didactic training will therefore contribute only a portion of the training necessary. Intensive mentorship in this area will be critical to achieving my career goals. I will participate in weekly lab meetings with Dr. \_\_\_\_\_ and will be involved in all aspects of this project's laboratory component. Participating in laboratory meetings will expose me to a wide variety of genetics topics and techniques, as well as analysis and publication issues. Technical proficiency with laboratory techniques will not be the learning emphasis, but rather an in-depth knowledge of the subject matter and the ability to

use that knowledge to forge productive research collaborations will be emphasized. Dr. \_\_\_\_\_ has mentored \_\_\_\_\_ young investigators (PhD and MD) with NIH-funded training awards. \_\_\_\_\_ has also participated on \_\_\_\_\_ graduate student advisory committees. Personnel in \_\_\_\_\_ laboratory have extensive experience working with young investigators, especially those with clinical backgrounds.

Dr. \_\_\_\_\_ will also advise me on genetic data analysis and statistical issues, as described above.

### **Training Aim 3: Develop additional skills in the design and implementation of clinical trials**

**Rationale** A goal of the genetics research for this project is to promote further basic science and genetics research aimed at determining the perinatal genetic and environmental factors that predispose fetuses to adverse neurodevelopmental outcomes. In this regard, this type of research is intended to act as a bridge to the eventual initiation of interventional and therapeutic clinical trials. In order to accomplish this longer term goal, an in-depth knowledge of the design and implementation of clinical trials will be necessary.

**Coursework** (Note that the first class will be completed prior to the award period)

1. **MDCRC 6040 Design and Implementation of Clinical Trials.** This course defines clinical trials and reviews drug registration trials, phase I, II, and III trials, clinical endpoints, surrogate endpoints, pharmacokinetics, drug-drug interactions, data and safety monitoring, criteria for closure and single versus multi-institutional trials. Fall 2008 (2 credit hours).
2. **Research Administration Training Series (RATS),** Sponsored by The Office of the Vice President for Research. **The Clinical Research Certificate** acknowledges study of **clinical trials management** and the conduct of human subject investigations. Each course is taught as a single 2-hour seminar. The certificate will be completed over the course of a year. Core Courses include: 'Overview of Research Administration', 'Introduction to the Office of Sponsored Projects (OSP)', 'Responsible Conduct in Research and Conflict of Interest', and 'Introduction to the IRB, the IACUC and the IBC'. Requisite Courses include: 'Basics of Good Clinical Practices', 'Financial Management in Clinical Trials: Budget Development, Negotiation and Oversight', 'Institutional Review Board (IRB) and Human Subject Research', and 'Source Documentation for Data Management'. Two elective courses of interest are also required.

### **Mentorship**

Drs. \_\_\_\_\_ will provide strong mentorship for Training Aim 3. These clinician investigators have extensive experience in the design and implementation of clinical trials within a single institution and multi-institution trials within collaborative networks, both modest and large. They are established clinician-scientists with a track record of successfully applying for extramural funding, forging collaborations, initiating clinical trials, and publishing results. They have each already played a critical role in my career development and research. Under their expert mentorship, I will learn these skills and will be prepared to play an active role in clinical trials research at the conclusion of this award, and ultimately initiate independent clinical trials aimed at reducing the incidence of CP and other adverse outcomes.

### **Training Aim 4: Develop skills for leadership of a multi-disciplinary research team**

**Rationale** To become a leader in neurodevelopmental disabilities research, I need to develop skills in effective and efficient multidisciplinary team collaboration and management. Skills in research administration are particularly relevant to this goal.

### **Coursework**

1. **Research Administration Training Series (RATS),** see also Training Aim 3, above. The **Pre-Award Certificate** acknowledges study of the proposal development and funding search process in research administration. The **Post-Award Certificate** acknowledges study of the accounting and project management aspects of research administration. Each course is taught as a single 2-hour seminar. Both certificates will be completed over the course of 2 years. Requisite Courses for the Pre-Award Certificate include: 'Researcher Resources and Funding Searches', 'Proposal Preparation', 'Processing and Review', and 'Budget Preparation and Development'. Requisite Courses for the Post-Award Certificate include: 'Project Management: Implementing the Award Process', 'Grants and Contracts Accounting', and 'Management Accounting and Analysis'.

2.

My participation in the program will consist of a year-long, 300-hour curriculum, consisting of classroom, clinical and leadership components. The classroom instructional methods used in this course include interactive seminars, trainee activities, role-plays, presentations by families, trainees, and panels of professionals, and discussion groups. Didactic sessions are conducted for 3-5 hours each Friday afternoon, with all participants expected to attend either in person or by video-link. The \_\_\_\_\_ curriculum is summarized in **Appendix A**.

Participants in this program characteristically come from a specialized or sub-specialized training program and generally do not have a full appreciation of the complexity of care that many of these children require. As a result, the clinical curriculum is individually constructed to provide exposure to settings outside those of the participant's own area of specialization, primarily at the \_\_\_\_\_ and at the \_\_\_\_\_

\_\_\_\_\_. Because the goal of this grant is to train the leaders for tomorrow, leadership skills are a key outcome for the program. Over the course of the year, each trainee completes a leadership project either individually, or as part of a team.

**It should be noted that, to this point in time, there has been no obstetric involvement in the \_\_\_\_\_, either as a participant or as faculty. My involvement in the \_\_\_\_\_ program would not only prepare me to be a leader in neurodevelopmental research, but would also provide this program with a resource for clinical expertise in perinatal medicine. The skills acquired during my participation in this program, and in my career development plan outlined above, will subsequently allow me the possibility of participating at a faculty level in the \_\_\_\_\_ program.**

#### **Mentorship**

Drs. \_\_\_\_\_, co-Directors of the \_\_\_\_\_, support my application for this program for 2009-2010 (see letters of support which follow) and will be my mentors on this leadership career development aim. Dr. \_\_\_\_\_ is a Neurodevelopmental Pediatrician with special interests in \_\_\_\_\_

\_\_\_\_\_. Dr. \_\_\_\_\_ has extensive leadership experience in developing successful interdisciplinary training programs and is conversant with strategies for securing and maintaining cross-institutional collaboration. Their skills in neurodevelopmental issues and leadership of interdisciplinary teams will be invaluable and will assist in forming long-term regional collaborative research relationships.

#### **1c3. Local Seminars and Conferences**

To supplement the mentorship and coursework outlined above, I will attend and present at regional research seminars and conferences relevant to my research and career development. For example, weekly research-in-progress seminars for the Department of Obstetrics and Gynecology, twice monthly School of Medicine Clinical Research and Methods seminars, and monthly \_\_\_\_\_ Program Research Seminars. I will participate in the annual \_\_\_\_\_ Department of OBGYN Postgraduate Course; I am currently scheduled to speak on Cerebral Palsy in \_\_\_\_\_. I will also present topics related to my research interest at the Department of OBGYN Grand Rounds at least semi-annually.

#### **1c4. National Meetings**

During the award period, I will attend and participate in three national meetings per year that are relevant to my research and career development. I will attend the annual meetings of the Society of Maternal Fetal Medicine, the Society of Gynecologic Investigation, and the Pediatric Academic Societies. As noted in the letters of support from Drs. \_\_\_\_\_, expenses for these meetings will be covered by \_\_\_\_\_ Department of Obstetrics and Gynecology funds.

#### **1c6. Level of Commitment and Proposed Allocation of Time**

During the award period, I will commit 75% effort towards the research project and related research and career development (**Table 3**).

**Table 3: Proposed Allocation of Time**

| Activity                                     | Year 1     | Year 2     | Year 3     |
|----------------------------------------------|------------|------------|------------|
| <b>K23 activities</b>                        |            |            |            |
| Research project                             | 40%        | 40%        | 40%        |
| Coursework & independent study with mentors  | 25%        | 20%        | 10%        |
| Meeting with mentors, conferences & seminars | 10%        | 10%        | 10%        |
| R-01 grant writing                           | 0%         | 5%         | 15%        |
| <b>Total K23 effort</b>                      | <b>75%</b> | <b>75%</b> | <b>75%</b> |
| <b>Clinical and Teaching Effort</b>          | <b>25%</b> | <b>25%</b> | <b>25%</b> |

My clinical responsibilities will not exceed 25% during the award period as guaranteed in the attached letters from Dr. \_\_\_\_\_, Dr. \_\_\_\_\_, and Dr. \_\_\_\_\_. During the \_\_\_\_\_ award period, my research time has been well-protected and this precedent will be continued for the duration of this proposed award. My clinical duties will involve Maternal-Fetal Medicine Clinic, approximately three weeks per year on-service as the Obstetrical and Perinatal Attending, and occasional night and weekend call (approximately 4 nights per month). This amount of clinical involvement is necessary to fulfill my MFM Board Certification requirements. My teaching duties include lectures and small group discussions with residents and medical students. I will have no administrative responsibilities.

**1c7. Timeline for Career Development and Research Plan**

In consultation with my mentors and advisors, I have proposed a career development and research plan designed to support my short and long-term career goals (Table 4). Although the didactic plan appears ambitious, many of the courses, including the RATS courses, are brief (2-3 hours), intense didactic efforts. I will never have more than 2 weekly courses per semester. The \_\_\_\_\_ time commitment is less than one-half day per week. This schedule will fulfill my specific training aims and will prepare me to be an independent clinical investigator by the completion of the award period.

**With the protection of research time guaranteed by Drs. \_\_\_\_\_, I believe that this plan, while ambitious, is feasible. Ongoing review of progress with mentors and advisors will assure appropriate allocation of time throughout the award period.**

**1d. Training in the Responsible Conduct of Research**

**Training Aim 5: Understand the ethical and legal implications of human subjects research, with emphasis regarding research in clinical genetics and research with vulnerable subjects**

**Rationale** Bioethics training is vital to a career in genetics research. In addition, specific ethics training in vulnerable subject research, such as research involving children and those with neurodevelopmental disability, is necessary. In fellowship, I completed a course in bioethical issues in clinical research. I will strengthen my bioethics training through the formal program outlined below, with emphasis on my area of research. I have completed the University's IRB training on the protection of human subjects (See Appendix B for certificate of training).

**Coursework** (Note that the first class will be completed pre-award)

- 1. MDCRC 6430 Bioethical Issues in Clinical Research.** Ethical issues and standards for scientific investigation are covered in-depth. Course-work emphasizes the history and evolution of research norms and practices, institutional expectations and standards, and the process of review and oversight for experimental protocols. Additional material covers ethical issues and public policy linked to genetic research. Completed Summer 2006 (1 credit hour).
- 2. PHIL 7570 Case Studies and Research Ethics.** An examination of research integrity and other ethical issues involved in scientific research. Objectives are to increase ethical sensitivity to issues regarding clinical research, aid in developing moral reasoning skills, and discuss relevant policies, procedures, and professional standards of ethical research. Topics include scientific fraud, conflicts of interest, plagiarism and authorship designation, and the role of science in formulating social

Program Director/Principal Investigator (Last, First, Middle): \_\_\_\_\_

policy. The Fall Semester curriculum emphasizes issues specific to medical and basic science research. The Spring Semester curriculum emphasizes issues specific to social science research. Fall and Spring Semesters 2011-2012 (1 credit hour each semester).

3. **Ethics of Research with Children.** This course is a seven-module case-based learning course involving the ethical conduct of research with children and is offered through the Office of Pediatric Clinical and Translational Research. It is generally available only to participants in the Pediatric Clinical and Translational (PCAT) Research Scholars Program. Although I am not eligible to be a PCAT scholar since I am not in the Department of Pediatrics, I have been invited to attend the PCAT Research Scholars Seminars on the basis of collaborative research projects that are ongoing with the Department of Obstetrics and Gynecology. A letter of support from the PCAT Research Scholars Program Director, Dr. \_\_\_\_\_, follows.
4. **Responsible Conduct in Research/ Conflict of Interest.** Offered as part of the Research Administration Training Series (RATS), Sponsored by the Office of the Vice President for Research. Addresses the proper conduct and reporting of research. Participants will become familiar with federal regulations, professional standards and University policies regarding research integrity and responsibilities, ethics and compliance obligations, and conflict of interest issues. A case history review, procedures for suspected abuse, potential sanctions for noncompliance, and instructions on completing a disclosure form will be discussed. One 2 hour seminar.

#### **Mentorship**

\_\_\_\_\_, MD, is a tenured Professor of Pediatrics at the \_\_\_\_\_ and has a long-standing clinical and research interest in bioethics. \_\_\_\_\_ currently serves as the University's Associate Vice President for Research Integrity and is also involved in various bioethics capacities both within the \_\_\_\_\_ and on a national level (AAP's Committee on Bioethics, NIH Human Genetics Study Section, and bioethics advisory capacities for the FDA and CDC). The study of genetic and environmental contributions to Cerebral Palsy and neurodevelopmental delay provides many opportunities to address issues in research ethics. Therefore, beyond the aforementioned specific educational objectives, I will meet with Dr. \_\_\_\_\_ on a quarterly basis, and whenever needed, to review and discuss the ethical issues that arise during my training and research. This is confirmed in Dr. \_\_\_\_\_'s letter of support.

**Table 4. Timeline for Career Development and Research Plan**

| Shading reflects intensity of effort                                                                               | Year 1 (2009-2010) | Year 2 (2010-2011) | Year 3 (2011-2012) |
|--------------------------------------------------------------------------------------------------------------------|--------------------|--------------------|--------------------|
| <b>Research Specific Aim 1: Identify genetic polymorphisms associated with Cerebral Palsy (CP)</b>                 |                    |                    |                    |
| Multiplex SNP assay: platform design                                                                               | ██████████         |                    |                    |
| Data acquisition                                                                                                   |                    | ██████████         |                    |
| Data analysis                                                                                                      |                    | ██████████         |                    |
| Manuscript preparation                                                                                             |                    | ██████████         | ██████████         |
| <b>Research Specific Aim 2: Identify genetic polymorphisms associated with neurodevelopmental delay (NDD)</b>      |                    |                    |                    |
| Multiplex SNP assay: platform design                                                                               | ██████████         |                    |                    |
| Data acquisition                                                                                                   |                    | ██████████         |                    |
| Data analysis                                                                                                      |                    | ██████████         |                    |
| Manuscript preparation                                                                                             |                    | ██████████         | ██████████         |
| <b>Research Specific Aim 3: Identify gene-gene and gene-environment interactions that contribute to CP and NDD</b> |                    |                    |                    |
| Data analysis                                                                                                      |                    | ██████████         |                    |
| Manuscript preparation                                                                                             |                    | ██████████         | ██████████         |
| <b>Coursework</b>                                                                                                  |                    |                    |                    |
| Biostatistics I                                                                                                    | ██████████         |                    |                    |
| Biostatistics II                                                                                                   |                    | ██████████         |                    |
| Epidemiology I                                                                                                     | ██████████         |                    |                    |
| Epidemiology II                                                                                                    |                    | ██████████         |                    |
| Perinatal Epidemiology                                                                                             |                    |                    | ██████████         |
| Linear and Logistic Regression Models                                                                              |                    |                    | ██████████         |
| Applied Multivariate Data Analysis                                                                                 |                    | ██████████         |                    |
| Medical Genetics for Clinical Investigators                                                                        | ██████████         |                    |                    |
| Genetics of Complex Diseases*                                                                                      |                    |                    |                    |
| Foundations of Genetic Epidemiology                                                                                |                    |                    | ██████████         |
| Design/Implementation of Clinical Trials*                                                                          |                    |                    |                    |
| Research Administration Training Series (RATS)                                                                     |                    |                    |                    |
| -The Clinical Research Certificate                                                                                 | ██████████         |                    |                    |
| -Pre-Award Certificate                                                                                             |                    | ██████████         |                    |
| -Post-Award Certificate                                                                                            |                    |                    | ██████████         |
| Case Studies and Research Ethics                                                                                   |                    |                    | ██████████         |
| Bioethical Issues in Clinical Research*                                                                            |                    |                    |                    |
| Ethics of Research with Children                                                                                   |                    |                    | ██████████         |
| Responsible Conduct in Research/<br>Conflict of Interest (RATS Series)                                             |                    | ██████████         |                    |
| <b>R Grant</b>                                                                                                     |                    |                    |                    |
| Preparation and Writing                                                                                            |                    | ██████████         | ██████████         |

\*will be completed pre-award

Program Director/Principal Investigator (Last, First, Middle): \_\_\_\_\_

**2. STATEMENTS BY MENTOR, CO-MENTOR AND ADVISORS**

**2a. Letters of Support from Mentors (following)**

Statement from Primary Mentor:

\_\_\_\_\_

Statement from Co-Mentors:

\_\_\_\_\_

**2b. Letters of Support from Advisors (following)**

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\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

**2c. Sealed Letters of Recommendation (attached to face page)**

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

**2e. Additional Letters of Support (following)**

\_\_\_\_\_

\_\_\_\_\_

Maternal-Fetal Medicine

September 22, 2008

Center for Scientific Review  
National Institutes of Health  
6701 Rockledge Drive  
Room 1040 - MSC 7710  
Bethesda, MD 20892-7710

re: \_\_\_\_\_, M.D.  
Mentored Patient-Oriented Research Career Development Award  
(K23) - \_\_\_\_\_

Dear Ladies and Gentlemen:

It is my privilege to serve as a primary mentor for Dr. \_\_\_\_\_ and to write this letter supporting \_\_\_\_\_ application for a NICHD Mentored Patient-Oriented Career Development Award (K23). Obstetrics and Gynecology faces a shortage of young physician scientists, and Dr. \_\_\_\_\_ is focusing on a very difficult area of our specialty - \_\_\_\_\_

\_\_\_\_\_ This is clearly an area without simple solutions and Dr. \_\_\_\_\_ is grappling with multiple complex factors that will doubtless impact the rate and severity of these devastating conditions. If I might sum up the remainder of this document: \_\_\_\_\_ MD is outstanding and is going to be a superstar!

