Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD)

Progress Reports

December 2004

Presented CIFASD Steering Committee Meeting
Indianapolis, IN
January 12-14, 2004

Table of Contents

Progress Report for Administrative Core

I. Principal Investigator: Edward Riley, PhD

II. Title of Project: Administrative Core U24 AA014811

III. Objectives:

The CIFASD coordinates basic, behavioral, and clinical investigators in a multidisciplinary research project to better inform approaches aimed at developing effective intervention and treatment approaches for FASD.

The Specific Aims of the Consortium are as follows.

- Establish procedures for better defining the range of outcomes from prenatal alcohol exposure.
- Build upon existing research programs on FASD to meet this outcome.
- Establish a basic science program assessing mechanisms of alcohol damage and translate this work into effective interventions.
- Attract new and innovative investigators to the study of FASD by recruiting individuals for the development of U01 grants and pilot projects.
- Secure additional funding to implement innovative interventions and treatment strategies on a wide scale.
- Make available the data collected through this consortium to all interested parties.

The Primary Goals of the Administrative Core are:

- To provide an infrastructure that will allow effective communication between the various cores, components, and projects and to ensure the overall success of the consortium.
- To provide administrative support to the Steering Committee. Provide progress reports of the individual projects and collectively, progress towards the goals of the consortium to the Steering Committee.
- Convene meetings of the Steering Committee.
- To coordinate with the Informatics Core to ensure that communication within the projects is effective.
- Generate reports to all of the projects and ensure the dissemination of information to researchers, as well as to groups and individuals with an interest in FASD.
- Maintain consortium web site

IV. Methods

- Utilizes web based information, regular emails, monthly conference calls and twice annual meetings.
- Maintains contact with NIAAA through regular phone calls.

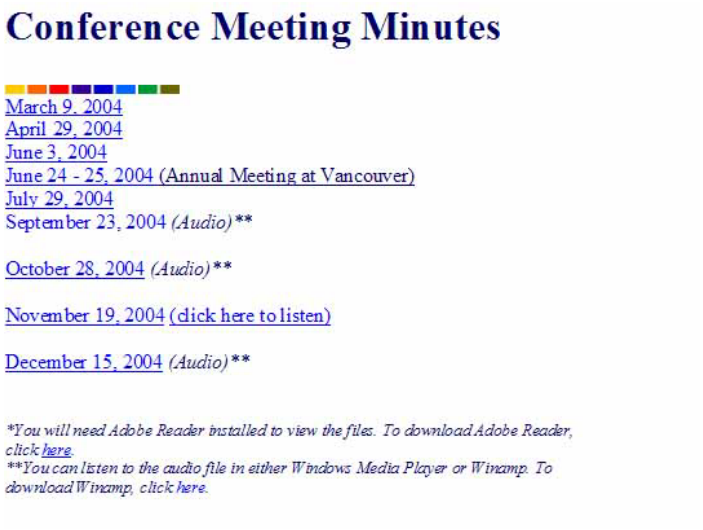
V. Accomplishments and Results

- A consortium website is functioning.
- Contains forms, manuals, database entry forms, tests, minutes, progress reports, etc.
- Regular monthly phone conference calls have been convened. These conference calls are available verbatim on the consortium website.
- Twice annual meetings of the consortium PIs have been convened. Minutes from these meetings are available on the website.
- Administrative core has financed parts of projects when funds were not available in the budget of the project

- The PI has given several talks about the consortium and has recruited new sites interested in joining the consortium or that will utilize the forms, tests, etc. created by the consortium.
- We have been approached by scientists in Australia, England, Germany, Japan, and the Reunion Islands about obtaining information about the consortium, our pilot project program, and to provide forms, manuals, etc. to the international research community.
- Pilot projects are being solicited and applications are available for online submission
  - NEW!

Apply for Pilot Project Grants

Click Here



- Publications are being put online
- Listservs are in place in cooperation with the Informatics Core.

VI. Discussion

The administrative core has helped to facilitate the goals of the consortium and some of the individual projects**.** Whereas some projects are clearly well underway, obtaining useful data, and clearly want to expand, others are just beginning to collect data or to provide services.

VII. Interrelation with Aims of the Consortium and Other Projects

By its very nature, the Administrative core interrelates with each of the projects.

VIII. Plans for the Next Year

The plans for the next year are to focus on the enhanced integration of the projects and to begin to analyze data in preparation for a contemplated renewal application to NIAAA.

IX. Publications

Not applicable

X. Posters, presentations

Not applicable

Progress Report from the Informatics Core

I. Principal Investigator: Craig A. Stewart, Ph.D.

II. Title of Project: Informatics Core U24 AA014818

III. Objectives:

The objective of the Informatics Core is to provide a data repository, data input tools, data verification tools, and data retrieval tools for the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD). In particular, we aim to provide reliable and HIPPA-compliant storage of data to be shared within the CIFASD, and tools that facilitate accomplishment of the scientific mission of CIFASD.

IV. Methods

CIFASD is collecting eight different suites of data: dysmorphology, 2D facial images, alcohol & control variables, neurobehavioral testing, 3D facial images, brain images, ultrasound, and laboratory analysis results. For each of these categories of data, the Informatics Core is working with the rest of the consortium to accomplish the following:

- Work with members of the consortium to define a data dictionary that precisely defines what data is to be stored.
- Create one or more input tools that allow projects to record their data.
- Expand the central repository to be able to store the data, and create methods to transfer data from the input tools to the central repository.
- Expand the methods for retrieving data to include the ability to retrieve each type of data in turn.

The result will be a combination of software tools that allow projects to locally store each of the types of data being collected for the consortium, upload/submit that data to a central repository, and query that central repository for results obtained across the projects in the consortium.

V. Accomplishments and Results

The development of data dictionaries is a particularly useful part of the Informatics Core services to the consortium as a whole. The need to create one data dictionary used for the data that are shared across the consortium, and the detailed consistency imposed by the use of shared database tools, has helped the consortium as a whole arrive at data definitions that are consistent. In fact, the degree of detail required to create shared data input tools has repeatedly helped us discover areas in which definitions were almost consistent rather than consistent. We have been very careful to leave it to CIFASD, and subcommittees appointed by the CIFASD leadership, to actually define the data items. This interplay has been very productive.

The status of the development of particular tools is as follows:

Dysmorphology Data Dictionary	finished
2D Facial Imaging Data Dictionary	in progress
Core Alcohol & Control Data Dictionary	finished
Expanded Alcohol & Control Data Dictionary	finished
Neurobehavior Data Dictionary	in progress
3D Facial Imaging Data Dictionary	finished
Dysmorphology MS Access Input tool	finished
Core Alcohol & Control MS Access Input tool	finished
Expanded Alcohol & Control MS Access Input tool	finished
Neurobehavior MS Access Input tool	in progress
Dysmorphology central database	in testing
Dysmorphology Upload tool	in testing
Dysmorphology Report tool	in testing

We have created a web page from which consortium researchers may download Microsoft Access input tools at http://discover.uits.indiana.edu:8250/downloads. Example screen images of data entry and retrieval tools we have created thus far are shown in Figures 1 and 2.