By way of personal introduction, I currently serve as the Vice-Chair for Research in the Department of Obstetrics and Gynecology at the \_\_\_\_\_ I am the Principal Investigator on two NICHD multi-center clinical research networks \_\_\_\_\_

\_\_\_\_\_ and the Co-PI on five others ( \_\_\_\_\_

\_\_\_\_\_). I also serve on the \_\_\_\_\_ I have served functionally as a research mentor to the majority of Maternal-Fetal Medicine fellows at the \_\_\_\_\_ over the past \_\_\_\_\_ years (100% of who have maintained full-time academic appointments), as a research advisor for the Department of Pediatrics' K-12 program and as Dr. \_\_\_\_\_'s primary mentor for the \_\_\_\_\_ that \_\_\_\_\_

obtained through the NICHD \_\_\_\_\_  
).

I have known Dr. \_\_\_\_\_ since \_\_\_\_\_ was a fourth-year medical student on our obstetric service. From that time to the present, \_\_\_\_\_ has demonstrated a consistent interest in a research career. As detailed elsewhere in this application as well as in numerous letters of support, \_\_\_\_\_ is off to a meteoric start in her research career. I continue to be impressed with \_\_\_\_\_ initiative, organization and intense commitment to research. \_\_\_\_\_ has proven \_\_\_\_\_ to be a 'quick study' and has created \_\_\_\_\_

\_\_\_\_\_ This work has been accepted for an oral presentation at the \_\_\_\_\_ meeting and an extension of the work is being pursued in anticipation of submission to the \_\_\_\_\_ meeting. I have been meeting with her at least twice monthly since the initiation of this program and will continue that schedule. My office is on the same hallway as Dr. \_\_\_\_\_'s and my door is always open for more informal mentoring.

You should understand that Dr. \_\_\_\_\_ award counts toward the five-year limit of K-award funding. Given that the \_\_\_\_\_ award is only a two-year award, that \_\_\_\_\_ is beginning \_\_\_\_\_ second year, and that Dr. \_\_\_\_\_ is an outstanding candidate for K23 funding, we have chosen to submit this application for three years of K23 funding in hopes that it can follow immediately upon the completion of \_\_\_\_\_ award. Although \_\_\_\_\_ is just now in \_\_\_\_\_ third year of MFM fellowship, I am confident that, by the time \_\_\_\_\_ finishes five years of mentored research training (and likely sooner), \_\_\_\_\_ will be able to successfully compete for R01 funding. As documented elsewhere in this submission, \_\_\_\_\_ has already been recruited as a co-investigator with a team from \_\_\_\_\_ on an R21 submission and I am confident that \_\_\_\_\_ collaborative skills and contacts will expand rapidly over the next few years.

I would like to particularly emphasize Dr. \_\_\_\_\_'s proposed participation in the \_\_\_\_\_ program. This will substantially expand \_\_\_\_\_ understanding of neurodevelopmental disabilities and will, in turn, significantly enhance that program's effectiveness if \_\_\_\_\_ then returns to it in a faculty context. It will also provide \_\_\_\_\_ with substantial training, both didactic and experiential, in team leadership.

As documented in Dr. \_\_\_\_\_'s letter, we are restructuring the neonatal follow-up program with the intent of making it a truly inclusive statewide program build on a collaboration of the \_\_\_\_\_, the \_\_\_\_\_ and all hospitals in the State with Neonatal Intensive Care Units. We envision Dr. \_\_\_\_\_ being able to play a prominent clinical and administrative role in this undertaking and the proposed K23 award will greatly enhance that possibility.



\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

September 22, 2008

Center for Scientific Review  
National Institutes of Health  
6701 Rockledge Drive  
Room 1040 - MSC 7710  
Bethesda, MD 20892-7710

re: \_\_\_\_\_  
Mentored Patient-Oriented Research Career Development Award  
(K23)

Dear Review Committee Members:

As the Maternal-Fetal Medicine Division Chief at the \_\_\_\_\_  
\_\_\_\_\_ for the past \_\_\_\_\_ years and the Principal Investigator on two  
NIH-funded multicenter research networks, I enthusiastically support Dr. \_\_\_\_\_  
s K23 application entitled \_\_\_\_\_

Dr. \_\_\_\_\_ is an outstanding  
addition to our Division, repeatedly demonstrating \_\_\_\_\_ dedication to improving  
outcomes for pregnant women and their children. \_\_\_\_\_ is a critical thinker and  
predictably makes valuable contributions to our clinical and research  
conferences. \_\_\_\_\_ has published three manuscripts and has two others under  
review.

Our Division is strongly committed to Dr. \_\_\_\_\_s success and will continue to  
protect the time \_\_\_\_\_ needs for research and career development. We have  
sufficient staffing and financial resources to ensure that Dr. \_\_\_\_\_ will have at  
least 75% protected time for research and career development upon receipt of  
this award. We also have research support staff to assist with project  
implementation, follow-through, and subsequent grant administration. We are  
committed to ensuring that the salary will be competitive with \_\_\_\_\_ academic rank  
and accomplishments. Our Division and Department will cover the expenses of  
professional meetings during the course of \_\_\_\_\_ training awards. We will  
continue to provide \_\_\_\_\_ with an office, administrative assistance, computer and  
support in developing agreements/contracts as needed. \_\_\_\_\_ does not have any  
administrative responsibilities at present and will not receive any for the duration  
of \_\_\_\_\_ post-fellowship training. We will, of course, abide by all financial and  
other requirements stipulated by the National Institutes of Health.

It should be noted that, in the course of the first year of \_\_\_\_\_ two-year \_\_\_\_\_  
award, \_\_\_\_\_ has developed productive relationships with \_\_\_\_\_

Program Director/Principal Investigator (Last, First, Middle): \_\_\_\_\_

Network staff and personnel as well as with research personnel at throughout our University, our community and elsewhere. This ability to develop and maintain collegial and collaborative relationships is an intrinsic asset that will serve Dr. \_\_\_\_\_ well in \_\_\_\_\_ subsequent research career:

I believe that Dr. \_\_\_\_\_'s career development plan is well-designed and feasible. In contrast to many other K-award submissions, Dr. \_\_\_\_\_'s research proposal is not overly ambitious. It is focused and provides an excellent platform for acquisition of research techniques.

We will continue to prioritize the support and protection of Dr. \_\_\_\_\_'s time to ensure that \_\_\_\_\_ can accomplish the research and career goals proposed. \_\_\_\_\_ success will support the mission of our Division and contribute to the growth of our own research capabilities.

In summary, \_\_\_\_\_ is an outstanding candidate for a research career development award. I will do anything I can to help \_\_\_\_\_ become a successful career scientist.

Sincerely,

\_\_\_\_\_  
Professor and Maternal-Fetal Medicine Division Director  
Department of Obstetrics and Gynecology

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Program Director/Principal Investigator (Last, First, Middle): \_\_\_\_\_

September 9, 2008

Center for Scientific Review  
National Institutes of Health  
6701 Rockledge Drive  
Room 1040 - MSC 7710  
Bethesda, MD 20892-7710

re: \_\_\_\_\_, M.D.  
Mentored Patient-Oriented Research Career Development Award (K23)

Dear Review Committee Members:

This letter confirms my enthusiastic willingness to serve as an educational and research advisor and consultant to Dr. \_\_\_\_\_'s K23 program. By way of introduction, I am a tenured Professor of Pediatrics at the \_\_\_\_\_ and have a long-standing clinical and research interest in bioethics. I currently serve as the \_\_\_\_\_'s Associate Vice President for Research Integrity and am also involved in various capacities in bioethics both within our Institution and on a national level. I am a member of the \_\_\_\_\_  
I chair the \_\_\_\_\_  
I serve on the \_\_\_\_\_  
, and I am on the \_\_\_\_\_

I have reviewed Dr. \_\_\_\_\_'s bioethics training to date and have made several specific recommendations to Drs. \_\_\_\_\_ about appropriate additional bioethics training opportunities within the \_\_\_\_\_. In particular, Professors \_\_\_\_\_ offer an excellent graduate level biomedical ethics course through the Department of Philosophy each fall and my office offers several medical ethics modules within our Research Administration Training Series. In addition, \_\_\_\_\_ participation in the \_\_\_\_\_ Program will provide additional hands-on ethics exposure both to involved families and to the perspectives of the multidisciplinary health care providers with whom these families interact.

I have discussed with Dr. \_\_\_\_\_ and Dr. \_\_\_\_\_ the objectives of this grant submission. The study of genetic and/or environmental contributions to neurodevelopmental delay will with certainty provide numerous intersections with biomedical ethics. Beyond the aforementioned specific educational objectives, I am willing and able to meet with Dr. \_\_\_\_\_ on a quarterly basis, and whenever needed, to review and discuss the bioethical aspects of \_\_\_\_\_ training and research.

Program Director/Principal Investigator (Last, First, Middle): \_\_\_\_\_

Dr. \_\_\_\_\_'s K23 proposal clearly complies with the bioethics training requirements of the RFA and I strongly encourage you to consider \_\_\_\_\_ application favorably.

Sincerely,

\_\_\_\_\_  
Professor of Pediatrics  
Associate Vice President for Research Integrity,  
Research Administration Building

Phone: \_\_\_\_\_  
Email address: \_\_\_\_\_

**3. ENVIRONMENT AND INSTITUTIONAL COMMITMENT TO THE CANDIDATE**

**3a. Description of Institutional Environment**

**3a1. Supportive Environment**

**3a2. Resources for Research within the**

**Maternal Fetal Medicine Fellowship**

**3a3. Resources within the \_\_\_\_\_**

**K30 Training Program in Clinical Investigation (TPCI)**

The TPCI is a two to three-year post-graduate training program in clinical investigation with an emphasis on the inherited basis of human disease. The TPCI is funded by an NIH Clinical Research Curriculum Award \_\_\_\_\_. The program consists of formal didactic coursework, a longitudinal seminar series, and a mentored clinical research project. Trainees who successfully complete the program are awarded a Masters Degree in Clinical Investigation from the \_\_\_\_\_. This course is designed to teach physician-scientists the essential concepts in performance of clinical and/or laboratory-based research. The primary goals of this program are to produce independent clinical investigators who are able to successfully apply for extramural grant support by the completion of their training.

The specific aims of the TPCI program are outlined on pages 25-26 of the Resource Section. The program at the \_\_\_\_\_ is under the excellent direction of Dr. \_\_\_\_\_, Professor of Medicine and Principal Investigator and Director of the \_\_\_\_\_, and Dr. \_\_\_\_\_, Professor Medicine at the \_\_\_\_\_ and Chief of the Division of Clinical Epidemiology. The recent acquisition by the \_\_\_\_\_ award will ensure continuation of this important program for fostering the development of independent clinical researchers.

**Research Administration Training Series (RATS)**

The Office of the Vice President for Research offers several continuing education and training opportunities designed to support, develop and maintain a standardized body of knowledge and best practice methodology for all research personnel at the \_\_\_\_\_. The Research Administration Training Series (RATS) provides a comprehensive curriculum for overall research administration at the \_\_\_\_\_. The curriculum includes traditional classes and lectures, interactive workshops, online instruction and educational resources provided to ensure compliance with federal regulations and to enhance the overall productivity of researchers.

The program goals are:

1. To facilitate a trained workforce in research administration supporting the academic mission of the \_\_\_\_\_
2. To provide coordinated education and training programs which assist faculty and staff in the management of research activities
3. To advance professional development and high performance standards for all research administrators at the \_\_\_\_\_
4. To promote a culture of compliance and research integrity at the \_\_\_\_\_

While all members of the University research community are invited to participate in individual classes of interest, the Office of the Vice President for Research recognizes the completion of certain specialized tracks of instruction by awarding a Certificate of Achievement. Each Certificate of Achievement is designed to address the specific and immediate needs of the participant through Core Courses, Requisite Courses and Elective Courses. The Certificate of Achievement Program offers participants the opportunity to earn and be awarded the following official documents of recognition: the **Pre-Award Certificate** acknowledges study of the proposal development and funding search process in research administration; the **Post-Award Certificate** acknowledges study of the accounting and project management aspects of research administration; the **Clinical Research Certificate** acknowledges study of clinical trials management and the conduct of human subject investigations.

Program Director/Principal Investigator (Last, First, Middle): \_\_\_\_\_

**Center for Clinical and Translational Sciences (CCTS)**

The \_\_\_\_\_ has been awarded a Clinical and Translational Science Award \_\_\_\_\_ to develop a Center for Clinical and Translational Sciences (CCTS). This Center builds upon the University's strengths in genetics and biomedical informatics and will incorporate the existing General Clinical Research Center (GCRC) and K30 Clinical Research Curriculum into the CCTS. The CCTS will be housed in \_\_\_\_\_ of space renovated specifically for the Center, comprising an entire floor of a building attached to the School of Medicine. Most CCTS members will be faculty in the School of Medicine, the College of Nursing, the College of Pharmacy, the College of Health, and the College of Engineering. Other CCTS members have primary appointments in other colleges, schools and departments located on the campus of the \_\_\_\_\_ and in our partner institutions of the \_\_\_\_\_

The goals of the \_\_\_\_\_ CCTS are to establish an academic home, located in a dedicated space, to support clinical and translational research by consolidating resources, promoting interdepartmental and interdisciplinary research, and facilitating increased interactions between basic scientists and clinical investigators. The Center will also promote the development of a new generation of clinical and translational investigators through a variety of educational programs and, most importantly, through a mentoring program to provide support and promote recognition of these investigators.

**Pediatric Clinical and Translational (PCAT) Research Scholars Program**

The Department of Pediatrics' commitment to the success of clinical investigators, particularly during the early years of career development, is seen in the establishment of the Grants and Research Support Office for Clinical and Translational Research ( \_\_\_\_\_ ) and the development of the Pediatric Clinical and Translational (PCAT) Research Scholars Program. Both are directed by Dr. \_\_\_\_\_ . The goal of the PCAT program is to support the career development of promising clinician scientists. The program provides biostatistical support, research project coordination and administrative assistance, and scientific and career development mentorship from senior investigators in the Department of Pediatrics. As part of the increasingly collaborative and overlapping research interests of the Departments of Pediatrics and Obstetrics and Gynecology, PCAT resources are now available to promising clinician-scientists from both Departments.

**3a4. Resources Outside the \_\_\_\_\_**



**3b. Institutional Commitment to Candidate's Research Career Development**

\_\_\_\_\_, MD, \_\_\_\_\_ Professor and Chair of the Department of Obstetrics and Gynecology, \_\_\_\_\_ (letter following)

**Please also see the preceding letters of support from Drs. \_\_\_\_\_ which reference the strong institutional commitment to Dr. \_\_\_\_\_.**

As documented throughout this application, the \_\_\_\_\_ has a strong, well-established research and training program in Dr. \_\_\_\_\_'s areas of interest (maternal-fetal medicine, clinical genetics, human neurodevelopment). These programs offer high-quality research environments in which \_\_\_\_\_ will interact on a daily basis with productive and collaborative faculty. The \_\_\_\_\_ is unequivocally committed to the development of Dr. \_\_\_\_\_ into a productive, independent investigator and will comply with the spirit and all details of the K23 award program. The \_\_\_\_\_ support of Dr. \_\_\_\_\_ is NOT contingent upon receipt of this award.

As documented in Dr. \_\_\_\_\_'s letter of support, Dr. \_\_\_\_\_ will have unrestricted access to research opportunities in the Department of Human Genetics. Drs. \_\_\_\_\_ have clearly documented their commitment to Dr. \_\_\_\_\_'s clinical training. This application amply documents that the \_\_\_\_\_ is a national leader in both maternal-fetal medicine and human genetics, with experienced and successful senior mentors standing ready to actively assist in Dr. \_\_\_\_\_'s career development. The \_\_\_\_\_ program provides an additional unique multidisciplinary training opportunity for Dr. \_\_\_\_\_. \_\_\_\_\_ also has an impressive team of advisors who are willing and able to facilitate numerous aspects of \_\_\_\_\_ career development and research goals.

Drs. \_\_\_\_\_ all confirm that \_\_\_\_\_ will have 75% of \_\_\_\_\_ time protected for this award and that the Department of Obstetrics and Gynecology stands willing and able to cover any salary and research expenses beyond the amount of this proposed award. It is clearly stated and understood that Dr. \_\_\_\_\_ will be released from normal clinical, teaching and administrative responsibilities.

September 8, 2008

Scientific Review Committee  
National Institutes of Health  
Bethesda, Maryland 20892

RE: Mentored Patient-Oriented Research Career Development  
Award (K23) for \_\_\_\_\_, M.D.

Dear Review Committee Members:

I enthusiastically support Dr. \_\_\_\_\_ K23 application entitled, \_\_\_\_\_ Dr.

\_\_\_\_\_ has been a member of our Department for the past six years, the first four as a resident and the past two in \_\_\_\_\_ three-year Maternal-Fetal Medicine fellowship. During this time \_\_\_\_\_ has quietly, but relentlessly, pursued \_\_\_\_\_ quest for excellence in clinical medicine and research. \_\_\_\_\_ enthusiasm for research is recognized by \_\_\_\_\_ colleagues and fully supported by our Department. I have met with \_\_\_\_\_ on several occasions to discuss \_\_\_\_\_ research and career development goals. \_\_\_\_\_ goal of becoming an independent investigator with expertise in genetic and environmental predisposition to neurodevelopmental delay is very well suited to the vision of our Department, our University, and our community.

Dr. \_\_\_\_\_ has developed strong relationships with \_\_\_\_\_ mentors, who, in turn, have the expertise and experience to guide \_\_\_\_\_ research and career development. Our Obstetrics and Gynecology Research Network is led by Dr. \_\_\_\_\_, Dr. \_\_\_\_\_ primary mentor, and will provide an ideal setting for Dr. \_\_\_\_\_ to pursue \_\_\_\_\_ goals.

I am pleased to offer all the resources of the Department to support the research proposed in \_\_\_\_\_ application. I will ensure that \_\_\_\_\_ has the protected time from clinical responsibilities to conduct research and pursue \_\_\_\_\_ career development plan as outlined in this application. I also confirm that \_\_\_\_\_ salary will be consistent with our established Departmental salary structure and that the remaining 25% of \_\_\_\_\_ effort is to be consistent with the plan developed by Drs. \_\_\_\_\_ to develop Dr. \_\_\_\_\_ into an independent investigator in patient-oriented research. \_\_\_\_\_ will have a

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Program Director/Principal Investigator (Last, First, Middle): \_\_\_\_\_

Scientific Review Committee  
Page Two  
September 8, 2008

full-time faculty appointment at the \_\_\_\_\_ during the course of this proposed training program.

I look forward to continuing to advise and support Dr. \_\_\_\_\_ as \_\_\_\_\_ develops into an independent investigator. The support of the Department is not contingent upon Dr. \_\_\_\_\_ receiving this K23 award.

I support \_\_\_\_\_ application without reservation. If I can provide any further information, please do not hesitate to contact me.

Sincerely

\_\_\_\_\_  
Department of Obstetrics & Gynecology

Phone: \_\_\_\_\_  
Fax: \_\_\_\_\_  
email: \_\_\_\_\_

## 4. RESEARCH PLAN

### 4a. Specific Aims

Cerebral palsy (CP) and other forms of neurodevelopmental delay are leading causes of disability among preterm infants. Genetic polymorphisms in inflammation, coagulation, and vascular response pathways may increase vulnerability to CP. However, only a small number of polymorphisms in these pathways have been assessed, and polymorphisms associated with CP have been inconsistent among studies. Further investigation of the association between CP and polymorphisms in the inflammation, coagulation, and vascular regulation pathways is therefore necessary. In addition, whether polymorphisms in these pathways contribute to other forms of neurodevelopmental delay is unknown.

We hypothesize that preterm infants with CP are more likely than those with normal neurodevelopment to have polymorphisms in genes involved in inflammation, coagulation, and vascular regulation pathways. We also hypothesize that polymorphisms in these pathways are associated with other forms of neurodevelopmental delay. These polymorphisms may contribute to cerebral injury in motor and non-motor pathways, and therefore confer susceptibility to both CP and other forms of neurodevelopmental delay.

We will test our hypotheses using a custom, multiplex approach to identifying single-nucleotide polymorphisms (SNPs). The research proposed will utilize samples in the NICHD Neonatal Research Network's (NRN's) Anonymized DNA Bank to evaluate genetic polymorphisms associated with CP and neurodevelopmental delay in premature infants. This DNA bank was formed from samples obtained in the NRN's Cytokines Study. The samples are currently housed at The Duke University Center for Human Genetics. De-identified clinical data are linked to the DNA Bank. The study cohort includes 1,074 infants who were enrolled in the Network's Cytokines Study. Samples consist of filter paper cards and whole genomic DNA. It is estimated that DNA samples and 18-22 month follow-up are available for 701 subjects. Of these subjects, 494 have neurodevelopmental delay, with an MDI or PDI <85, 102 have CP, and 207 have normal neurodevelopment.

**Specific Aim 1.** Identify genetic polymorphisms that are associated with CP in preterm infants using a custom, multiplex SNP assay designed to assess ~1300 SNPs in pathways related to white matter brain injury.

**Specific Aim 2.** Identify genetic polymorphisms that are associated with neurodevelopmental delay in preterm infants using the same custom multiplex SNP assay. Neurodevelopmental delay will be defined by the Bayley II Infant Neurodevelopment Screen.

**Specific Aim 3.** Perform analyses to identify unique gene-gene and gene-environment interactions that contribute to susceptibility to CP and/or neurodevelopmental delay among preterm infants.

This proposal represents a unique opportunity for correlation of genetic polymorphisms with well-characterized neurodevelopmental outcomes in a large cohort. It is also a unique opportunity to gain multi-disciplinary training and research experience to support my career goal of becoming a nationally-recognized authority on the genetic and environmental factors that contribute to adverse neurodevelopmental outcomes. This work will contribute to an increased understanding of the genetic risk factors predisposing a fetus to cerebral injury, and will thereby identify possible prevention strategies and provide a basis for future therapeutic intervention trials. In addition, this K23 award will provide me with advanced skills in research methods, statistical analysis, and genetic techniques critical to my development into an independent clinical investigator.

My future plans include the collaborative initiation of a comprehensive \_\_\_\_\_ neonatal follow-up program that will provide detailed population-based outcomes for children with recognized perinatal risks factors for adverse neurodevelopmental outcomes who were born and currently residing in \_\_\_\_\_. This would include a DNA Repository linked to long-term neurodevelopmental outcomes and the \_\_\_\_\_. This unique resource will allow for detailed genotype-phenotype correlation and hypothesis testing and will provide the basis for a future R award submission.

## **4b. Background and Significance**

### **4b1. Definition of Cerebral Palsy**

Cerebral palsy (CP) is well-recognized neurodevelopmental condition beginning in childhood and persisting throughout the lifespan. CP describes a group of disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain.<sup>2</sup> The motor disorders of CP are often accompanied by disturbances of sensation, cognition, communication, perception, and/or behavior, and/or by seizure disorder.<sup>2</sup> The components of CP classification include: motor abnormalities, associated impairments, anatomic and radiological findings, and causation and timing.<sup>2</sup> CP can be antenatal or postnatal (e.g. trauma, meningitis) in origin. The majority of cases of CP in term infants originate in the prenatal period. In premature infants, both prenatal and postnatal causes contribute. In the majority of cases, a specific cause is not identified.

### **4b2. Definition of neurodevelopmental delay**

Like CP, neurodevelopmental delay is a complex, heterogeneous disorder that can result from multiple etiologies. Abnormal early cognitive development in infants can be diagnosed on the basis of several different infant neurodevelopmental screens. One of the most widely used screening tools is the Bayley Scales of Infant Development-II (BSID-II).<sup>3</sup> This screening tool consists of a mental developmental index (MDI) and psychomotor developmental index (PDI). Scores of <70 by either index represent 2 SD below the mean (moderate-severe delay). Scores =70 and <85 represent 1 SD below the mean (mild delay).

### **4b3. The scope of Cerebral Palsy and other neurodevelopmental disabilities**

Neurodevelopmental disabilities, including CP, are among the leading childhood morbidities and are responsible for significant cost and life-long healthcare utilization. CP is a leading cause of permanent neurological impairment world-wide. In Western countries, the prevalence of CP is approximately 2 per 1000 live births.<sup>4-7</sup> It is estimated that CP affects more than 100,000 children in the US. Up to one-quarter of people with CP are severely affected and unable to walk, and approximately one-third are mentally handicapped.<sup>8</sup> It is estimated that CP costs society \$5 billion dollars each year.<sup>8</sup>

The prevalence of CP is inversely related to gestational age, and studies of preterm infants suggest rates of 15-200 per 1000 live births depending on gestational age, year of birth, and study population.<sup>9,10</sup> Controversy exists as to whether the prevalence of CP among preterm infants is increasing<sup>6,11-16</sup> or decreasing.<sup>9,17-20</sup> However, it is clear that CP and neurodevelopmental delay remain leading causes of permanent disability in preterm infants, despite ongoing research and efforts to reduce preterm birth rates.

The preterm birth rate in the US is now 12.7% and rising.<sup>21</sup> Preterm births <28 weeks represent approximately 2% of all births but represent 25% of all children with CP.<sup>22</sup> In a late 1990's population-based survey, children with birth weights <2500 grams (who constituted 5.3% of survivors) contributed 47% of all CP cases, and those with birth weights <1000 grams (0.2% of survivors) contributed 8%.<sup>23</sup> Cohorts of very preterm infants suggest that approximately one quarter of very preterm survivors have significant long-term neurosensory disability.<sup>24,25</sup> Furthermore, very preterm children without early disability remain at risk for a range of motor, cognitive, behavioral, and psychological deficits during childhood.<sup>26</sup>

### **4b4. The etiologies of Cerebral Palsy and neurodevelopmental disabilities in preterm infants**

The traditional view that CP was most often caused by intrapartum asphyxia and fetal brain damage as a result of poor obstetric management has been refuted in epidemiologic studies.<sup>27, 28</sup> These studies indicate that the majority of cases are not associated with intrapartum asphyxia, but rather maternal and fetal antenatal factors such as prematurity, fetal growth restriction, intrauterine infection, fetal coagulation disorders, multiple gestation, antepartum hemorrhage, and congenital abnormalities. There is increasing evidence that a majority of CP is antenatal in origin. Intrauterine infections, inherited thrombophilias, cytokine polymorphisms, and apolipoprotein E genotypes are recognized susceptibility factors.<sup>29-35</sup>

The etiology of CP differs according to different gestational age groups and different CP subtypes.<sup>36</sup> Prematurity may confer risk for adverse neurodevelopmental outcomes in several ways, including increased risk of hemorrhagic brain injury (intra or periventricular hemorrhage, or hemorrhagic parenchymal infarction) and injury to the periventricular white matter (periventricular leukomalacia [PVL]). In addition, the conditions complicating pregnancy (maternal or fetal) which lead to premature delivery, such as chorioamnionitis, may themselves have important consequences for neurodevelopment. A distinction exists between injuries that occur in the mature and immature brain, which may be explained by differences in free-radical generation and management, glutamate receptor expression or programmed cell death.<sup>36</sup> Characteristics of preterm infants, including immature cerebrovascular autoregulation, fragility of

blood vessels, and the presence of the germinal matrix, also increase vulnerability to neurologic injury. Both motor and cognitive dysfunction may result from these injuries.<sup>37,38</sup>

In premature infants, spastic diplegia is the dominant form of CP, and its cause, as indicated by neuroimaging<sup>39</sup> and neuropathology,<sup>40</sup> is most often periventricular white matter brain injury, or periventricular leukomalacia (PVL). This type of injury may be found as the etiologic determinant of CP, and a spectrum of cognitive and behavioral disorders, in preterm infants. While this lesion is sometimes described as ischemic, the most prominent risk factors identified for white matter disorders are inflammatory conditions and cytokine exposure.<sup>41</sup> Overexpression of inflammatory cytokines has been observed in areas of leukomalacia.<sup>42</sup> There also may be potentiation of hypoxic-ischemic insults by prenatal exposure to inflammation or infection.<sup>43</sup> This injury may result from the vulnerability of the immature oligodendrocytes before 32 weeks gestation. Oxidative stress and excitotoxicity resulting from excessive stimulation of ionotropic glutamate receptors on immature oligodendrocytes are the most prominent molecular mechanisms for PVL in premature infants.<sup>44</sup>

#### **4b5. The association of Cerebral Palsy with intrauterine inflammation**

Intrauterine infection/inflammation has been identified as a major contributor to CP in term and near-term infants.<sup>45-49</sup> Although the association is less strong, intrauterine infection/inflammation has also been associated with CP in preterm infants.<sup>45,46,50</sup> Conflicting study results for preterm infants may be related to differences in study populations and differences in susceptibility by stages of neurologic development.