Data for the consortium are stored in duplicate – one copy in a robotic tape storage system in Indianapolis, IN, and a second copy in a robotic tape storage system in Bloomington, IN. This ensures that the consortium's valuable data will be kept reliably even in the event of a disaster at one of Indiana University's two computer rooms. Because it is inherently impossible to de-identify the facial image data, these data will be written to tape in an encrypted format. Even if an unauthorized person were to gain physical control of a tape with encrypted facial image data on it, it would be impossible to break the encryption and actually see the images.

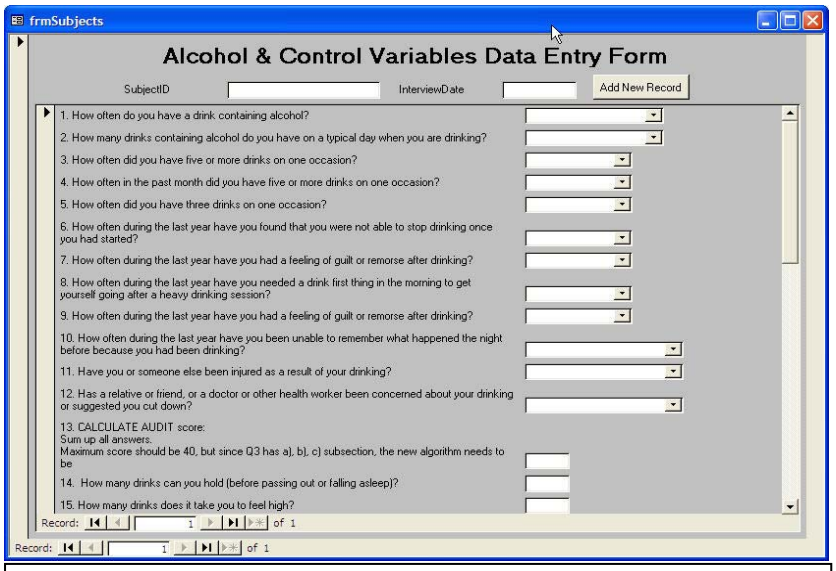


Figure 1. Alcohol and Control Variable Data Entry Form. This data entry form includes range checking that makes it impossible to enter a datum value outside the logically permissible range identified in the data dictionary.

VI. Discussion

The consortium leadership decided in early September that the expanded version of the Alcohol and Control variables (which include alcohol questions, maternal questions, other drug questions, confounders, and controls) being used by the Moscow project should become required for any new projects joining the consortium in the future. This decision meant that two different versions of the Alcohol and Control variables needed to be considered: the Core variables that the existing projects already have in common and the expanded variables, being used in Moscow. The result was the creation of separate data dictionaries and Access input tools for each. The work required close to three months of full time effort by the Informatics Core's lead programmer, Christina Deximo.

We are happy to report that the Informatics Core has been able to dedicate more of Christina Deximo's time to this project than the amount paid for by the NIH grant. This has enabled the Informatics Core to make rapid progress during the past several months. Our status in general is that we are implementing data tools in a reasonably short period of time after the CIFASD scientists have come to consensus on data dictionaries.

VII. Interrelation with Aims of the Consortium and Other Projects

The Informatics Core is essential infrastructure for CIFASD as a whole. The structure of a separate Informatics Core has facilitated the collaborative processes that have enabled the consortium's scientists to come to consensus on data definition and measurement issues that are essential to the broader goals of the CIFASD as a whole. We believe that

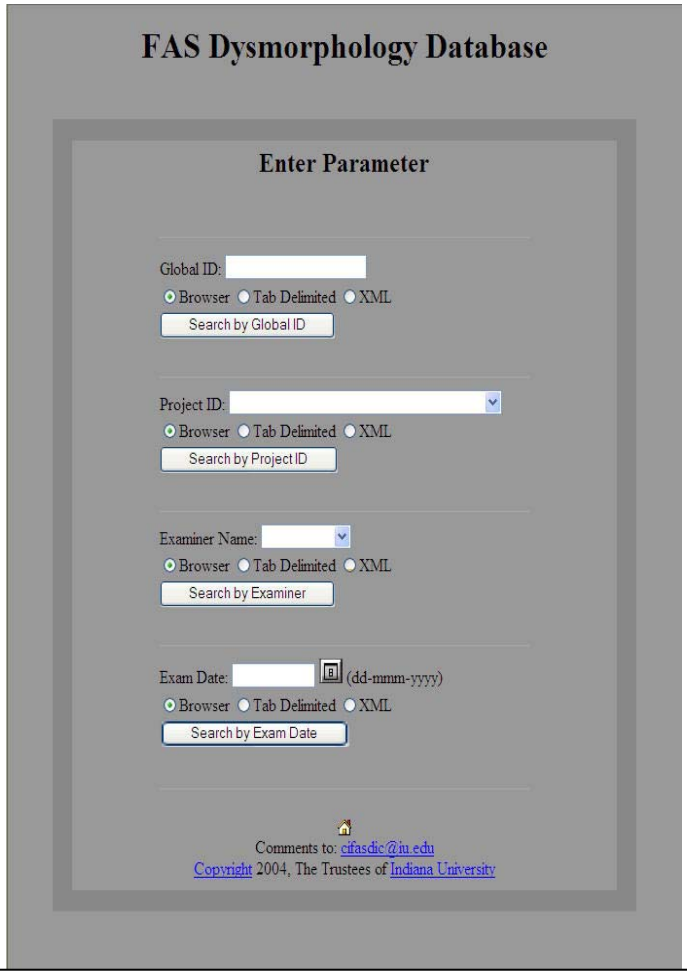


Figure 2. Web-based Dysmorphology data access and download tool.

this model may be for other large NIH-funded consortia in the future. The work of the Informatics Core has led to the creation of data dictionaries that will ensure that the common data collected by the consortium are usable and understandable indefinitely, and the suite of computer tools we have created will ensure that the data are accessible indefinitely.

VIII. Plans for the Next Year

Once the full suite of Dysmorphology tools (input, upload, and reporting) is available, we are looking forward to working with the consortium as a whole to ensure that the tool meets the needs of the consortium researchers. So far, feedback on the tools we have developed has been positive in general. The report tool that is currently being tested includes the essential functionality required to give feedback quickly to researchers about how well the input and upload tools are working. The report tool will be expanded to allow more elaborate queries and to include download types for SAS and SPSS formats.

One of the highest priorities for the coming year will be creating upload and report tools for 2D images. We've heard a strong need amongst researchers who want to begin looking at these images in conjunction with the Dysmorphology data.

We are looking forward to working through the remaining portions of the Neurobehavior data dictionary so the Neurobehavior Access input tool can be finished and we can begin working on the upload and report tools. The Neurobehavior data is complicated by the fact that many of the variables are calculated by other programs. We are searching for ways to include these variables directly and avoiding any need to rekey results.

We have already worked closely with the 3D Facial Imaging Core to understand their needs and consult on their process for organizing data before submitting it to the central repository. We're looking forward to creating the upload and reporting web tools for 3D facial images.

For brain images, we worked briefly with the Brain Imaging Core to understand their needs and look forward to building a data dictionary with them when it becomes clearer how the brain images will be used.

If the consortium decides to include Ultrasound and/or Lab results in the central repository and identifies who will take responsibility for these areas, we will begin working with those people to create data dictionaries, input tools, etc.

IX. Publications

Stewart, C.A., R. Repasky, and A. Arenson. 2004. Open source tools for computational biology. Tutorial handbook. SC2004 conference, November 2004, Pittsburgh, PA. Tutorial notes may be downloaded from rac.uits.indiana.edu

Stewart, C.A. 2004. Bioinformatics: transforming biomedical research and medical care. Communications of the ACM 47(11): 31-33.

X. Posters, presentations

CIFASD Informatics Core. 2004. Indiana University display at Indiana Health Industry Forum, June 2004. Indianapolis, IN

CIFASD Informatics Core. 2004. Electronic poster included as part of Indiana University display at the SC2004 Conference, November 2004. Pittsburgh, PA.

Progress Report for Pilot Project Core

I. Principal Investigator: Edward Riley, PhD

II. Title of Project: Pilot Project Core U24 AA014828

III. Objectives:

The Pilot Project Core provides a mechanism by which the consortium acts as a dynamic entity. It provides a flexible means for developing and exploring new research activities and directions for the Consortium and a mechanism by which new sites can be added or projects can evolve into independently funded research projects. Four pilot projects are currently funded and a call has been sent out for the support of new projects. There was to be an emphasis on adding new sites with unique resources or attributes about the population under study that would contribute to the overall functioning of the Consortium. However, under current discussion is the option of providing additional funding for ongoing pilot and regular projects that want to expand based upon current findings from the consortium.

IV. Methods

Four independent projects are described in the next section, each presenting its objectives, methods, accomplishments and goals. Solicitation of new projects is ongoing.

V. Accomplishments and Results

The major accomplishment in the administration of the Pilot Project Core has been the solicitation of new proposals. An announcement of possible funding for new projects has gone out through the RSA Fetal Alcohol Study Group. Also, please see website for online submission of pilot projects. http://www.cifasd.org/projectgrants.htm

Each of the currently funded projects provides updates following this section.

VI. Discussion

The pilot project core seems to be making progress, with significant amounts of data collected at three of the sites, and the fourth site, delayed for a variety of bureaucratic and political reasons is beginning to collect data. This site expects to be up to speed and complete its goals during the current funding year. One R01 submission has resulted from the project at the University of Washington.

VII. Interrelation with Aims of the Consortium and Other Projects

See individual project descriptions

VIII. Plans for the Next Year

See individual project descriptions

IX. Publications

None

X. Posters, presentations

None

Progress Report on Pilot Project: Buffalo

I. Principal Investigator: Luther K. Robinson, MD

II. Title of Project: Comparison of Three Diagnostic Modalities in Fetal Alcohol Spectrum Disorders

III. Objectives:

1. To compare morphological data in racially diverse subjects with FASD, Williams syndrome, and a group of control subjects
2. To generate data from digital (2-D) and laser (3-D) imaging techniques in these groups of subjects
3. To compare standard morphological measures with digital (2-D) and laser (3-D) imaging in this racially diverse group of subjects with FASD, Williams syndrome, and control subjects

IV. Methods

1. We are comparing 1) clinical measures such as palpebral fissure length (PFL), inner canthal distance (ICD), philtral integrity utilizing the Likert scale of Astley and Clarren, and anthropometric measures such as maxillary and mandibular arcs with 2) data from digital photographs and 3) 3-D laser images in subjects with FASD, Williams syndrome, and a group of age and gender matched control subjects
2. We are using the diagnostic paradigm as set forth by the Dysmorphology Core
3. Subjects are offered an honorarium of $25.00

V. Accomplishments and Results

1. We have studied 90 subjects
2. 38/90 subjects have FASD (FAS and ARND), 36/90 subjects are age-matched controls, 16/90 have Williams syndrome
3. Morphological data and data from 3-D imaging have been submitted to the Bioinformatics and Facial Imaging cores, respectively, for analysis

VI. Discussion

1. We are about one month behind our proposed timeline; we have not begun to analyze our data
2. The project has been well received locally
3. We have experienced challenges
    a. Small room size enhances intensity of light and diminishes definition of important facial landmarks (e.g., philtral columns)
    b. Subject movement degrades quality of the image
    c. Delays in feedback from imaging core has not allowed for prompt response or changes in technique at our facility
    d. While we have successfully imaged younger subjects, age 5 years in our experience is the youngest age at which imaging data can be reliably obtained
4. Laser imaging, because of its potential for measurement in 3-dimensions, may permit more comparative measures with clinical morphology

VII. Interrelation with Aims of the Consortium and Other Projects

1. This project contributes data concerning the feasibility of computerized or laser imaging to the diagnosis of FASD. It may provide answers to the question "What does the dysmorphologist "see" when he examines the subject with craniofacial dysmorphology?"
2. Standardized digital photography will add consistency to clinical assessment

VIII. Plans for the Next Year

1. We have hired a statistician who will analyze our local data
2. Add subjects with Down syndrome as a second disease control category

IX. Publications To be submitted as data are analyzed

X. Posters, presentations. Data to be presented at Research Society on Alcoholism annual meeting June 2005 and at David W Smith annual meeting on Morphogenesis and Malformation

Progress Report on Pilot Project: University of Washington

I. Principal Investigator: Ann Streissguth, Ph.D.

II. Title of Project: Neonatal Ultrasound Study to Detect Fetal Alcohol Brain Damage

III. Objectives:

This project proposes to develop methods and procedures for the earliest detection of babies born with prenatal brain damage from alcohol. This pilot study proposes to demonstrate the effectiveness of an innovative new method for detecting not just those with the full FAS, but also those with the full spectrum of fetal alcohol damage. The project proposed here is a downward extension to infancy of our previous work on morphometric-neuropsychological deficits in adults and adolescents, which has over an 80% detection rate for patients with FAS or FAE vs. controls. Here we propose to use neonatal cranial ultrasound to obtain the images, rather than MRI as we did with older patients. Here the challenge is greater, as we must also make determinations about which newborns to scan for alcohol effects – in contrast to our MRI work, which involved diagnosed patients, all of whom were known to be alcohol-affected vs. controls. We have demonstrated in a small feasibility study that our procedures work, but we need a larger sample size to demonstrate quantifiable differences in the hypervariance signal between alcohol-exposed and unexposed infants.

IV. Methods

We proposed to locate, enroll, and scan 50 mother/child pairs: 25 recently postpartum mothers who are heavier drinkers and at risk of having alcohol-affected offspring according to BARC (our binge-alcohol rating criteria), and 25 non-drinking or lightly drinking mothers. Babies classified as "exposed" have mothers who were BARC+ (based on a weighted sum of: "monthly frequency of 5 or more drinks on an occasion plus the frequency of 3 to 4 drinks on an occasion for either time period: during pregnancy or in the month or so before pregnancy"). If this sum is equal to or greater than 4, the mother is BARC+. Babies were scanned before they were 4 months old.