Intrauterine infection/inflammation is also among the most identifiable causes of spontaneous preterm birth (SPTB). Maternal inflammatory cytokine polymorphisms in the TNF- $\alpha$ , IL-6, and IL-4 genes, as well as other inflammatory mediators, have been associated with SPTB.<sup>51-58</sup> Fetal inflammatory genes have also been evaluated, and at least six polymorphisms have been found to be more prevalent in fetuses delivered prematurely. An increased incidence of TNF- $\alpha$ , IL-1 receptor, IL-4 receptor, matrix metalloprotease-9 (MMP-9), MMP-1, and toll-like receptor-4 (TLR-4) gene mutations have been found more commonly among infants who were delivered prematurely, when compared to infants delivered at term.<sup>58</sup>

When microorganisms or their products gain access to the fetus they stimulate the production of cytokines and a systemic response termed the Fetal Inflammatory Response Syndrome (FIRS). The fetal inflammatory response to intra-amniotic infection is biologically important, perhaps even more so than the maternal inflammatory response.<sup>59</sup> In fact, some investigators believe that chorioamnionitis primarily involves a fetal inflammatory response.<sup>60</sup> The onset of spontaneous preterm labor +/- preterm premature rupture of membranes may be preceded by a systemic proinflammatory cytokine response in the fetus, which may be a fetal response to the presence of microbial products.<sup>61</sup> FIRS may result in SPTB and has been implicated as a cause of perinatal injury that leads to CP. A multicenter cohort of 1078 preterm infants <1500 grams showed that fetal inflammatory responses contribute to cerebral white-matter damage and that maternal infection can damage the fetal brain without the presence of fetal brain infection.<sup>59</sup>

Several authors have found an increase in the risk for CP in preterm infants born to mothers with clinical chorioamnionitis.<sup>45,46,50</sup> A relationship between CP and intra-amniotic inflammation has also been demonstrated. Concentrations of cytokines such as TNF- $\alpha$ , interleukin (IL)-6, and IL-1 $\beta$  are increased in the amniotic fluid of fetuses that later develop CP<sup>62,63</sup> and IL-6 concentrations in umbilical cord plasma are elevated in neonates with white matter lesions associated with PVL.<sup>64</sup> Intrauterine infection leads to release of proinflammatory cytokines into the fetal circulation. These products may cross the blood-brain barrier (BBB), either as a result of immaturity of the BBB, or as a result of proinflammatory cytokines which disrupt its integrity. Cytokine-mediated white matter brain injury may then occur secondary to excitotoxic-induced brain damage mediated by the glutamate system. It remains unclear whether the cytokines themselves mediate or cause white-matter damage or whether damage occurs from the infection that initiated the response, or some combination thereof. The association of polymorphisms which increase proinflammatory cytokines with CP would suggest the former. It is hypothesized that cytokines act as a final common pathway for injury to the CNS and that this pathway may be initiated by a variety of insults, including infection, hypoxic-ischemic injury, reperfusion injury, and toxin-mediated injury.<sup>65,66</sup>

#### **4b6. The link between genetic polymorphisms in the inflammation pathway and Cerebral Palsy**

Fetal genetic mutations that predispose to inflammation may confer an increased risk for cerebral palsy.<sup>33-35</sup> Inflammatory cytokine polymorphisms could be detrimental by causing cytokine overproduction, which could result in inflammatory damage to fetal brain white matter. Alternatively, cytokine underproduction could leave the fetus susceptible to infection and/or white matter damage.

An exploratory case-control study of 96 very preterm infants and 119 controls showed several fetal polymorphisms associated with the development of CP, including a polymorphism in the lymphotoxin A (LTA) gene (thr26asn), a gene involved in cytokine function.<sup>33</sup> In a significantly larger case-control study of 443 infants with CP compared to 883 control infants (187 preterm infants <37 weeks gestation; 128 very preterm infants <32 weeks gestation), homozygous or heterozygous TNF- $\alpha$  polymorphism -308G/A was associated with hemiplegic CP for babies born <32 weeks gestation (OR 2.38, 95% CI 1.02-5.58).<sup>34</sup> At all gestational ages, a mannose-binding lectin (MBL) polymorphism in exon-1 codon-54 increased the risk of developing diplegia (OR 1.55, 95% CI 1.03-2.32).

TNF- $\alpha$  is an example of a gene with several well-characterized polymorphisms that has been linked to CP in preterm infants. The TNF-2 polymorphism is an allele in the promotor region of the gene and is represented as a single nucleotide G? A base-pair substitution at nucleotide -308 relative to the transcriptional start site. This polymorphism is associated with high levels of TNF- $\alpha$ . It has been suggested that regulatory polymorphisms of cytokines such as TNF- $\alpha$  may influence the outcomes of severe infection in adults.<sup>67</sup> Evidence suggests that increased levels of TNF- $\alpha$  also can be detrimental to the fetus, and directly or indirectly cause fetal brain damage. The combination of increased levels of TNF- $\alpha$  secondary to a promoter-region polymorphism, and the physiological upregulation as a result of infection, may contribute to the pathogenesis of white matter damage. TNF- $\alpha$  has been shown to be directly toxic to neurons and may cause white-matter damage by disturbing developmental transitions from the oligodendrocyte precursor to the mature oligodendrocyte.<sup>68</sup> TNF- $\alpha$  and other inflammatory cytokines can also activate endothelial cells by promoting a shift from an antithrombotic to a prothrombotic state.<sup>69</sup> Cytokines also may cause increased permeability of the BBB, allowing toxic mediators into the sensitive developing fetal brain.<sup>70</sup> Cytokines may also initiate their effects through initiation of preterm birth, an independent risk factor for the development of CP.

MBL is also an intriguing gene with regards to its association with neurodevelopmental outcomes. MBL plays an anti-infectious role, identifying and removing potentially infectious pathogens from the body.<sup>71</sup> Because the MBL pathway plays an important role in eliminating pathogens, especially in neonates and infants, in whom immune responses are immature, low levels of MBL could be deleterious in fetuses exposed to infection, because fetuses deficient in MBL may be more susceptible to subclinical infections and/or inflammatory events in-utero. The human MBL gene has a number of polymorphic sites capable of altering circulating levels. Two SNPs within the promoter region of exon-1 are G? C base pair substitutions in codons -54 and -57. These are associated with low MBL serum concentration compared to the wild-type allele.<sup>72</sup> Decreased circulating MBL levels result in an impaired ability to defend against infectious pathogens and an increase in susceptibility to infection. It is important to note that 90% of MBL-deficient individuals do not acquire repeated infections, possibly because of the redundant complement system.<sup>71</sup> The phenotypic manifestations of MBL deficiency may become apparent only when combined with another immunodeficiency, either acquired or genetically determined. Associations between MBL haplotypes and CP after perinatal viral exposure have been demonstrated.<sup>32</sup>

Apolipoprotein E (APOE) genotype also has been linked to CP.<sup>35</sup> In a case-control study of 209 individuals with CP, carriage of the APOE  $\epsilon$ 4 or  $\epsilon$ 2 allele was associated with an increased risk for CP when compared with gender- and race-matched controls (OR 3.4, 95% CI 1.4-8.7; OR 12.0, 95% CI 1.6-247.2). APOE is produced in the brain,<sup>73</sup> where it serves to protect against injury through multiple mechanisms.<sup>74,75</sup> These alleles attenuate the inflammatory response to brain injury. The  $\epsilon$ 4 allele, in particular, is less effective at attenuating this inflammatory response. The APOE genotype also may be linked to vascular mechanisms of cerebral injury leading to CP. Meta-analyses and neuroradiologic studies suggest increased susceptibility to stroke in carriers of the  $\epsilon$ 4 allele.<sup>76,77</sup> Interestingly, transgenic mice carrying the human  $\epsilon$ 4 allele show increased herpes simplex 1 virus CNS invasion, compared to wild-type mice.<sup>78</sup> These results are consistent with a role for apoE in regulation of the blood-brain barrier,<sup>79</sup> potentially allowing access of neurotrophic viruses to the developing brain. The association of APOE genotype with CP in preterm infants has not yet been explored.

#### **4b7. The association of Cerebral Palsy with fetal thrombosis**

Although white matter disorders appear to be the most common cause of CP in preterm infants, perinatal stroke also may be an etiologic factor.<sup>80</sup> Fetal stroke has been associated with postnatal epilepsy, mental retardation, and cerebral palsy. The entity is caused by antenatal ischemic, thrombotic, or hemorrhagic injury. Because perinatal thrombotic stroke is a known etiology of CP, fetal thrombophilias

are of interest as a risk factor. Perinatal thrombotic stroke is defined as arterial ischemic stroke between 28 weeks gestation and 28 days after birth. When this occurs in term and near-term infants, hemiplegic CP is the most common neurologic disability. In very preterm babies, the etiology is more often hemorrhagic, and spastic diplegia most often results. As the etiology of fetal stroke varies according to gestational age, it might be expected that genetic polymorphisms which contribute to these outcomes might differ.

#### **4b8. The link between genetic polymorphisms in the coagulation pathway and Cerebral Palsy**

An association between inherited thrombophilic polymorphisms and CP has been demonstrated. Methylenetetrahydrofolate reductase (MTHFR) polymorphism 677C/T reportedly doubles the risk of CP in preterm infants.<sup>29</sup> A combination of this polymorphism and heterozygosity for prothrombin gene mutation has been shown to increase the risk of quadriplegia five-fold at any gestational age.<sup>29</sup> In addition, a polymorphism in plasminogen activator inhibitor I (PAI1) has been associated with CP in very preterm infants (OR 3.2, CI 1.2-8.7).<sup>33</sup>

The interaction of inflammation and coagulation appears to be important in the genesis of CP in very preterm infants. TNF- $\alpha$  can downregulate the expression of thrombomodulin and suppress its downstream anticoagulant effects, and upregulate tissue factor and suppress tissue factor inhibitor, causing prothrombotic effects.<sup>81</sup> Because of the prothrombotic effects of TNF- $\alpha$  and other cytokines, the additional presence of a thrombophilic polymorphism may have an additive effect on the risk of white matter damage and adverse neurodevelopmental outcomes. Some of the genes associated with CP are involved in anti-oxidant and anti-excitotoxic defense. eNOS and ADRB-2 influence regulation of vascular tone and can alter blood flow to and within the brain and placenta and alter responsiveness of the vasculature to infection, procoagulant state, hypoxia, or hyperoxia.<sup>33</sup>

#### **4b9. The role of other pathways in conferring risk for adverse neurodevelopmental outcomes**

The inflammation and coagulation pathways are obvious targets for further evaluation, as relatively few SNPs in these pathways have been evaluated, and results have not been consistent between studies. In addition, several pathways of interest, including vascular regulation, and anti-oxidant and anti-excitotoxic defense, are largely unexplored. One small case-control study showed evidence of association of endothelial nitric oxide synthase (eNOS) -922A/G and beta-2 adrenergic receptor (ADRB-2) gln27glu with preterm CP.<sup>33</sup> Genes such as eNOS3 and ADRB-2 influence regulation of vascular tone and can alter blood flow to and within the brain and placenta and alter responsiveness of the vasculature to infection, procoagulant state, hypoxia, or hyperoxia. Some genes in these pathways have actions that could influence the development and function of the nervous system by additional and less explored mechanisms, such as involvement of proteins in memory consolidation and synaptic plasticity, which could potentially affect motor and cognitive function.

Glutamate is the primary excitatory CNS neurotransmitter. While it contributes to neuronal communication, it is also involved in neuropathological damage through the activation of excitatory amino acid receptors. Divalent calcium influx mediates excitotoxicity with a cascade of events involving free radical generation, mitochondrial dysfunction, and the activation of many enzymes, including phospholipases, endonucleases, and proteases, all of which have multiple recognized polymorphisms. The etiologic contributions of these pathways to CP and neurodevelopmental delay in the preterm infant are poorly understood, understudied, and merit additional investigation.

#### **4b10. The contribution of genetic polymorphisms to other forms of neurodevelopmental delay**

Survivors of very premature birth are at risk for a variety of neurodevelopmental disabilities, both motor and cognitive. However, there are very few studies evaluating genetic contributions to outcomes in preterm infants beyond CP. The development of motor and cognitive deficits in the surviving very preterm infant may be associated with genetic polymorphisms in similar pathways.

In a population of surviving children (n=148) born <32 weeks gestation, the IL-6 -174 G/C polymorphism was associated with postnatal brain injury (hemorrhage and PVL), and neurodevelopmental disability.<sup>82</sup> A study of the same cohort (n=113) evaluated the IL-6 -572 G/C polymorphism and showed an association between this rare polymorphism and impaired cognitive development.<sup>83</sup> Both the IL-6 -174 and -572 functional polymorphisms are associated with increased IL-6 synthesis. This result is consistent with the reported association of IL-6, cerebral injury, and subsequent neurodevelopmental abnormalities.<sup>64,84-88</sup>

A polymorphism in the cyclooxygenase-2 (COX-2) gene (-765 G/C) also has been associated with cognitive outcome (n=156).<sup>89</sup> COX inhibition by indomethacin does not result in improvement in long-term neurocognitive outcomes despite reducing the incidence of severe IVH and PVL on ultrasound.<sup>90</sup> Although COX inhibition may be neuroprotective in some models, a principle COX-2 product, prostaglandin E2

(PGE2), may be neuroprotective.<sup>91</sup> Demonstration of an association of the -765 G/C polymorphism, which confers reduced COX-2 activity, with worse neurocognitive outcomes in preterm infants suggests that COX inhibition, and therefore indomethacin, could have detrimental effects on the preterm brain.

The above preliminary findings have not been substantiated in larger studies. Importantly, these studies have been limited in scope, and the association of additional inflammation pathway polymorphisms, and polymorphisms within other pathways of interest, with neurodevelopmental delay should be explored.

**Few studies have explored the association of genetic polymorphisms and CP in preterm infants, and the number of CP cases evaluated in these studies is small (96 to 187).<sup>29-32</sup> Collectively, these studies suggest that genetic polymorphisms in genes related to inflammation, coagulation, and vascular response may contribute to susceptibility to CP. However, few polymorphisms in these pathways have been assessed, and polymorphisms associated with CP have been inconsistent among studies. Further investigation of the association between CP in preterm infants and polymorphisms in the inflammation, coagulation, and vascular regulation pathways is therefore necessary. In addition, whether polymorphisms in these pathways contribute to other forms of neurodevelopmental delay is uncertain, although preliminary studies are intriguing.**

#### **4b11. Prevention of adverse neurodevelopmental outcomes among preterm infants**

Due to incomplete understanding of the etiologic determinants of CP in preterm infants, a lack of specific and sensitive markers for pregnancies at risk for adverse neurodevelopmental outcomes, and an absence of effective preventative therapies for those infants determined to be at risk, the prevention of CP and other forms of neurodevelopmental disability in preterm infants has remained an unrealized goal. The known causes of CP and neurodevelopmental disability account for only a minority of the cases. Even for those cases in which an etiology is known, evidence of preventability is usually lacking.

Epidemiologic studies have focused on reduction of the preterm birth rate as a primary means to decrease the rate of adverse neurodevelopmental outcomes associated with prematurity. Despite these efforts, available strategies, including weekly intramuscular administration of 17-alpha-hydroxyprogesterone caproate for those with a history of prior spontaneous preterm birth, have not been successful in significantly decreasing the overall rate of preterm birth in the US.<sup>92, 93</sup>

Among other primary prevention strategies for preterm infants, magnesium sulfate is perhaps the most promising. There is increasing evidence that magnesium sulfate may offer neuroprophylaxis against CP for preterm infants. Animal data and an observational clinical study suggested that administration of magnesium sulfate to women in very preterm labor may provide neuroprotection for the infant, and a lower rate of motor handicap.<sup>94</sup> A systematic review of randomized trials to date indicated no major maternal or fetal complications, and a 'significant reduction in the rate of substantial gross motor dysfunction.'<sup>95</sup> The results of a large (2241 women), randomized, controlled trial of magnesium sulfate in preterm infants was recently published.<sup>96</sup> There was no significant difference in the composite primary outcome of stillbirth or infant death by 1 year of corrected age or moderate/severe CP at or beyond 2 years of corrected age. However, in a pre-specified secondary analysis, moderate or severe cerebral palsy occurred significantly less frequently in the magnesium sulfate group (1.9% vs. 3.5%; RR, 0.55; 95% CI, 0.32 to 0.95). This appeared to be dominated by the effect in the <28 week strata (2.7% vs. 6.0%, RR 0.45, 95% CI 0.23-0.87). Of particular interest are the genetic and environmental factors that might modify this effect. Polymorphisms in pathways leading to white matter injury (including glutamate release and receptor binding, calcium channels, lipid peroxidation, and generation of free radicals through multiple mechanisms) and the modifying effect of antenatal magnesium sulfate should be explored.