An HDL Ultrasound Scanner using a C8-5 Pediatric Cephalic Transducer with cine memory is used to obtain approximately 50 trans-fontanelle freeze frame images of each baby's corpus callosum (CC) in the midsagittal plane. The raw images are transported to our Unit in JPEG format, where the images are sorted by subject, converted to grayscale images, and color inverted to black on white for better visualization of the CC during image averaging. Identifying information is erased from the image, the study ID number inserted, and the images are saved into a .tiff format and transported electronically to Fred Bookstein for data preparation and analysis using an existing module of the Edgewarp program package. A system of *unwarped image averaging* was developed by Bookstein as follows: on each frame of the selected subset for each infant, reference points were placed at the presumptively fixed location of the transducer (the center of the visible fan beam) and at visually characteristic points in the vicinity of genu and of splenium. (Thus, these points are not landmarks in the morphometric sense.) The resulting stack of fiducially marked images was processed by the averaged image unwarping method of Bookstein (1999) which, for the simplest fiducial configuration here (three points only), reduces to (1) an operator-determined affine transformation (translation, rotation, and shear) applied to each image in turn to superimpose its three points over their average positions, followed by (2) a pixel-by-pixel averaging of the image information after all transformations have been applied as shown in Figure 1.

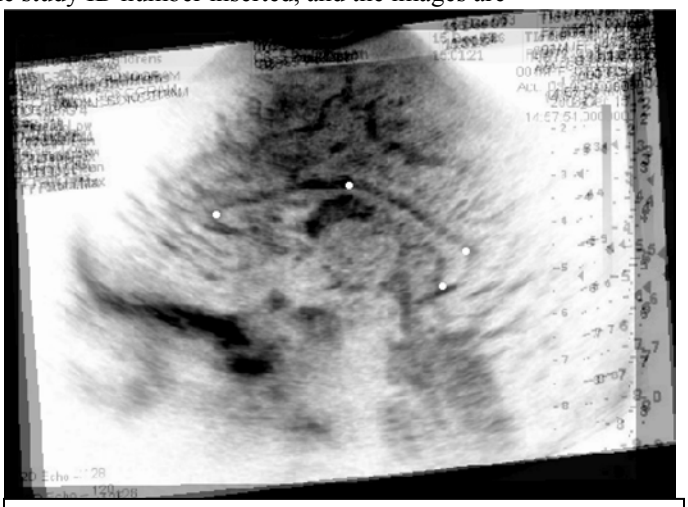


Figure 1. Four-point representation of arch form, unwarped averaged images. Clockwise around arch from left: tip of genu (a landmark); top of arch (a semilandmark taken exactly halfway between tip of genu and tip of splenium); "angle-of-splenium point" (a semilandmark taken where the long axis of splenium intersects the upper arch margin); tip of splenium (a landmark).

An additional $20,000 grant was received from the University of Washington Alcoholism and Drug Abuse Institute (ADAI), utilizing the same design as the CIFASD study, in order to 'jump-start the study while a hospital

screening study was ongoing through our Unit, funded by another source for a different project. Human Subjects approval and 11 modifications were obtained and a confidentiality certificate from NIAAA. Additionally, mother/baby pairs were recruited via flyers, advertisements, and referrals by programs for high-risk mothers. Kristi Baldwin, Research Coordinator, met with mothers to obtain informed consent, administered the one-page Hospital Screening Questionnaire, the Pregnancy Drinking Calendar, and the UW Addiction Severity Index, and transported mothers and babies to CHRMC for the scan.

V. Accomplishments and Results

The code was broken after 20 infants were scanned. Two of these had too few comparable original ultrasound frames to be processed by this method. (One of these two was the very first baby imaged, when fewer images were obtained; the other was the oldest baby, possibly with too small a fontanelle.) The protocol was adjusted accordingly. In this comparison of eleven controls to seven alcohol-exposed infants, a single quantitative feature of these averaged unwarped ultrasound images separates five of the seven alcohol-exposed from the other two and from all but one of the unexposed (Figure 2). A formal t-test of the coordinate in this direction returns a p-value of less than 0.0012 for the mean comparison. But also, the *variance* of this relative orientation to the alcohol-exposed group is nearly four times that in the unexposed ($p = 0.04$), confirming the general finding of hypervariability of callosal form that we reported in our adolescent-adult samples (Bookstein et al, 2002). This quantification, the angle between the long axis of splenium and the segment from tip of genu to tip of splenium on the callosal outline as a whole may be the long-sought biomarker for damage from prenatal alcohol exposure.

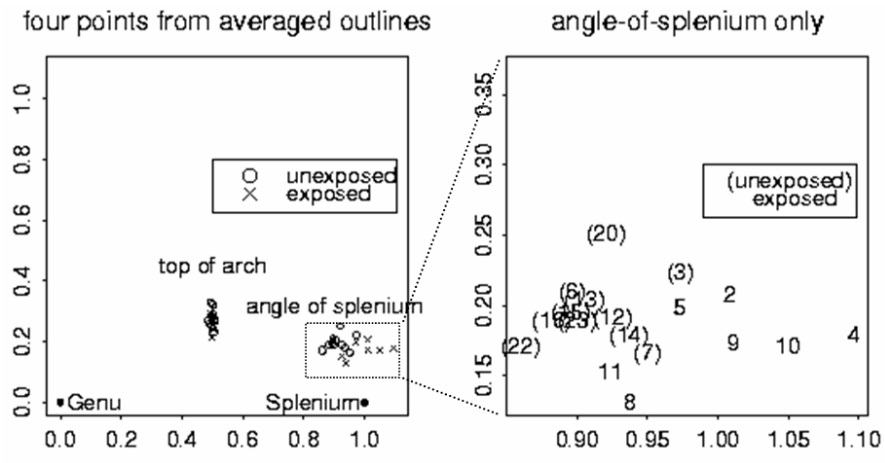


Figure 2. Two-point shape coordinates of top of arch and the angle of the splenium to a genu-splenium baseline. Left: all shape coordinates. Right: enlargement of the distribution of the angle-of-splenium semilandmark. Shape coordinate pairs for babies in the exposed group are set without parentheses.

VI. Discussion

Since the above analyses were carried out, we have scanned 13 more babies (undesignated as code has not been broken), bringing our total count to 33 babies scanned. We have overcome innumerable problems. It has been an almost impossible task to run a study of this complexity on a $30,000 per year budget. Yet, we remain enthusiastic about the results, the response of community professionals to this early identification project, and the impacts of the program on the mothers and babies. We have been surprised to discover the number of early intervention community resources in existence, which the babies of heavy-drinking mothers are not now eligible for because of the lack of an early diagnosis.

VII. Interrelation with Aims of the Consortium and Other Projects

This small pilot project is totally congruent with the aims of the Consortium and other projects within it, such as those in South Africa, Ukraine, and Finland, where the capacity to study newborns and their mothers has already been demonstrated. The goal of our Seattle project is to develop a novel and cutting-edge protocol that can be used cross-culturally for the earliest neonatal detection of babies across the whole spectrum of fetal alcohol disorders. The brain-based model we are developing can be carried out in any modern neonatal unit in almost any country of the world, using existing personnel with little extra training, and will be suitable even for babies of those highest risk mothers who fail to receive prenatal care. The public health significance of this new brain-based neonatal detection model lies in its ability to detect not only alcohol-abusing mothers who can be enrolled in alcohol treatment and advocacy programs before they bear another alcohol-affected child, but also simultaneously to detect alcohol-affected newborns who can be enrolled in early intervention programs many years before they might otherwise have been detected.