#### **4b12. Rationale for study of genetic polymorphisms associated with CP and neurodevelopmental delay in preterm infants**

Genomic analysis has been applied with success to a wide range of complex disorders, including asthma, cystic fibrosis, cancer, schizophrenia, and autism. There is a paucity of known prenatal predictors of CP in very preterm infants and only 4 studies<sup>29-32</sup> have evaluated genetic predictors of CP in preterm infants, and only one study focused solely on a population of very preterm children born at <32 weeks.<sup>33</sup>

Preliminary studies and the observation of apparent differences in vulnerability to adverse neurodevelopmental outcomes in preterm infants suggest that genetic factors play an important role in determining susceptibility. The association of genetic polymorphisms with adverse neurodevelopmental outcomes provides a possible explanation for the observed differences in outcomes of preterm infants

exposed to similar risk factors for white matter injury. These findings could ultimately provide a basis for preventative strategies, such as expanded pre-pregnancy immunization, anticoagulant therapy, or immunomodulating therapies. The interactions of viral or bacterial infections during pregnancy, normal or abnormal fetal cytokine responses, and hereditary fetal polymorphisms as antenatal causes of adverse neurodevelopmental outcomes are an important research priority.<sup>97</sup>

**Current strategies for prevention of adverse neurodevelopmental outcomes among preterm infants have yielded only modest results. Further investigation of genetic risk factors is necessary in order to identify new prevention and intervention strategies.**

#### **4b13. Rationale for study of gene-gene and gene-environment interactions**

The developmental outcome of a preterm infant appears to be determined by a complex interaction of genetic and environmental factors acting on the developmentally vulnerable preterm brain. Interest is increasing in the role of genetic polymorphisms in modifying the effect of exposures to environmental health hazards (often referred to as gene-environment interaction) which render some individuals or populations more or less likely to develop disease after exposure. It has been recognized that many human diseases arise from the complex interplay of environmental factors and host susceptibilities. Several recent studies have represented the first step in investigating how genetic susceptibility modulates risk of adverse pregnancy outcomes from environmental exposures and host susceptibilities.

There are many proposed causes and contributors to the development of CP and other forms of neurodevelopmental disability. Although it is important to focus on individual risk factors and causes of this disorder, it is also critically important to look at the effects of combined gene-environment risk factors, such as the presence of a cytokine polymorphism and intrauterine infection, and neurodevelopmental risks associated with these combinations. A recent study demonstrated an association between MBL haplotypes and CP after perinatal viral exposure.<sup>32</sup>

In addition, a combination of gene polymorphisms may be necessary for expression of an adverse phenotype. Cytokines produced in response to infection are able to mediate intravascular cell adhesion, coagulation, and vasoconstriction. The presence of an existing thrombophilia or cytokine polymorphism that results in a pathologically increased response or increased susceptibility to infection may result in cytokine-mediated damage to the fetal brain and cause an insult sufficient to cause an adverse neurodevelopmental outcome, such as CP. It has been suggested that most thrombophilias need another risk factor to express the adverse phenotype and cause vascular thrombosis. The interaction of genotypic risk factors in the inflammation and coagulation pathways with environmental risk factors, such as intrauterine infection/inflammation, may ultimately play the most important role in the pathogenesis of adverse neurodevelopmental outcomes. Analysis of gene-gene and gene-environment interactions will therefore be crucial to understanding the pathogenesis of these disorders and identifying possible prevention and intervention strategies.

#### **4b14. The limitations of single gene approaches**

The complex nature of both CP and neurodevelopmental delay, which likely involves interactions between environmental and genetic risk factors, limits the effectiveness of single-gene research approaches. It is unlikely that a single gene mutation or risk factor will explain a substantial portion of the cases of neurodevelopmental delay or CP. Thus, research efforts that focus on single candidate genes are unlikely to identify strong associations in mixed phenotypic populations. Improved methods that allow for the evaluation of multiple candidate genes simultaneously are most likely to be successful in identifying genetic influences that increase the risk for poor neurodevelopmental outcomes and CP.

Genomic techniques, such as high-throughput genotyping technologies, make it possible to assess a single patient sample for hundreds of thousands of known gene alterations, such as single nucleotide polymorphisms (SNPs), and to identify potential candidate genes or localize regions of the chromosome that may have a special role in the etiology of the phenotype of interest. Microarray chips are now available that contain up to 1,000,000 unique SNPs that may be used to screen for mutations that are associated with specific disease states.<sup>98</sup> However, these chips remain very expensive and are often cost prohibitive. Fortunately, it is now also possible to create multiplex assays with smaller numbers of polymorphisms that focus on particular suspected mechanisms or biologic pathways. Such 'designer assays' can be constructed at reasonable cost and can provide valuable mechanistic insight into common clinical syndromes.

**4b15. Why not conduct a Genome-Wide Association Study?**

A project within the Neonatal Research Network (NRN) is currently planned to perform a genome-wide association study (GWAS) on the samples that will be used for this study. **While the GWAS will provide a genome-wide view, this K23 grant would provide a valuable and specific supplement to this more comprehensive study, and will assess candidate genes and regions that are not sufficiently covered by the markers on the GWAS platform.** The best GWAS platforms cover 80% of the genome in Caucasians, and less in other races. These platforms also tend to ignore SNPs with lower minor allele frequencies and, therefore, are unable to assess many disease-associated SNPs. I will select SNPs for our focused array by densely covering candidate genes with a bias towards those SNPs that may be rarer in the population, but are likely to cause functional changes (coding, donor sites, near promoters), and those regions not covered by genome platform selected by the NRN. **The strength of my smaller, more specific, multiplex SNP assay is its ability to assess for SNPs within specific pathways of interest (e.g. inflammation and coagulation) and over areas and markers in the genome that will be missed by current GWAS.**

**4c. Preliminary Studies**

I recently completed a secondary analysis of the Randomized Placebo-Controlled Trial of Antenatal Corticosteroid Regimens (BEARS Trial) that was initiated by the NICHD MFMU Network in 2000.<sup>99,100</sup> The goal of the BEARS Trial was to determine the clinical efficacy of repeated doses of corticosteroids in pregnancies at risk for spontaneous preterm delivery and to quantify the maternal, neonatal and infant risks. A secondary aim of the study was to evaluate the long-term outcomes of infants exposed to repeated courses of steroids as compared to those who received a single course. The objective of this case-control secondary analysis was to determine if infants with neurodevelopmental delay at age 2 years are more likely to have SNPs in inflammation, coagulation, and vascular regulation pathway genes. Cases were defined as children with mental developmental and/or psychomotor delay (Bayley II Infant Neurodevelopmental Screen MDI and/or PDI <85) at age 2 years. CP cases (7) were excluded from the analysis. Controls were children in the BEARS Trial that had normal neurodevelopment (MDI and PDI = 85). DNA from placenta and/or fetal cord serum was required for inclusion (available for 125/218 cases). Univariate analyses were used to compare allele and genotype frequencies. Because this was an exploratory study, no multiple comparison correction was made. 125 cases and 147 controls were analyzed. There were 31 sets of twins among the cases/controls, and one twin from each pair was randomly selected for inclusion in the analysis.

**As part of this study effort, I worked closely with \_\_\_\_\_ to create a custom multiplex SNP assay that evaluates 48 candidate gene polymorphisms involved in inflammation, coagulation, and vascular response (Appendix C).** The secondary analysis study samples were evaluated with this assay and the 48-Plex GenomeLab™ SNPstream® Genotyping System (Beckman Coulter, Fullerton, CA) was used for the analysis. On univariate analysis, several fetal inflammation pathway SNPs were associated with neurodevelopmental delay at age 2 (Table 2).

**Table 2. Genotype and Allele Frequencies, BEARS Trial Secondary Analysis\***

| Gene   | SNP      | Genotype/Allele | Cases, n(%) | Controls, n(%) | P     |
|--------|----------|-----------------|-------------|----------------|-------|
| IL-1 β | -511     | GG              | 28(36.3)    | 50(50.5)       | 0.029 |
|        |          | GA              | 33(42.9)    | 41(41.4)       |       |
|        |          | AA              | 16(20.8)    | 8(8.1)         | 0.009 |
|        |          | G               | 89(57.8)    | 141(71.2)      |       |
| IL-4R  | ile50val | A               | 65(42.2)    | 57(28.8)       | 0.030 |
|        |          | G               | 131(66.2)   | 132(55.9)      |       |
| IL-6   | -174     | A               | 67(33.8)    | 104(44.1)      | 0.041 |
|        |          | GG              | 35(72.9)    | 44(50.6)       |       |
|        |          | GC              | 11(22.9)    | 37(42.5)       | 0.020 |
|        |          | CC              | 2(4.2)      | 6(6.9)         |       |
| IL-6   | -176     | G               | 81(84.4)    | 125(71.8)      | 0.007 |
|        |          | C               | 15(15.6)    | 49(28.2)       |       |
| IL-6   | -176     | G               | 101(50.5)   | 159(63.1)      | 0.007 |
|        |          | C               | 99(49.5)    | 93(36.9)       |       |

\*P<0.05

Genotype frequencies for IL-1 $\beta$  and IL-6 were significantly different between cases and controls, as were allele frequencies for IL-1 $\beta$ , IL-4 receptor (IL-4R), and IL-6. I hypothesize that these SNPs may contribute to cerebral injury in motor and non-motor pathways, and therefore confer susceptibility to both CP and other forms of neurodevelopmental delay. As this is an exploratory study, confirmation in a larger cohort is necessary. The remainder of the analysis, including multivariate analyses and evaluation for gene-gene and gene-environmental interactions, is ongoing at the time of this submission.

#### 4d. Research Design and Methods

**Specific Aim 1. Identify genetic polymorphisms that are associated with CP in preterm infants using a custom, multiplex SNP assay designed to assess ~1300 SNPs in pathways related to white matter brain injury.**

**Specific Aim 2. Identify genetic polymorphisms that are associated with neurodevelopmental delay in preterm infants using the same custom multiplex SNP assay. Neurodevelopmental delay will be defined by the Bayley II Infant Neurodevelopment Screen.**

##### 4d1a. Study overview

We hypothesize that preterm infants with CP are more likely than those with normal neurodevelopment to have gene polymorphisms in pathways related to white matter brain injury. In addition, we hypothesize that preterm infants with neurodevelopmental delay are also more likely to have polymorphisms in these pathways. Polymorphisms of interest are in genes related to inflammation, coagulation, vascular regulation, glutamate release and receptor binding, calcium channels, lipid peroxidation, and free radical generation. We will test this hypothesis using the GoldenGate Assay, which will allow us to genotype a total of 1,536 SNPs per sample. This assay will allow us to be highly inclusive of candidate genes and candidate SNPs, as well as allow the genotyping of Ancestry Informative Markers (AIMS) to allow us to control and adjust for admixture. We will choose ~1300 candidate SNPs and ~200 AIMS selected to be informative for diverse ancestry. Currently, we are planning on using the multiethnic AIM SNP panel of Kosoy et al.,<sup>101</sup> but may change if better marker panels are developed. I will use HapMap to select the SNPs with assistance from Drs. \_\_\_\_\_.

**Compared to the prior custom 48-plex platform, the proposed custom multiplex assay takes advantage of the decreasing cost of genotyping to markedly increase the number of SNPs analyzed.** The new platform will be constructed at minimal to no increased cost compared to the previous platform, and will therefore be more fiscally and scientifically efficient. A list of the 48 polymorphisms and 27 genes on the original microarray platform are presented in **Appendix C**.

##### 4d1b. Subjects

This genetic association study is case-control in design and will utilize samples in the NICHD Neonatal Research Network's Anonymized DNA Bank. This DNA Bank was formed from samples obtained in the NRN's Cytokines Study. The samples are currently housed at The Duke University Center for Human Genetics. De-identified clinical data, including 30 month follow-up, are linked to the DNA Bank. The study cohort includes 1,074 infants who were enrolled in the Network's Cytokines Study. It is estimated that DNA samples and 18-22 month follow-up are available for 701 subjects. Of these subjects, 494 have neurodevelopmental delay and/or CP. Of these 494, 102 subjects have cerebral palsy and will be analyzed in Aim 1; 392 cases of neurodevelopmental delay (excluding subjects with CP) will be analyzed in Aim 2. For both Aims 1 and 2, controls will be gestational age distribution and racial group matched from the 207 subjects with normal neurodevelopment. For Aim 1, cases (n=102) are defined as those with a diagnosis of Cerebral Palsy, as diagnosed by standard clinical criteria used in the NRN's Cytokines Study, at 18-22 month follow-up.<sup>2</sup> For Aim 2, cases (n=392) are defined as those with neurodevelopmental delay, as defined by the Bayley II Infant Neurodevelopment Screen at 18-22 month follow-up. Mild neurodevelopmental delay is defined as an MDI and/or PDI <85; moderate-severe delay is defined as an MDI and/or PDI <70. Controls are subjects with normal neurodevelopment, defined as Bayley PDI and MDI =85.

##### 4d1c. Outcomes

The demographic, clinical, and outcome data from the NICHD NRN's Cytokines Study were collected in a uniform manner from neonatal intensive care units at institutions participating in the NICHD NRN and are housed at the Network's Data Coordinating Center (DCC), Research Triangle Institute (RTI). The data are

not meant to be a representative regional sample, but instead represent a sample population from major tertiary care academic centers. Although the centers serve varying populations, they exemplify the spectrum of neonatal morbidity problems. Data from the neonatal hospitalization and up to 30 months post-discharge are included in the database. Definitions of terms and diagnoses are included in the Manuals of Operations for the Cytokines Study. These manuals can be located on the NICHD Neonatal Research Network website (neonatal.rti.org). All definitions are consistent with definitions used for the Generic Database between January 1, 1998 and December 31, 2002. Maternal and neonatal demographics and neurologic data are shown in **Appendix D**.

#### 4d1d. Predictors

DNA extraction and whole genome amplification (WGA) will be done at the Duke Center for Human Genetics per the DNA Bank protocol. Samples consist of 3 mm punches from filter paper cards (up to 4 for any individual infant) obtained from infants during the study. The samples are stored at the Duke Center for Human Genetics (Duke CHG) DNA bank, and are the sole property of the NICHD Neonatal Research Network. DNA will be extracted from all proposed study samples and amplified to yield sufficient quantities for the proposed genotype assays. Recent studies demonstrate that WGA methods provide a reliable approach for increasing the amount of DNA in samples for use with SNP genotyping arrays.<sup>102-104</sup> These studies demonstrate that, although call rates may be slightly lower, current WGA methods have minimal allelic bias and good concordance with genomic DNA samples.

The multiplex SNP analysis will be performed in collaboration with Dr. \_\_\_\_\_, PhD, at the \_\_\_\_\_, Dr. \_\_\_\_\_ is an expert in this type of genetic analysis and, as my co-mentor, will ensure that the creation and utilization of the new microarray is successful. It must be noted that the previous collaborations with \_\_\_\_\_ have been forged primarily as a contract service arrangement. These collaborations have been very successful in this capacity. However, this grant proposal also requires an intense mentoring experience exposure to a top-notch genetics research program capable of promoting my research career development. Therefore, in order to maximize the mentorship experience, the decision was made to collaborate with Dr. \_\_\_\_\_ for this project rather than continue with the previously established collaboration with \_\_\_\_\_.

#### 4d1e. Study design rationale

Compared to the prior secondary analysis of the BEARS Trial, this case-control study involves a much larger cohort with similarly well-characterized neurodevelopmental outcomes at 18-22 months. In addition, the number of cases permits analysis of genetic polymorphisms associated with CP, which was not possible in the prior analysis. This study design represents the most efficient and cost-effective method for an exploratory study evaluating genetic factors predisposing to cerebral injury in the very preterm neonate.