VIII. Plans for the Next Year

We are carrying over funds from Year 1 in order to pay for ultrasounds and maternal remuneration on the 17 additional babies to be located and scanned in year 2. The Year 2 money ($30,000) will continue to pay Kristi Baldwin's part-time salary for locating, screening, and transporting the additional 17 mother/baby pairs to reach our goal of 50 babies scanned; the rest of the year 2 money will go to Fred Bookstein for analysis. We have also applied for another ADAI grant (around $18,000) to cover transportation and per diem for Ken Jones to make 4 trips to Seattle in 2005 to conduct dysmorphology examinations of our scanned babies, and to pay an outreach worker for locating, obtaining consents, and transporting mothers and babies for the dysmorphology exams. We have applied for an R01 to continue this work and will be resubmitting this in 2005. Additionally, we are negotiating how to obtain some neurobehavioral testing on a small group of our scanned babies, but at the present have no funding for this. If there were some additional funds available from the Consortium for a pilot neurobehavioral study of some of our scanned infants, it would enhance the likelihood of a successful R01 for the larger cross-cultural study.

IX. Publications

Submitted 12/30/04: "Prenatal Alcohol Damage Can Be Visible in Averaged Ultrasound Images of the Neonatal Corpus Callosum." by Fred L. Bookstein, University of Washington, University of Vienna; Paul D. Connor, Kristi D. Covell, Helen M. Barr, Christine A. Gleason, Ann P. Streissguth, University of Washington; Raymond W. Sze, Jenny A. McBroom, Children's Hospital and Regional Medical Center, Seattle, Washington.

X. Posters, presentations

1. Platform presentation at the June 2004 Teratology Society Meetings in Vancouver, Canada: "Ultrasound Imasging of the Neonatal Corpus Callosum is Feasible and Useful" presented by Fred L. Bookstein. (Although the earlier printed abstract described only pilot data, the oral presentation
included the first analysis and findings from the CIFASD project.)

2. Presentation at the Fetal Alcohol Study Group Meeting of the Research Society on Alcoholism June 2004 Meeting in Vancouver, Canada: "Detecting FASD with Prenatal Ultrasound". Presentor: Fred L. Bookstein. (other authors: Ann Streissguth).

3. Presentation at the 25th Anniversary Celebration of the University of Washington Department of Statistics. Sept 2004, Seatle, Washington. Presentor: Fred L. Bookstein. Hour-long talk of which 1/4 was on our CIFASD project

4. Presentation at the Konrad Lorenz Institute for Evolution and Cognition Research, Altenberg, Austria. October 2004. Presentor: Fred L. Bookstein. Hour-long talk of which 1/4 was on our CIFASD project.

Progress Report on Pilot Project: University of New Mexico

I. Principal Investigator: Philip A. May, PhD; Mauro Cecanti, M.D. (co-P.I.)

II. Title of Project: FASD in Italy

III. Objectives:

This study is examining the prevalence and characteristics of FAS and other FASD in a European population where drinking is much more frequent among the female population, yet drinking patterns are more formally prescribed by cultural norms and associated with meals and a general pattern of hospitality and familial sociability. The specific aims of the study are: 1) to determine the number, rate, and characteristics of children with fetal alcohol syndrome (FAS) and other fetal alcohol spectrum disorders (FASD) entering school each year in select towns in the Province of Rome Italy through active case ascertainment and random sampling methods; 2) to screen suspects for FASD in schools with both normal and special needs children; 3) to determine if FAS and other FASD exist in Italy and if FASD is more prevalent among certain ethnic groups and immigrants, particularly those of low socioeconomic status; 4) to detail the specific physical, mental, and neurodevelopmental characteristics of Italian children with FAS, Partial FAS, ARBD, and ARND; 5) to identify children on whom culturally appropriate neuropsychological tests can be applied to best diagnose and characterize Italian children with FAS, Partial FAS, and other FASD; and 6) to provide epidemiologic data of FASD in a select region of Italy that are uniquely applicable to targeted prevention programs. Such an approach has not been reported in European populations.

IV. Methods

We are using active case ascertainment both in first grade classes of representative schools (using both growth parameter screening and teacher referral of those with suspected disabilities) and in active outreach throughout the community in institutions for the severely retarded. Random selections of schools and capture/recapture methods to produce a complete prevalence of FAS in a prescribed age cohort (approximately 5 to 7 years of age) have been utilized. A two tier screening system is implemented based on: 1) growth and 2) the referral of children identified by teachers as having developmental problems.

V. Accomplishments and Results

In the fall of 2003, schools were randomly selected from a list of 68 elementary schools within the 15 regional study communities. The total number of first grade students in the participating schools was 1086. The total number of children enrolled in the study as a result of informed consent was 543. These 543 children were entered into the first tier of screening and their height, weight, and head circumference were measured by school nurses and University of Rome medical staff. As a result of the Tier 1 screening, 181 children were identified for dysmorphology examinations because of growth and/or behavioral concerns. Added to that number were 72 randomly selected controls children. As a result, a total of 253 children were initially sought for dysmorphology examination. Of those 253 children, ultimately 233 children were seen by the dysmorphology team (17 of the randomly selected children were also referred for growth or behavioral problems, 2 were chronically absent, and one was refused parental permission).

The families of children enrolled in the study were contacted prior to the dysmorphology screening and asked about family characteristics that would help “rule out” genetic and size factors that might produce phenotypes similar to FAS. Also, a structured maternal interview containing 300 items was conducted with 517 of the 543 consenting families. These interviews explored: demographic data, childbearing pattern; drinking patterns (and estimation of peak BAC) before, during, and after the index pregnancy; marital and cohabitation pattern; SES; nutrition, and social psychological factors such as self esteem, locus of control, fatalism, and social environment.

After these 233 children were given a preliminary diagnosis by the dysmorphology team, 147 were identified to receive basic psychological testing. Of those 147 children, 140 received psychological testing. The test instruments used for that group were the Ravens Colored Matrices and the Rustioni’s Evaluation of Language Comprehension.

V. Discussion

The project has gone much as anticipated, except for a low rate of consent to participate overall (50%). But within the sample of those who are participating, there has been a high rate of success in capture. For example over 95% of

the mothers have consented to a maternal interview, and over 97% of the mothers suspected of having given birth to a child with FASD have consented to interviews.

VI. Interrelation with Aims of the Consortium and Other Projects

The data and results from this study will be compared with the other sites involved in the epidemiology portion of the consortium. The Italian team used the CIFASD dysmorphology-core forms to record the data for the children seen in the first year of screening in Italy. The dysmorphology core (Drs. Jones, Robinson, and Hoyme) were the dysmorphologists who diagnosed the children in this first round of screening. They were joined by Miguel Del Campo, M.D., from Spain and Luigi Teranni and Agatino Battaglia, both Italian geneticist/dysmorphologists. This ensures that the cases diagnosed from Italy will be gold standard cases worthy of comparison anywhere else.

The data from this study will be included in the data set of children diagnosed across the international consortium sites so that a greater understanding of the spectrum of alcohol exposure (FASD) across populations can be discovered. Furthermore, if additional funding is secured, a complete battery of neuropsychological tests can be administered to Italian children with symptoms of FASD and a sub-sample of the randomly-selected Italian controls.

VIII. Plans for the Next Year

The Italian team will meet with the dysmorphology team and epidemiology research team in Albuquerque on the third week of January, 2005. There they will review in a formal case conference the findings for all children who were given a preliminary diagnosis, whose diagnosis was deferred, and the randomly selected controls. A final diagnosis of each child seen will be assigned at that time.

In March, 2005, the second wave on screening will take place in the Lazio Region of Italy following the same methods that were used in the first wave of screening in 2004.

IX. Publications

None

X. Posters, presentations

A formal presentation of these preliminary research progress findings were made at the RSA meetings in Vancouver in June.

Because FAS and FASD have not been popular issues in Western Europe and because this is the first population-based study of FAS and other FASD ever undertaken in Western Europe, results from this study must be treated with great sensitivity. It has, therefore, been agreed upon by both Italian and American researchers that no findings from this study will be released prior to the finalization of data and first aggregate analyses from both waves one and two (approximately June of 2006).

Progress Report on Pilot Project: UCSD

I. Principal Investigator: Kenneth Lyons Jones, Ph.D.

II. Title of Project: Prenatal Ultrasonographic Markers of FASD in Ukraine

III. Objectives:

To use a standard prenatal ultrasound technique at 4 specified time points in pregnancy to compare somatic and brain growth in fetuses of pregnant women with moderate to heavy alcohol consuming behaviors to the fetuses of pregnant women who drink low amounts or no alcohol at all.

IV. Methods

Early prenatal screening will be undertaken in several large obstetric services in two oblasts or regions of Ukraine. Specially trained interviewers will administer structural interviews 4 times during pregnancy focused on alcohol consumption. These will take place at the time of each ultrasound scan. The goal is to achieve an exposed sample of approximately 80 subjects resulting in live births. An equal number of low or non-drinking subjects will be recruited over the same time period. Physical examinations will be performed on each subject by trained Neonatologists, and those determined to be affected (FAS) or deferred will be subsequently examined by Dr. Jones or Dr. Robinson.

V. Accomplishments and Results

All interviewers, ultrasonographers and Neonatologists were trained by Drs. Chambers, Hull and /or Jones. Interview forms and physical exam forms were translated into Ukrainian. Standard forms for entering ultrasound data were developed. Data collection will begin in mid-January 2005. After 3 months, we will look at the recruitment numbers. Based on these numbers we will determine how long recruitment will continue.

VI. Discussion

Despite significant problems relating to civil unrest in Ukraine, we have been encouraged by the extent to which our Ukrainian collaborators have enthusiastically moved forward with this project.

VII. Interrelation with Aims of the Consortium and Other Projects

Since the initiation of this study and the Prospective Study of Factors associated with FASD in the Moscow Region, the potential value of prenatal ultrasonographic markers of FASD has become more evident. A study using a similar methodology using the same ultrasonographer, Dr. Andy Hull, is being discussed.

These 3 studies are now being done in 3 different consortium sites, all using the same methodology in order to identify prenatal markers of FASD which would be a major step forward regarding early diagnosis of this disorder.

VIII. Plans for the Next Year

Over the next year, this pilot study will be completed. Hopefully, all pregnant subjects will be recruited over the next 3 months and all deliveries will have occurred in the next 9 months.

IX. Publications

None

X. Posters, presentations

None

Progress Report for Dysmorphology Core

I. Principal Investigator: Kenneth Lyons Jones, M.D.

II. Title of Project: Dysmorphology Core U24 AA014815

III. Objectives:

1. To insure consistency as well as accuracy in diagnosis of FASD at all project sites where children are being evaluated throughout the world.
2. To explore the extent to which various degrees of deficient ($\leq 3^{rd}$% vs. $\leq 10^{th}$ %) anthropomorphic measurements, including length, weight, head circumference (OFC), palpebral fissures, inner canthal distance and philtrum should be used to enhance specificity of diagnosis without loss of sensitivity.
3. To explore strategies for diagnosis of FASD in the newborn period or at least during the first year of life.
4. To delineate the full range of structural anomalies in children with FASD and identify clinical features most indicative of future problems in neurobehavioral development.
5. To correlate the clinical diagnosis of FASD with that determined by 3-D photography.

IV. Methods

Accomplishments and Results

1. To insure consistency and accuracy of diagnosis.
   a. Implementation of standard protocol
   b. Development of manual to explain and standardize the methods used in performing the physical exam
   c. Development of a Dysmorphology Access Data Base
   d. Translation of the Dysmorphology Core Physical Exam Form into Russian and Ukrainian
   e. Training of Local Physicians
      • 2 Neonatologists completed training at each of the 4 birthing hospitals in the Moscow Region
      • 4 Pediatricians completed training to examine children at Boarding Schools and Orphanages in Moscow
      •16 Pediatricians, Neonatologists and/or Geneticists completed training in Ukraine
      • 4 Pediatricians and/or Neurologists completed training in Rome
   f. Travel to Consortium sites to verify diagnosis and update training.
      • Prospective Study – Moscow Region
        Jones - 4 trips
        Robinson - 2 trips
      • Neurobehavioral Development of children with FASD in Moscow
        Jones - 2 trips
        Robinson - 1 trip
        70 children examined
      • Prenatal U/S markers of FASD in Ukraine
        Jones - 2 trips
      • FASD Epidemiology in Italy
        Jones, Hoyme, Robinson, del Campo – 1 trip each
        - <u>Results -</u>
        1086 - Total Numbers in 1^{st} grade classes
        547 - Total Providing Consent to participate
        181 - Total Children screened because of growth or behavioral problems
        75 - Randomly selected Controls
        256 - Total to be provided full Dysmorphology Exams.
        Results Total Examined - 231
        FAS - 15/1086 (1.4%)
      • Detecting FASD from Neonatal U/S – Seattle
        No Trips
      • South Africa
        No Trips
      • American Indian reservation in Northern Plains States

Hoyme - 2 trips
Robinson - 2 trips

- Neuroimaging Studies in Finland
  Hoyme - 1 trip
    Diagnosis according to Consortium Dysmorphology Scoring Sheet
    FAS = 32
    Deferred = 9
    Not FAS = 4 (included in the four are two with possible ARND and two with other unknown malformation syndromes)
- 3-D Photography Studies in Buffalo
  92 Images Completed
  40 with FAS
  52 with Williams Syndrome and normal controls

2. To explore the extent to which various degrees of deficient ($\leq 3^{rd}$ % vs. $\leq 10^{th}$ %) anthropometric measurements should be used to enhance specificity of diagnosis without loss of sensitivity, we are gathering this information. Analysis of these data will require completion of the data acquisition.

3. Explore strategies for diagnosis of FASD in the newborn period or at least during the first year of life. No newborn babies have delivered at any of the consortium sites over the first year. This will become a major focus of the Dysmorphology Core over the third year.

4a. Delineation of the full spectrum of defects seen in children prenatally exposed to alcohol. By significantly broadening the criteria necessary for a child to be categorized as "deferred", we have significantly increased the number of children who are being deferred. For example, in Wave I of the Epidemiology of FASD in the Italy study, 78 of the 256 (26%) children seen were deferred by the expert dysmorphologist. As noted in question 66(2), of the Dysmorphology Core Physical Exam Form, the definition of Deferred is much broader than has been previously used in order to provide the opportunity to determine if the physical features traditionally used to diagnose FAS are too narrow and that less restrictive diagnostic criteria might be indicative of prenatal alcohol exposure. In order to most successfully accomplish this aim, it will be necessary to evaluate children in the newborn period who have been ascertained prenatally by virtue of their mother's alcohol intake during pregnancy. This will be a major focus of the Dysmorphology Core over the third year.