#### 4d1f. Analysis

Statistical analysis will occur at the Data Coordinating Center for the NRN, Research Triangle Institute (RTI, Bethesda, MD), in compliance with the biologic sample secondary analysis procedures established by the NRN. I will test for association between each of the ~1300 candidate SNPs typed on the NRN samples and CP (Aim 1) and NDD (Aim 2) using logistic regression  $\text{Logit}(p(\text{CP or NDD})) = \beta_0 + \beta_1 x + \beta_2 G + e$ , where  $p(\text{CP or NDD})$  represents prevalent CP or neurodevelopmental delay,  $x$  is a vector of background covariates (with regression parameter estimates of  $\beta_1$ ), which will be used in Aim 3, such as ancestry, other genotypes, maternal variables, neonatal variables, birth weight, gestational age, and interaction terms associated with CP and NDD. Interaction terms of interest include gestational age, birth weight, gender, race, steroid exposure, chorioamnionitis, growth restriction, placental abruption and tobacco use, among others.  $G$  represents the SNP being tested for association yielding parameter  $\beta_2$ . Here the genetic model could be additive, dominant, recessive or general. I will use the additive which has the best power when the underlying model is unknown.<sup>105</sup> In an additive model the  $e^{\beta_2}$  from the logistic model provides an odds ratio (OR) estimate for CP or NDD due to each minor allele, with the standard error used to obtain a 95% confidence intervals. When there is more than 1 case per family I will use all individuals. This is somewhat contrary to conventional belief, but recently Visscher et al<sup>106</sup> wrote, "contrary to common belief, there is hardly any loss in power when using relatives to conduct an association study." That being said, I am aware that this may cause some biases. I am not planning on imputing any genotypes.

#### 4d1g. Sample size and power

Power calculations for Aim 1 are shown in **Table 3**. Power has been calculated assuming a moderate adjustment for multiple testing ( $\alpha = 0.001$ ). For Aim 1, we will have low power at lower allele frequencies (<~3%), but have good power to detect common alleles (>10%), with an effect size down to a

genotype relative risk (GRR) of 2.3, and at higher frequencies, down to a GRR of 1.77. Power calculations for Aim 2 are shown in **Table 4**. The power for Aim 2 is considerably higher than Aim 1 due to the near tripling of the sample size. There is 80% power to detect a GRR down to 1.9 at a DAF of 0.02, and down to a GRR of 1.65 at a DAF of 0.2.

**Table 3. Power Calculation for Aim 1**

Assuming LD=1 between marker and trait locus and additive model. Power calculated at  $\alpha=0.001$  for case=102 and controls=207. Columns are the disease allele frequency (DAF) and rows are the genotype relative risk (GRR).

| GRR\DAF | 0.02 | 0.1  | 0.2    | 0.5    |
|---------|------|------|--------|--------|
| 1.5     | ~0   | 0.04 | 0.11   | 0.74   |
| 2       | ~0.1 | 0.32 | 0.59   | 0.99   |
| 2.5     | 0.04 | 0.72 | 0.90   | 0.99   |
| 3       | 0.15 | 0.93 | 0.98   | >0.999 |
| 4       | 0.40 | 0.99 | >0.999 | >0.999 |

**Table 4. Power Calculations for Aim 2**

Assuming LD=1 between marker and trait locus and additive model. Power calculated at  $\alpha=0.001$  for case=392 and controls=207. Columns are the disease allele frequency (DAF) and rows are the genotype relative risk (GRR).

| GRR\DAF | 0.02   | 0.1    | 0.2    | 0.5    |
|---------|--------|--------|--------|--------|
| 1.5     | 0.28   | 0.38   | 0.53   | 0.99   |
| 2       | 0.88   | 0.95   | 0.99   | 0.99   |
| 2.5     | 0.99   | 0.999  | >0.999 | >0.999 |
| 3       | >0.999 | >0.999 | >0.999 | >0.999 |
| 4       | >0.999 | >0.999 | >0.999 | >0.999 |

**4d1h. Expected outcomes**

Aim 1 will contribute to an increased understanding of the genetic risk factors predisposing a preterm infant to CP, and will therefore identify possible prevention strategies and may provide a basis for future therapeutic intervention trials. Whether polymorphisms in the inflammation, coagulation, vascular regulation and other pathways contribute to other forms of neurodevelopmental delay in preterm infants is uncertain. Preliminary work by myself and others provides compelling preliminary data that suggests this might be the case.<sup>82,83,89</sup> Aim 2 will contribute further understanding of the genetic risk factors predisposing a preterm infant to neurodevelopmental delay.

**4d1i. Genotype Quality Control (QC)**

I will implant several layers of QC to ensure optimal results, including: 1) quantifying dsDNA, 2) identifying chips with poor call rates, 3) identifying incorrect subject gender, 4) identifying sample mix-up, 5) identifying duplicate samples or cryptic relatedness, 6) identifying non-Mendelian inheritance (not possible in this study), and 7) identifying SNPs that perform poorly.

**4d1j. Admixture**

The individuals in the NRN are drawn from several racial groups. Genetic ancestry can be effectively identified using high density GWAS SNP panels by using AIM markers.<sup>107</sup> Recent work suggests that applying admixture adjustments improve statistical power and reduce the type 1 error rate.<sup>108</sup> To account for potential confounding substructure or admixture in these samples, principal component analyses (PCA) will be computed.<sup>109</sup> We will use the AIM markers typed on the 1534 SNP chip to estimate the admixture of the individuals and use it as a stratifying covariate in our association studies.

**4d1k. Limitations and alternate approaches**

This study is limited to the evaluation of preterm infants. Term and near-term infants comprise over half of all CP cases.<sup>110</sup> SNPs significant in this analysis will be important candidates for exploration in future studies of term and near-term infants.

Questions may arise regarding the adequacy of the sample size despite the favorable power calculations presented above. The sample size for Aim 1 is comparable to other similar case-control studies evaluating the association of genetic polymorphisms with CP. In fact, the only previous case-

control study evaluating genetic polymorphisms and CP in very preterm infants had fewer CP cases (96 versus 102).<sup>33</sup> In the study referenced, 21 genes and 31 polymorphisms were evaluated. These polymorphisms were in the nitric oxide synthase 3 gene, 5 coagulation pathway genes, 2 cytokine genes (lymphotoxin- $\alpha$  and TNF- $\alpha$ ), and 13 genes involved in hypertension. For Aim 2, the sample size is four-fold larger than the secondary analysis which I completed with the MFMU Network and is 2- to 3-fold larger than prior genetic association studies of individual SNPs with neurodevelopmental delay.<sup>82,83,89</sup> **There is clearly a need to reassess these polymorphisms within a different cohort and to explore additional genes in pathways involved in white matter injury. This study represents a very unique opportunity to evaluate a large cohort of very preterm infants, appropriate controls, and well-characterized clinical outcomes.** Although a prospective trial would be scientifically superior, it would be cost-prohibitive. This case-control design is efficient, cost-effective, and appropriate for an exploratory study of its nature.

This study is limited to evaluation of SNPs in candidate genes of interest and copy number variants (CNVs) will not be able to be assessed. CNVs occur commonly in the human genome, often affect genes, contribute to genomic evolution and genetic diversity, and influence a number of human diseases.<sup>111-114</sup> Relative to SNPs, CNVs may have larger phenotypic effects.<sup>114</sup> While it is likely that future genetic studies of CP would benefit from analyzing CNVs in addition to SNPs, this study will not assess CNVs, as a genome-wide SNP assay would be necessary for evaluation.<sup>115</sup> **Genome-wide association studies and evaluation of CNVs contribute to our growing understanding of the genetic basis of health and disease. It is clear that these and other evolving research technologies will profoundly enhance our understanding of complex, multifactorial conditions such as CP and neurodevelopmental delay. These technologies are beyond the budget and scope of this K23 submission. However, I will be working with world-class mentors who will continue to expose me to these evolving technologies. With ongoing assistance and advice from my mentors, I plan to position myself to participate in these new opportunities as they arise.**

This study has a number of analytic challenges. Because of the multiple comparisons that will be made, there is an increased likelihood of committing a Type I error and appropriate adjustments will be made. We also acknowledge the possibility that associations may be statistically significant but not clinically meaningful. All analyses will be guided by biologically plausible models and will be interpreted with attention to clinically meaningful associations as opposed to those that simply reach statistical significance. Given the nature of SNP analysis, it is also possible that it will be genes in linkage disequilibrium with those that are identified, rather than the targeted genes or SNPs, that are associated with CP and neurodevelopmental delay. Only further studies will clarify this issue.

The NICHD is a member of the Center for Inherited Disease Research (CIDR) at Johns Hopkins. Although genotyping through CIDR is available for NICHD-funded research and is a very cost-effective option, it is not optimal for the purposes of this training grant, as hands-on experience and intense genetics mentoring in the local environment is preferable. Given the documented successes of Dr. \_\_\_\_\_ and genetics research at \_\_\_\_\_, we feel this is the optimal environment in which to achieve the training and research aims outlined in this grant. In addition, any additional expenses incurred as a result of this approach will be covered by the Departments of Human Genetics and Obstetrics and Gynecology. The budget is based on current prices, and historically these prices decrease from year-to-year as technologies continue to advance. We fully expect that we will be able to complete the proposed work over the three years of the award for less than today's current price. If, however, prices are higher than projected at the time of the analysis, we are aware of the CIDR resource and will utilize it for genotyping.

#### **4d1i. Relationship to training aims**

These aims provide experience in acquisition, management, and analysis of complex genetic data. Formal coursework in biostatistics, epidemiology, and genetics will complement the research experience. Collaboration with Dr. \_\_\_\_\_, an accomplished genetics researcher, and Dr. \_\_\_\_\_ who is experienced with genetic epidemiology and statistical analysis, will augment the formal didactic training.

**Specific Aim 3. Perform multivariable analyses to identify unique gene-gene and gene-environment interactions that contribute to susceptibility to CP and/or neurodevelopmental delay among preterm infants.**

#### **4d2a. Study overview**

As outlined in Specific Aims 1 and 2, we hypothesize that genetic polymorphisms in inflammation

coagulation, vascular regulation, and other pathways contribute to susceptibility to CP and neurodevelopmental delay in preterm infants. The complex nature of both CP and neurodevelopmental delay strongly suggests that interactions between environmental and genetic risk factors are important. It is unlikely that a single gene mutation or risk factor will explain a substantial portion of adverse neurodevelopmental outcomes. The evaluation for possible gene-gene and gene-environment interactions contributing to CP and neurodevelopmental delay is therefore a compelling area of research.

#### **4d2b. Subjects**

Study subjects will be the same as outlined in Sections 4d1b and 4d2b.

#### **4d2c. Outcomes**

For Aim 3, gene-gene and gene-environment interactions associated with CP and neurodevelopmental delay are the outcomes of interest.

#### **4d2d-e. Predictors and study design rationale**

The predictors and study design rationale are discussed in Sections 4d1d-e.

#### **4d2f. Analysis**

Gene-gene (G-G) and gene-environment (G-E) interactions associated with CP and/or neurodevelopmental delay will be assessed via logistic regression (Aims 1-2). This analysis will give an OR for the G-G or G-E interactions of interest. This design assumes independence between genotype and environment and cannot evaluate the independent effects of exposure and genotype. Its primary utility is in identification of interactions for further exploration. This is an efficient approach for looking at G-G and G-E interactions. SNPs which are determined to be significantly associated with CP and/or neurodevelopmental delay in the univariate analysis will be included as covariates in the logistic regression, as will environmental factors such as chorioamnionitis. Recursive partitioning and multifactor dimensionality reduction will also be considered to explore possible G-G interactions that confer risk of CP and neurodevelopmental delay.<sup>116-119</sup> For the G-G and G-E analyses, neurodevelopmental delay will be examined as a dichotomous variable, defined as a score of <85 on the Bayley MDI and/or PDI. Additional analysis will be performed for moderate-severe neurodevelopmental delay, defined as a score of <70 on the Bayley MDI and/or PDI.

#### **4d2g. Sample size and power**

Due to the highly exploratory nature of these studies, the unknown number of possible tests and heavy dependence on a number of uncertain variables, power is difficult to determine. We will thus conduct analyses in an exploratory mode and use estimates of false discovery rate rather than p-values to select models for the next stage of analysis. All studies in Aim 3 will be considered exploratory, and the p-values for all the hypotheses tested in this aim will be joined and Story's q-value used to adjust for multiple testing. Only results with a q value <0.1 will be considered meritorious of further studies.

#### **4d2h. Expected outcomes**

This research aim will contribute understanding of the G-G and G-E interactions that might predispose preterm infants to adverse neurodevelopmental outcomes. This is arguably the most intriguing aspect of genetic association studies in this area of research. Few such studies in this area exist and this work will likely result in the identification of new and novel G-G and G-E interactions on which to base future studies.

#### **4d2i. Limitations and alternate approaches**

Evaluation of G-G and G-E interactions will be exploratory in nature and any results will need to be confirmed in subsequent studies. However, analysis of these interactions will be crucial to understanding the pathogenesis of CP and neurodevelopmental delay in preterm infants and to identifying possible prevention and intervention strategies. This specific aim represents a unique opportunity to explore these interactions in a large cohort with well-characterized clinical outcomes.

#### **4d2j. Relationship to training aims**

Specific Aim 3 provides experience in analysis of complex genetic data for G-G and G-E interactions. Formal coursework in biostatistics, epidemiology, and genetics will complement the research experience. Collaboration with Dr. \_\_\_\_\_, an accomplished genetics researcher, and Dr. \_\_\_\_\_, who is experienced with genetic epidemiology and statistical analysis, will augment the formal didactic training.

#### **4e. References Cited**

1. Weiss RB, Baker TB, Cannon DS, et al. A Candidate Gene Approach Identifies the *CHRNA5-A3-B4* Region as a Risk Factor for Age-Dependent Nicotine Addiction. *PLoS Genet* 2008;4:e1000125.