4b. Identify Clinical Features most indicative of future problems in neurobehavioral development. This can be accomplished only after the completion of the neurobehavioral studies.

5. Correlation of the Clinical Diagnosis with that determined by 3-D photography. Both Drs. Hoyme and Robinson received training in the use of the 3-D laser camera. Dr. Robinson has been using the camera in Buffalo in the pilot project entitled Comparison of three Diagnostic Modalities in FASD and related Disorders in African-Americans.
   Dr. Hoyme has begun using the 3-D laser camera in the pilot project being performed in Helsinki.

VI. Discussion

VII. Interrelation with Aims of the Consortium and Other Projects

1. We have demonstrated our ability to train Pediatricians, Neonatologists and Geneticists to diagnose fetal alcohol syndrome through training programs we held in Russia in which the diagnosis was validated by one of two Dysmorphologists.

2. Through this methodology that we used in South Africa and, most recently, in Rome, we have demonstrated the effectiveness of ascertaining children with Fetal Alcohol Syndrome in 1^{st} grade classes for normal children. It may be that this is a far better way to diagnose this disorder than in physician's offices and clinics run by Dysmorphologists and Geneticists.

3. We have developed a highly effective physical examination form that has been "field tested" and can be used in a variety of different countries throughout the world.

4. We have developed a manual used in conjunction with the physical examination form.
5. It is still too early to comment on the success or failure of most of the aims we set out to accomplish in the Dysmorphology Core.

VIII. Plans for the Next Year

Over the next year, we plan to increase the number of trips to most of the consortium sites.
Two of us will go on 2 separate occasions to evaluate children in the boarding Schools and orphanages in Moscow.
Two of us will go on 3 separate occasions to evaluate newborn infants in the 4 birthing hospitals in the Moscow Region of Russia.
Two of us will go on 1 occasion to evaluate adults and adolescents (both subjects and controls) in Helsinki.
Two of us will go on 3 separate occasions to evaluate newborn infants in 2 birthing hospitals in Ukraine.
Two of us will go on 2 separate occasions to South Africa.
Two of us will go on 2 separate occasions to Seattle.

IX. Publications

X. Posters, presentations

Bakhireva, L., Jones, K. Robinson, L., Riley E., Mattson, S., Marintcheva, G., Chambers, C. (2004). Effective Training of Pediatricians to Diagnose Features of the Fetal Alcohol Syndrome in Russia Sample. Research Society on Alcohol. Vancouver,.

Progress Report of Brain Imaging Core

I. Principal Investigator: Elizabeth R. Sowell, Ph.D.

II. Title of Project: Cross-cultural FASD: Brain Imaging U24 AA014808

III. Objectives:

Specific Aim 1: Among the first of the original specific aims of this project was to scan a human volunteer and a mechanical phantom on all magnets that will be used to collect structural MRI data in order to establish the protocols and parameters for scanner calibration to correct scanner-specific geometric distortion. We proposed to calibrate data collected from each scanner, calculating the deviation in spatial registration between phantom (human and mechanical) images collected at each site, and apply spatial correction algorithms to ensure that they are anatomically (and geometrically) matched. We will work with other Consortium members to help them best perfect their image acquisition protocol that may vary somewhat by magnet manufacturer.

Specific Aim 2: We also proposed to adapt automated image analysis tools for dissemination to the various sites collecting structural brain imaging data to assess CC shape and other brain structural information. All software adapted and created for the purposes of this Consortium is platform independent, and user-friendly.

Specific Aim 3: We proposed to assess relationships between the data collected and analyzed within the Imaging Core, and data collected by the other projects and cores in this Consortium such as the Dysmorphology Core, the Neurobehavioral Core, and the Facial Imaging Core.

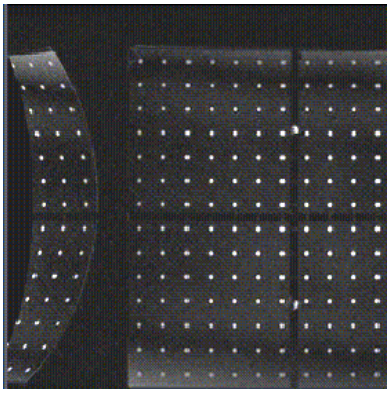
IV. Methods

V. Accomplishments and Results

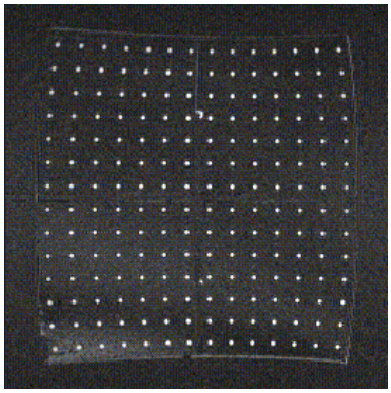
Specific Aim 1: We have scanned the mechanical phantom at both UCSD and UCLA. A human volunteer who has been scanned at UCSD will travel to UCLA in early January. Following is a description of progress on analyzing the mechanical phantom data, which can then be applied to the human volunteer.

Confirmation of phantom dimensions

The characteristic dimensions of the phantom were measured (cylinder diameter, plate thickness, hole diameters, adjacent hole spacing) and compared with the phantom manufacturer's specifications. No significant differences from the manufacturer's specifications were found.



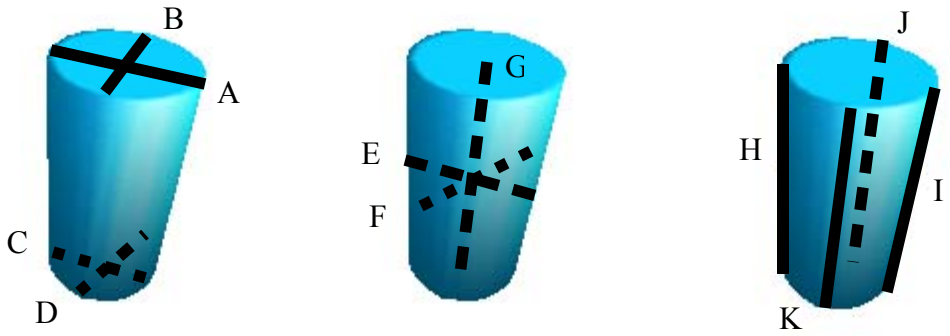
San Diego sample phantom scan



UCLA sample phantom scan.

Measurement of acquired image volume dimensions

For each of the San Diego and UCLA phantom scans, 11 measurements were made (see below) to determine the nature of the geometric distortions.