2. Bax M, Goldstien M, Rosenbaum P, et al. Proposed definition and classification of cerebral palsy, April 2005. *Dev Med Child Neurol* 2005;47:571-576.
3. Bayley N. *Bayley Scales of Infant Development-II*. San Antonio, TX: The Psychological Corporation;1993.
4. Himmelmann K, Hagberg G, Beckung E, et al. The changing panorama of cerebral palsy in Sweden, IX: prevalence and origin in the birth-year period 1995-1998. *Acta Paediatr* 2005;94:287-294.
5. Hagberg B, Hagberg G, Bechung E, et al. The changing panorama of cerebral palsy in Sweden, VIII: prevalence and origin of the birth-year period 1991-1994. *Acta Paediatr* 2001;90:271-277.
6. Stanley FJ, Watson L. Trends in perinatal mortality and cerebral palsy in Western Australia, 1967 to 1985. *BMJ* 1992;304:1658-1663.
7. Robertson CM, Svenson LW, Joffre MR. Prevalence of cerebral palsy in Alberta. *Can J Neurol Sci* 1998;25:117-122.
8. Kuban KC, Leviton A. Medical progress – cerebral palsy. *N Engl J Med* 1994;330:188-195.
9. Robertson CM, Watt M, Yasui Y. Changes in the prevalence of cerebral palsy for children born very prematurely within a population-based program over 30 years. *JAMA* 2007;297:2733-2740.
10. Vohr BR, Wright LL, Poole, WK, et al. Neurodevelopmental outcomes of extremely low birth weight infants <32 weeks gestation between 1993-1998. *Pediatrics* 2005;116:635-643.
11. Pharoah PO, Platt MJ, Cooke T. The changing epidemiology of cerebral palsy. *Arch Dis Child Fetal Neonatal Ed* 1996;75:F169-F173.
12. Pharoah PO, Cooke T, Cooke RWI, et al. Birthweight specific trends in cerebral palsy. *Arch Dis Child* 1990;65:602-606.
13. Winter S, Autry A, Boyle C, et al. Trends in the prevalence of cerebral palsy in a population-based study. *Pediatrics* 2002;110:1220-1225.
14. Lorenz JM, Paneth N, Jetton JR, den Ouden L, Tyson JE. Comparison of management strategies for extreme prematurity in New Jersey and the Netherlands: outcome and resource expenditure. *Pediatrics* 2001;108:1269-1274.
15. Vincer MJ, Allen AC, Joseph KS, et al. Increasing prevalence of cerebral palsy among very preterm infants: a population-based study. *Pediatrics* 2006;118:e1621-e1626.
16. Bhushan V, Paneth N, Kiely JL. Impact of improved survival of very low birth weight infants on recent secular trends in the prevalence of cerebral palsy. *Pediatrics* 1993;91:1094-1100.
17. Sturman G, Newdick H, Johnson A; Oxford Registry of Early Childhood Impairments Group. Cerebral palsy rates among low-birthweight infants fell in the 1990s. *Dev Med Child Neurol*. 2003;45:456-462.
18. O'Shea TM, Preisser JS, Klinepeter KL, Dillard RG. Trends in mortality and cerebral palsy in a geographically based cohort of very low birth weight neonates born between 1982 to 1994. *Pediatrics* 1998;101:642-647.
19. Cooke RW. Trends in incidence of cranial ultrasound lesions and cerebral palsy in very low birth-weight infants 1982-93. *Arch Dis Child Fetal Neonatal Ed* 1999;80:115-17.
20. Grether JK, Nelson KB. Possible decrease in prevalence of cerebral palsy in preterm infants. *J Pediatr* 2000;136:133.
21. Hamilton BE, Martin JA, Ventura SJ. Births: preliminary data for 2005. *Natl Vital Stat Rep* 2006;55:1-18.
22. Dammann O, Leviton A. Brain damage in preterm newborns: Might enhancement of developmentally regulated endogenous protection open a door for prevention? *Pediatrics* 1999;104:541-550.
23. Cummings SK, Nelson KB, Grether JK, Velie EM. Cerebral palsy in four northern California counties, births 1983-1985. *J of Pediatr* 1993;123:230-237.
24. Wood NS, Marlow N, Costeloe K, et al. EPICURE Study Group. Neurologic and developmental disability after extremely preterm birth. *N Engl J Med* 2000;343:378-384.
25. Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. *Lancet* 2008;317:261-269.
26. Botting N, Powls A, Cooke RW, et al. Cognitive and educational outcome of very-low-birthweight children in early adolescence. *Dev Med Child Neurol* 1998;40:652-660.
27. Nelson KB. What proportion of CP is related to birth asphyxia? *J Pediatr* 1988;112:572-574.
28. Yudkin PL, Johnson A, Clover LM, et al. Assessing the contribution of birth asphyxia to cerebral palsy in term singletons. *Paediatr Perinat Epidemiol* 1995;9:156-170.

29. Gibson CS, MacLennan AH, Hague WM, et al. Associations between inherited thrombophilias, gestational age, and cerebral palsy. *Am J Ob Gyn* 2005;193:1437.
30. Senbil N, Yuksel D, Yilmaz Deniz, et al. Prothrombotic risk factors in children with hemiplegic CP. *Pediatr Int* 2007;49:600-602.
31. Gibson CS, MacLennan AH, Goldwater PN, Haan EA, Priest K, Dekker GA. Neurotropic viruses and cerebral palsy: a population based case-control study. *BMJ* 2006;332:76-80.
32. Gibson CS, MacLennan, AH, Goldwater PN, et al. Mannose-binding lectin haplotypes may be associated with cerebral palsy only after perinatal viral exposure. *American Journal of Obstetrics and Gynecology* 2008;198:509.e1-509.e8.
33. Nelson KB, Dambrosia JM, Iovannisci DM, et al. Genetic Polymorphisms and Cerebral Palsy in Very Premature Infants. *Ped Rsch* 2005;57:494.
34. Gibson CS, MacLennan AH, Goldwater PN, et al. The association between inherited cytokine polymorphisms and cerebral palsy. *Am J Ob Gyn* 2006;194:674.
35. Kuroda MM, Weck ME, Sarwark JF, et al. Association of apolipoprotein E genotype and cerebral palsy in children. *Pediatrics* 2007;119:306-313.
36. McQuillen PS, Ferriero DM. Selective vulnerability in the developing central nervous system. *Pediatr Neurol* 2004;30:227-235.
37. Low J. Motor and cognitive development of infants with intraventricular hemorrhage, ventriculomegaly, or periventricular parenchymal lesions. *Am J Obstet Gynecol* 1986;155:750-756.
38. Pinto-Martin JA, Whitaker AH, Feldman JF, et al. Relation of cranial ultrasound abnormalities in low-birthweight infants to motor or cognitive performance at ages 2, 6, and 9 years. *Dev Med Child Neurol* 1999;4:826-833.
39. Krageloh-Mann I, Horber B. The role of magnetic resonance imaging in elucidating the pathogenesis of cerebral palsy: a systematic review. *Dev Med Child Neurol* 2007;49:144-151.
40. Folkerth RD. The neuropathology of acquired pre and perinatal brain injuries. *Semin Diagn Pathol* 2007;24:48-57.
41. Bashiri A, Burstein E, Mazor M. Cerebral palsy and fetal inflammatory response syndrome: a review. *J Perinat Med* 2006;34:5-12.
42. Kadhim H, Khalifa M, Deltenre P, et al. Molecular mechanisms of cell death in periventricular leukomalacia. *Neurology* 2006;67:293-299.
43. Larouche A, Roy M, Kadhim H, et al. Neuronal injuries induced by perinatal hypoxic-ischemic insult are potentiated by prenatal exposure to lipopolysaccharide: animal model for perinatally acquired encephalopathy. *Dev Neuro Sci* 2005;27:134-142.
44. Johnston MV, Hoon AH. Cerebral Palsy. *Neuromolecular Med* 2006;8:435-450.
45. Willoughby RE Jr, Nelson KB. Chorioamnionitis and brain injury. *Clin Perinatol* 2002;29:603-621.
46. Neufeld MD, Frigon C, Graham AS, et al. Maternal infection and risk of cerebral palsy in term and preterm infants. *J Perinatol* 2005;25:108-113.
47. Wu YW, Escobar GJ, Grether JK, et al. Chorioamnionitis and cerebral palsy in term and near-term infants. *JAMA* 2003;290:1677-1684.
48. Greenwood C, Yudkin P, Sellers S, et al. Why is there a modifying effect of gestational age on risk factors for cerebral palsy? *Arch Dis Child Fetal Neonatal Ed* 2005;90:F141-146.
49. Walstab J, Bell R, Reddihough D, Brennecke S, Bessell C, Beischer N. Antenatal and intrapartum antecedents of cerebral palsy: a case-control study. *Aust N Z J Obstet Gynaecol* 2002;42:138-146.
50. Jacobsson B, Hagberg G, Hagberg B, et al. Cerebral palsy in preterm infants: a population-based case-control study of antenatal and intrapartum risk factors. *Acta Paediatr* 2002;91:946-951.
51. Kalish RB, Vardhana S, Gupta M, Perni SC, Witkin SS. Interleukin-4 and -10 gene polymorphisms and spontaneous preterm birth in multifetal gestations. *Am J Obstet Gynecol* 2004;190:702-706.
52. Roberts AK, Monzon-Barbonaba F, Van Deerlin PG, Holder MD, Macones GA, Morgan MA, Strauss III JF, Parry S. Association of polymorphism with the promoter of the tumor necrosis factor a gene with increased risk of preterm premature rupture of the fetal membranes. *Am J Obstet Gynecol* 1999;180:1297-1302.
53. Moore S, Ide M, Randhawa M, Walker JJ, Reid JG, Simpson NA. An investigation into the association among preterm birth, cytokine gene polymorphisms and periodontal disease. *BJOG* 2004;111:125-132.
54. Simhan HN, Krohn MA, Roberts JM, Zeevi A, Caritis SN. Interleukin-6 promoter -174 polymorphism and spontaneous preterm birth. *Am J Obstet Gynecol* 2003;189:915-918.

55. Landau R, Xie HG, Dishy V, Stein CM, Wood AJ, Emala CW, Smiley RM. beta2-Adrenergic receptor genotype and preterm delivery. *Am J Obstet Gynecol* 2002;187:1294-1298.
56. Papazoglou D, Galazios G, Koukourakis MI, Kontomanolis EN, Maltezos E. Association of -634G/C and 936C/T polymorphisms of the vascular endothelial growth factor with spontaneous preterm delivery. *Acta Obstet Gynecol Scand* 2004;83:461-465.
57. Hao K, Wang X, Niu T, Xu X, Li A, Chang W, Wang L, Li G, Laird N, Xu X. A candidate gene association study on preterm delivery: application of high-throughput genotyping technology and advanced statistical methods. *Hum Mol Genet* 2004;13:683-691.
58. Crider KS, Whitehead N, Buus, BM. Genetic variation associated with preterm birth: A HuGE review. *Genet Med* 2005;7:593-604.
59. Leviton A, Paneth N, Reuss ML, et al. Maternal infection, fetal inflammatory response, and brain damage in very low birthweight infants. *Developmental Epidemiology Network Investigators. Pediatr Res* 1999;46:566-575.
60. Gaudet LM, Smith GN. Cerebral palsy and chorioamnionitis: The inflammatory cytokine link. *Obstet Gynecol Surv* 2001;56:433-436.
61. Romero R, Gomez R, Ghezzi F, et al. A fetal systemic inflammatory response is followed by spontaneous onset of preterm parturition. *Am J Obstet Gynecol* 1998;179:186-193.
62. Baud O, Emilie D, Pelletier E, et al. Amniotic fluid concentrations of interleukin-1beta, interleukin-6 and TNF-alpha in chorioamnionitis before 32 weeks of gestations: histological associations and neonatal outcome. *Br J Obstet Gynaecol* 1999;106:72-77.
63. Yoon BH, Jun JK, Romero R, et al. Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-1beta, and tumor necrosis factor-alpha), neonatal brain white matter lesions, and cerebral palsy. *Am J Obstet Gynecol* 1997;177:19-26.
64. Yoon BH, Romero R, Yang SH, et al. Interleukin-6 concentrations in umbilical cord plasma are elevated in neonates with white matter lesions associated with periventricular leukomalacia. *Am J Obstet Gynecol* 1996;174:1433-1440.
65. Dammann O, Leviton A. Infection remote from the brain, neonatal white matter damage, and cerebral palsy in the preterm infant. *Semin Pediatr Neurol* 1998;5:190-201.
66. Foster-Barber A, Ferriero DM. Neonatal encephalopathy in the term infant: neuroimaging and inflammatory cytokines. *Ment Retard Dev Disabil Res Rev* 2002;8:20-24.
67. Knight JC, Kwiatkowski D. Inherited variability of tumor necrosis factor production and susceptibility to infectious disease. *Proc Assoc Am Physicians* 1999;111:290-298.
68. Kahn MA, De Vellis J. Regulation of an oligodendrocyte progenitor cell line by the interleukin-6 family of cytokines. *Glia* 1994;12:87-98.
69. Jurd K, Stephens C, Black M, et al. Endothelial cell activation in cutaneous vasculitis. *Clin Exp Dermatol* 1996;21:28-32.
70. Dammann O, Leviton A. Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn. *Pediatr Res* 1997;42:1-8.
71. Peterson SV, Thiel S, Jensen L, et al. Control of the classical and the MBL pathway of complement activation. *Mol Immunol* 2000;37:803-811.
72. Garred P, Voss A, Madsen HO, et al. Association of mannose-binding lectin gene variation with disease severity and infections in a population-based cohort of systemic lupus erythematosus patients. *Genes Immun* 2001;2:442-450.
73. Elshourbagy N, Liao W, Mahley R, et al. Apolipoprotein E mRNA is abundant in the brain and adrenals, as well as in the liver, and is present in other peripheral tissues of rats and marmosets. *Proc Natl Acad Sci USA* 1985;82:203-207.
74. Horsburgh K, McCullough J, Nilsen M, et al. Increased neuronal damage and apoE immunoreactivity in human apolipoprotein E<sub>4</sub> isoform-specific, transgenic mice after global cerebral ischemia. *Eur J Neurosci* 2000;12:4309-4317.
75. Blackman J, Worley G, Conaway M, et al. Apolipoprotein E genotype and outcome after traumatic brain injury in children [abstract]. *Dev Med Child Neurol* 2004;46:26.
76. Liu Y, Laakso M, Karonen J, et al. Apolipoprotein E polymorphism and acute ischemic stroke: a diffusion- and perfusion-weighted magnetic resonance imaging study. *J Cereb Blood Flow Metab* 2002;22:1336-1342.

77. McCarron M, Weir C, Muir K, et al. Effect of apolipoprotein E genotype on in-hospital mortality following intracerebral haemorrhage. *Acta Neurol Scand*. 2003;107:106-109.
78. Burgos J, Ramirez C, Sastre I, et al. ApoE4 is more efficient than E3 in brain access by herpes simplex type I. *Neuroreport* 2003;14:1825-1827.
79. Lynch J, Tang W, Wange H, et al. APOE genotype and an Apo-E-mimetic peptide modify the systemic and central nervous system inflammatory response. *J Biol Chem* 2003;278:48529-48533.
80. Benders MJ, Groenendaal F, Uiterwaal CS, et al. Maternal and infant characteristics associated with perinatal arterial stroke in the preterm infant. *Stroke* 2007;38:1759-1765.(29) Gibson CS, MacLennan AH, Hague WM, et al. Associations between inherited thrombophilias, gestational age, and cerebral palsy. *Am J Obstet Gyn* 2005;193:1437.
81. Joseph L, Fink LM, Hauer-Jensen M. Cytokines in coagulation and thrombosis: a preclinical and clinical review. *Clot Coagul Fibrinolysis* 2002;13:105-116.
82. Harding DR, Sukhbir D, Whitelaw A, et al. Does interleukin-6 genotype influence cerebral injury or developmental progress after preterm birth? *Pediatrics* 2004;114:941-947.
83. Harding DR, Brull D, Humphries SE, et al. Variation in the interleukin-6 gene is associated with impaired cognitive development in children born prematurely: A preliminary study. *Pediatric Research* 2005;58:117-120.
84. Duggan PJ, Maalouf EF, Watts TL, et al. Intrauterine T-cell activation and increased proinflammatory cytokine concentrations in preterm infants with cerebral lesions. *Lancet* 2001;358:1699-1700.
85. Fotopoulos S, Pavlou K, Skouteli H, et al. Early markers of brain damage in premature low-birth-weight neonates who suffer from marked perinatal asphyxia and/or infection. *Biol Neonate* 2001;79:213-218.
86. Martin-Ancel A, Garcia-Alix A, Pascual-Salcedo D, et al. Interleukin-6 in the cerebrospinal fluid after perinatal asphyxia is related to early and late neurological manifestations. *Pediatrics* 1997;100:789-794.
87. Yoon BH, Jun JK, Romero R, et al. Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-1beta, and tumor necrosis factor-alpha), neonatal brain white matter lesions, and cerebral palsy. *Am J Obstet Gynecol* 1997;177:19-26.
88. Yoon BH, Romero R, Kim CJ, et al. High expression of tumor necrosis factor-alpha and interleukin-6 in periventricular leukomalacia. *Am J Obstet Gynecol* 1997;177:406-411.
89. Harding DR, Humphries SE, Whitelaw A, et al. Cognitive outcome and cyclo-oxygenase-2 gene (-175 G/C) variation in the preterm infant. *Arch Dis Child Fetal Neonatal Ed* 2007;92:F108-F112.
90. Fowlie PW, Davis PG. Prophylactic intravenous indomethacin for preventing mortality and morbidity in preterm infants. *Cochrane Database, Syst Rev* 2002; 3:CD000174.
91. McCullough L, Wu L, Haughey N, et al. Neuroprotective function of the PGE2 EP2 receptor in cerebral ischemia. *J Neurosci* 2004;24:257-268.
92. Simhan HN, Caritis SN. Prevention of preterm birth. *N Engl J Med* 2007;357:477-487.
93. Thornton JG. Progesterone and preterm labor: still no definite answers. *N Engl J Med* 2007;357:477-487.
94. Nelson KB, Grether JK. Can magnesium sulfate reduce the risk of cerebral palsy in very low birthweight infants? *Pediatrics* 1995;95:263-269.
95. Doyle L, Crowther C, Middleton P, et al. Magnesium sulphate for women at risk of preterm birth for neuroprotection of the fetus. *Cochrane Database Syst Rev* 2007;3:CD004661.
96. Rouse DJ, Hirtz DG, Thom E, et al. A randomized, controlled trial of magnesium sulfate for the prevention of cerebral palsy. *New Engl J Med* 2008;359:895-905.
97. Gibson CS, MacLennan AH, Goldwater PN, et al. Antenatal causes of cerebral palsy: Associations between inherited thrombophilias, viral and bacterial infection, and inherited susceptibility to infection. *Obstetrics and Gynecologic Survey* 2003;58:209-220.
98. Di X, Matsuzaki H, Webster TA, Hubbell E, Liu G, Dong S, Bartell D, Huang J, Chiles R, Yang G, Shen MM, Kulp D, Kennedy GC, Mei R, Jones KW, Cawley S. Dynamic model based algorithms for screening and genotyping over 100 K SNPs on oligonucleotide microarrays. *Bioinformatics* 2005;21:1958-63.
99. Wapner RJ, Sorokin Y, Thom EA, et al. Single versus weekly courses of antenatal corticosteroids: Evaluation of safety and efficacy. *Am J Obstet Gynecol* 2006;195:633-642.
100. Wapner RJ, Sorokin Y, Mele L, et al. Long-term outcomes after repeat doses of antenatal corticosteroids. *N Engl J Med* 2007;357:1190-1198.

101. Kosoy R, Nassir R, Tian C, et al. Ancestry informative marker sets for determining continental origin and admixture proportions in common populations in America. *Hum Mutat* 2008 [Epub ahead of print].
102. Sorensen KM, Jespersgaard C, Vuust J, et al. Whole genome amplification on DNA from filter paper blood spot samples: an evaluation of selected systems. *Genet Test* 2007;11:65-71.
103. Xing J, Watkins WS, Zhang Y, et al. High fidelity of whole-genome amplified DNA on high-density single nucleotide polymorphism arrays. *Genomics* 2008 [Epub ahead of print].
104. Pan X, Urban AE, Palejev D, et al. A procedure for highly specific, sensitive, and unbiased whole-genome amplification. *Proc Natl Acad Sci USA* 2008 [Epub ahead of print].
105. Lettre G, Lange C, Hirschhorn JN. Genetic model testing and statistical power in population-based association studies of quantitative traits. *Genet Epidemiol* 2007;31:358-362.
106. Vissher PM, Andrew T, Nyholt DR. Genome-wide association studies of quantitative traits with related individuals: little (power) lost but much to be gained. *Eur J Hum Genet* 2008;16:387-390.
107. Tang H, Coram M, Wang P, et al. Reconstructing genetic ancestry blocks in admixed individuals. *Am J Hum Genet* 2006;79:1-12.
108. Lee WC. Case-control association studies with matching and genomic controlling. *Genet Epidemiol* 2004;27:1-13.
109. Price AL, Patterson NJ, Plenge RM, et al. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 2006;38:904-909.
110. Nelson KB. The epidemiology of cerebral palsy in term infants. *Ment Retard Dev Disabil Res Rev*. 2002;8:146-150.
111. Sebat J, Lakshmi B, Troge J, et al. Large-scale copy number polymorphism in the human genome. *Science* 2004; 305:525-528.
112. Redon R, Ishikawa S, Fitch KR, et al. Global variation in copy number in the human genome. *Nature* 2006;444:444-454.
113. Kidd JM, Cooper GM, Donahue WF, et al. Mapping and sequencing of structural variation from eight human genomes. *Nature* 2008;453:56-64.
114. Cooper GM, Nickerson DA, and Eichler EE. Mutational and selective effects on copy-number variants in the human genome. *Nat Genet* 2007;39:S22-S29.
115. Cooper GM, Zerr Troy, Kidd Jeffrey M, et al. Systematic assessment of copy number variant detection via genome-wide SNP genotyping. *Nat Genet* 2008;40:1199-1203.
116. Young SS, Ge N. Recursive partitioning analysis of complex disease pharmacogenetic studies. I. Motivation and overview. *Pharmacogenomics* 2005;6:65-77.
117. Zaykin DV, Young SS. Large recursive partitioning analysis of complex disease pharmacogenetic studies. II. Statistical considerations. *Pharmacogenomics*. 2005;6:77-89.
118. Hahn LW, Ritchie MD, Moore JH. Multifactor dimensionality reduction software for detecting gene-gene-and gene-environment interactions. *Bioinformatics* 2003;19:376-382.
119. Moore JH, Gilbert JC, Tsai CT, et al. A flexible computational framework for detecting, characterizing, and interpreting statistical patterns of epistasis in genetic studies of human disease susceptibility. *J Theor Biol* 2006;241:252-261.

**4f. Human Subjects Research**

Please see **Appendix B** for the \_\_\_\_\_ IRB training on the protection of human subjects.

**4f1. Risks to the Subjects**

**4f1a. Human subjects involvement and characteristics**

This proposal concerns only human subjects. The study population will be comprised of those who had consented to be in the NICHD Neonatal Research Network's Anonymized DNA Bank. Research participants whose samples are used for this secondary analysis project will be protected ethically with utmost consideration for their privacy. All samples to be utilized for Specific Aims 1-3 have been de-identified. DNA samples included in the NICHD Neonatal Research Network's (NRN's) Anonymized DNA Bank have been de-identified prior to distribution to investigators. The NRN retains sole custody of the de-identified clinical information linked to the DNA Bank. Neither I, nor any other investigators participating in this study, will have access to identifiable genotypic or phenotypic information for study samples.

**4f1b. Sources of materials**

Specific Aims 1-3 all utilize biologic samples (whole genomic DNA) obtained from the NICHD Neonatal Research Network's (NRN's) Anonymized DNA Bank. This DNA bank was formed from samples obtained

in the NRN's Cytokines Study. The samples are currently housed at The Duke University Center for Human Genetics. De-identified clinical data are linked to the DNA Bank and will be obtained from the NRN Biostatistics Coordinating Center only as specified by an Institutional Review Board (IRB)-approved protocol. Evaluation of existing specimen records and/or data will be undertaken under strictly-enforced IRB policies.

**4f1c. Potential risks to subjects**

There are no anticipated risks for participants in this study. De-identified samples will be sent to the \_\_\_\_\_ labeled only with an NRN study number assigned by the NRN Data Coordinating Center (DCC). \_\_\_\_\_ researchers will not have access to the identification records.

The proposed study will be reviewed by the Institutional Review Board at the \_\_\_\_\_. DHHS regulations regarding human subjects research (e.g., 45 CFR 46, including Subparts B and D) are followed. HIPAA regulations will also be strictly adhered to in this study.

All research personnel comply with all IRB educational requirements. The principal investigators and all key personnel involved with this research protocol will have completed human subjects training per IRB policy. Currently, the IRB at the \_\_\_\_\_ requires at least one of the following online training courses that present information about the rights and welfare of human participants in research: 1) University of Miami Collaborative IRB Training Initiative (CITI); National Cancer Institute (NCI); or VA Good Clinical Practice Training. These training programs are all designed to improve the knowledge of the Common Rule, HIPAA Privacy Rule, and Good Clinical Practices for individuals involved in human subjects research. Training is repeated approximately every five years, per IRB policy.

Key personnel will also be required to complete annual HIPAA Privacy Rule training as outlined by their respective institutions \_\_\_\_\_. These records are kept on file in the Obstetrics and Gynecology Research Network (OGRN) office.

**4f2. Adequacy of protection against risks**

Identifiable clinical information will not be available to me, or other investigators on this proposal. Results of the genotyping will be sent back to the NRN's DCC, where correlation with phenotypic data and data analysis will take place.

**4f3. Potential benefits of the proposed research to the subjects and others**

No direct benefits are anticipated for the individuals whose biologic samples and clinical outcomes are being studied in this research proposal. The identify of those individuals will not be known to \_\_\_\_\_ researchers, and the consent process used in recruitment to the NRN's Anonymized DNA Bank included a statement that participants would not be contacted with results from any subsequent research. This research will benefit others as described in Section 4f4 below.

**4f4. Importance of knowledge to be gained**

This study will provide a better understanding of the genetic risk factors associated with adverse neurodevelopmental outcomes in very preterm infants. Identification of polymorphisms that either predispose to, or protect against, cerebral injury in preterm infants could lead to identification of prevention strategies and therapeutic intervention trials intended to prevent or minimize cognitive or neuromuscular handicaps. Likewise, identification of possible protective markers could provide considerable reassurance to families with at-risk children.

**Sections 4f1-4 confirm that criteria for Protection of Human Subjects from Research Risk, as described in Federal Regulations 45 CFR 46.120, are acceptable.**

**4f5. Inclusion of women**

The proposed research includes no gender-based inclusion or exclusion criteria. The proportion of male and female preterm infants included in the NICHD Neonatal Research Network's (NRN's) Anonymized DNA Bank is approximately equal.

**4f6. Inclusion of minorities**

The proposed research included no race or ethnicity-based inclusion or exclusion criteria. The race and ethnicity of preterm infants included in the NICHD Neonatal Research Network's (NRN's) Anonymized DNA Bank reflects the racial and ethnic diversity of the populations recruited by institutions participating in the NRN's cytokines study.

Program Director/Principal Investigator (Last, First, Middle): \_\_\_\_\_

**4f7. Targeted/Planned Enrollment**

Not applicable. No patient recruitment is proposed in this research plan.

**4f8. Inclusion of children**

As my research interest is the genetic factors which predispose preterm infants to adverse neurodevelopmental outcomes, children are the focus of this study. The subjects are preterm infants that were enrolled in the NICHD NRN's Cytokines Study.

**4g. Vertebrate Animals**

Not applicable

**4h. Select Agent Research**

Not applicable

**4i. Consortium/Contractual Agreements**

None

**4j. Resource Sharing**

Our plan to share materials and our management of intellectual property will adhere to the \_\_\_\_\_  
\_\_\_\_\_s policies and the NIH Grant Policy on Sharing of Unique Research Resources for all resources developed during this project.

September 30, 2008

Center for Scientific Review  
National Institutes of Health  
6701 Rockledge Drive  
Room 1040 - MSC 771808  
Bethesda, MD 20892-7710

re: \_\_\_\_\_ M.D.  
Mentored Patient-Oriented Research Career Development Award (K23)

Dear Review Committee Members:

This letter serves to confirm my enthusiastic support for Dr. \_\_\_\_\_ Mentored Patient-Oriented Research Career Development Award (K23) application entitled \_\_\_\_\_

\_\_\_\_\_ I am an Associate Professor in the Maternal-Fetal Medicine Division of the Department of Obstetrics and Gynecology at the \_\_\_\_\_ and am also the Principal Investigator for the \_\_\_\_\_ site in the NICHD-funded Maternal-Fetal Medicine Units (MFMU) Network. I have worked closely with Dr. \_\_\_\_\_'s proposed primary mentor, Dr. \_\_\_\_\_ second co-mentor, Dr. \_\_\_\_\_, is an internationally renowned human geneticist and has been one of the major architects of the \_\_\_\_\_ program.

I have met Dr. \_\_\_\_\_ and have found \_\_\_\_\_ to be knowledgeable, efficient, articulate and committed to a translational research career. It is obvious that \_\_\_\_\_ has made excellent progress in \_\_\_\_\_ project, having overcome several formidable political and laboratory obstacles. \_\_\_\_\_ presented \_\_\_\_\_ findings to date at the most recent MFMU Network Steering Committee Meeting and I was impressed with \_\_\_\_\_ understanding of the implications and limitations of \_\_\_\_\_ findings. I am not surprised to learn that \_\_\_\_\_ work has been accepted for an oral presentation at the \_\_\_\_\_ SMFM Meeting.

I enthusiastically support \_\_\_\_\_ research proposal as outlined and hope you will favorably consider \_\_\_\_\_ candidacy for a K23 award. It will be an excellent investment.

September 25, 2008

NOV 9 2008

Center for Scientific Review  
National Institutes of Health  
6701 Rockledge Drive  
Room 1040 - MSC 7710  
Bethesda, MD 20892-7710

re: \_\_\_\_\_, M.D.  
Mentored Patient-Oriented Research Career Development Award  
(K23) - \_\_\_\_\_

Dear Ladies and Gentlemen:

I am writing to offer my most enthusiastic support to \_\_\_\_\_'s K23 application. I have been a faculty member of the Maternal-Fetal Medicine Division of the Department of Obstetrics and Gynecology at the \_\_\_\_\_ since finishing my fellowship there in \_\_\_\_\_ and currently serve as a Full Professor with Tenure. I am also the Director of the Maternal-Fetal Medicine Fellowship Program at \_\_\_\_\_ and am immensely proud of the accomplishments of the men and women who have completed our program.

Dr. \_\_\_\_\_ has an outstanding background. \_\_\_\_\_ completed \_\_\_\_\_ undergraduate degree in \_\_\_\_\_ with flying colors (\_\_\_\_\_) in \_\_\_\_\_, where \_\_\_\_\_ obtained \_\_\_\_\_ MD at the \_\_\_\_\_, and, once again, \_\_\_\_\_ residency in Ob-Gyn was here in \_\_\_\_\_, and, once again, \_\_\_\_\_ received numerous accolades, including two Resident Teaching Awards, the Gold Foundation Humanism and Excellence in Teaching Award, and a Best Resident Paper Award. Dr. \_\_\_\_\_ is now in the 3<sup>rd</sup> year of \_\_\_\_\_ Fellowship in Maternal-Fetal Medicine at the \_\_\_\_\_, and \_\_\_\_\_ performance on both the clinical front and the research front has been nothing short of spectacular. \_\_\_\_\_ is already an author on \_\_\_\_\_ peer-reviewed papers, has several reviews to \_\_\_\_\_ name, and has presented several abstracts at national meetings. \_\_\_\_\_ abstract entitled \_\_\_\_\_

\_\_\_\_\_ is accepted for an oral presentation at the \_\_\_\_\_ Society for Maternal-Fetal Medicine meeting.

Dr. \_\_\_\_\_ has chosen to focus \_\_\_\_\_ research attention on genetic and genetic-environmental factors associated with neurodevelopmental delay. Having observed \_\_\_\_\_ evolution in this area and heard \_\_\_\_\_ lectures and presentations, I can attest to Dr. \_\_\_\_\_'s command of this important area of perinatal medicine. \_\_\_\_\_ primary mentor is \_\_\_\_\_, M.D. \_\_\_\_\_ serves as the PI for Dr. \_\_\_\_\_'s Eunice Kennedy Shriver award, a secondary analysis of a MFMU clinical trial. Dr. \_\_\_\_\_ has also aided Dr. \_\_\_\_\_'s submission as a Co-PI on a R21 proposal.

\_\_\_\_\_ M.D. and \_\_\_\_\_ Ph.D. are also mentors for Dr. \_\_\_\_\_ and share my enthusiasm for \_\_\_\_\_ academic potential. Dr. \_\_\_\_\_ is the Chief of Maternal-Fetal Medicine at the \_\_\_\_\_ and a funded NIH investigator. Dr. \_\_\_\_\_ is Distinguished Professor and Chair of Human Genetics at our institution; \_\_\_\_\_ role in Dr. \_\_\_\_\_ is an obvious one.

In summary, Dr. \_\_\_\_\_ is an exemplary candidate for this Career Development Award and one of the best I've seen in years. \_\_\_\_\_ has a very strong background and already has established an enviable track record in maternal-fetal medicine and \_\_\_\_\_ chosen field of interest. I strongly support \_\_\_\_\_ application – \_\_\_\_\_ is genuinely poised to be a leader in academic perinatal medicine.

Sincerely,

\_\_\_\_\_  
Professor  
Division of Maternal-Fetal Medicine  
Department of Obstetrics & Gynecology