11 Phantom Measurements (A-K)

- All measurements were made in mm
- A,C,E measurements were made across 10 consecutive holes
- G-K measurements were made across 14 consecutive holes
- H,I measurements were made closer to the phantom cylinder axis than J,K

Results:

1. The imaged interior of the phantom is not geometrically distorted
2. Geometric distortions shrunk the circular ends of the imaged phantom by about 8-10 mm in diameter (about 5%)
3. Geometric distortions elongated one side of the imaged phantom by about 5-6 mm (about 3%)
4. Over an 8 month period, the geometric distortions produced by the UCLA scanner did not appreciably change

Conclusion: Linear measurements of brain structures near the skull (approx 100 mm from the center of the head) may be incorrect by as much as 5% in UCLA image volumes. Future work will focus on manually creating a "gold standard" image volume whose dimensions match the physical phantom, translating and scaling each acquired image volume with respect to the gold standard, non-linearly warping the translated and scaled image volumes to the gold standard, and using the warping parameters to correct for distortions in human subject scans.

Specific Aim 2: Further work has been conducted which will allow us to semi-automatically define cortical sulcal landmarks on surface renderings of the brain. These methods will considerably improve reproducibility of the results and dramatically reduce time spent analyzing individual image data sets. David Shattuck (a co-investigator on the project) has also been developing similar methods for our continued work on cerebellar structures. Further, Dr. Shattuck has worked on automated methods for separating cerebral from non-cerebral structures within the imaged volumes and left from right hemispheres.

Specific Aim 3: Elizabeth O'Hare (Dr. Sowell's Ph.D. graduate student working on the project) has continued her work on mapping cerebellar structures in archived data (collected by Drs Riley and Mattson). She has found correlations between cerebellar vermal dysmorphology and cognitive measures, and has recently submitted a manuscript describing these results.

VI. Discussion

Progress has been made towards 3 of the 4 specific aims. We have begun to calculate distortion parameters from the phantom scans, have continued development of image analysis tools which will help improve brain structural measurements, and have submitted a manuscript describing correlative results from imaging data and neurobehavioral test results.

VII. Interrelation with Aims of the Consortium and Other Projects

As described in the initial application, we currently have statistical tools which allow us to map linkages (via correlation, multiple regression, multivariate, non-linear regression) between brain morphology (i.e., gray matter

density, local brain size) at every brain surface point and any measure collected by the various other projects and cores such as the Neurobehavioral Core and the Facial Imaging Core.

VIII. Plans for the Next Year

During the next year, we will begin to apply the methods developed to image data collected at various sites. At UCLA, we are close to obtaining IRB approval for subject recruitment, and San Diego is poised to begin data collection in the near future. Dr. Sandra Jacobson is also in the process of planning brain image data for her projects in Detroit and Cape Town, South Africa. Ms. O'Hare will continue her work on the archived data and as part of her dissertation work will map frontal and cerebellar surface structural abnormalities using the image analysis tools recently developed by Dr. Shattuck.

IX. Publications

O'Hare, ED, Kan, E, Yoshii, J, Mattson, SN, Riley, EP, Thompson, PM, Toga, AW, and Sowell, ER (2005). Mapping Cerebellar Vermal Morphology and Cognitive Correlates in Prenatal Alcohol Exposure. Submitted.

Riley EP, McGee CL, and Sowell ER. (2004) Teratogenic effects of alcohol: a decade of brain imaging. American Journal of Medical Genetics Part C (Semin. Med. Genet.) 127C:35–41.

X. Posters, presentations

O'Hare ED, Kan E, Yoshii J, Mattson SN, Riley EP, Thompson PM, Toga AW, Sowell ER (2004) Localized dysmorphology of the anterior vermal lobule and primary fissure in severe prenatal alcohol exposure (abstract). 11th Annual Meeting of the Organization for Human Brain Mapping, Budapest, 2004 (In Press).

O'Hare, ED, Kan, E, Yoshii, J, Mattson, SN, Riley, EP, Thompson, PM, Toga, AW and Sowell, ER. Cognitive Correlates of Anterior Cerebellar Vermal Dysmorphology in Heavy Prenatal Alcohol Exposure. 34th Annual Meeting, Society for Neuroscience, San Diego, CA.

Progress Report Facial Imaging Core

The Facial Imaging Project has moved forward to process and analyze images being collected from two sites (Buffalo and Finland). U24 AA014809

A. Data Collection

1. Buffalo

A camera has been in Buffalo since March. A total of 92 images have been collected. These include FASD, Williams syndrome patients and unaffected controls. The exact number of each type of patient is still in the local database stored at Buffalo. We are working with the Informatics Core to have dysmorphology data transferred to our core, so we can attempt to identify differences between the 3 groups.

There have been several issues related to the collection of data at this site, including problems with ridges on the images, subject placement and position, and subject movement. Many of the images with ridges can be used for anthropometric style analyses by Elizabeth Moore, however, the ridging will limit the usefulness of the images for the work proposed by Shiaofen Feng and Jeffrey Huang. A total of 50 images were sent to Shiaofen Feng earlier this week.

2. Finland

A camera has been in Finland since June, although the camera was only in place in August. A total of 60 images have been collected, although only 44 have been shipped to Indiana. All subjects scanned thus far are affected. No control images have yet been collected.

There have been issues related to the collection of data at this site. The main issue has been severe ridging. The adaptor for the lights was illegal so they could not use the lighting that we sent. Instead, they decided to use Halogen lights. According to Minolta, it is likely that the Halogen lighting interfered with the laser which caused the severe ridging. The ridging is also caused by too much lighting for the size of the room. The other issue was the use of surgical caps. It was suggested that they use wave caps, but they were not able to find any wave caps. On many of the subjects, the ears are not completely visible because the cap is covering them. However, this will only hamper ear measurements.

The severe ridging will most likely make the images useless to Shiaogen's group. Currently, what is preventing any analysis of data for Elizabeth is the lack of control subjects, many of the images collected have not been sent, and lack of access to the dysmorphology data.

B. Data Processing

Data from both Buffalo and Finland have been successfully transferred and processed by Elizabeth Moore. Images that were usable have been merged into one 3-D image. Measurements have been completed on most merged images, however, there have been problems with the Rapidform software and the plug-in that is used to pick the landmarks and record the measurements. Technicians from the company that makes Rapidform have been and are currently working on some of the Rapidform problems. Most of the problems have to do with registration of the points when trying to merge the 3 views into one image. The final version of the plug-in should be available in a few days. Jeff Rogers has been wonderful and has worked diligently in designing the plug-in. He has had to make several modifications to meet our ongoing needs.

C. Data Analysis

Once sufficient numbers of samples are collected to compare the FASD cases with normal controls, analyses will begin to compare the images and identify variables which have significant discriminatory power. We will also compare the clinical measurements to the indirect anthropometric measurements taken from the 3-D image.

Progress Report Neurobehavioral Core

I. Principal Investigator: Edward Riley, PhD

II. Title of Project: Neurobehavioral Core U24 AA014830

III. Objectives:

Essential to the diagnosis of the full range of effects of FASD is the assumption that specific neurobehavioral patterns or profiles will emerge to assist in this diagnosis, particularly in those cases where no apparent dysmorphology is present. Of course there may be multiple patterns or profiles because different features might occur due to differences in time of exposure, patterns of exposure or because of interactions with other factors, such as IQ. Several research groups, including many involved in this consortium, have been trying to help define these behavioral profiles, although at this time, there are no definitive tests for FASD.

The goals of this Neurobehavioral Core are to assist individual sites in the appropriate neurobehavioral assessment of FASD and to provide for common behavioral assessment instruments to be used across sites so that converging data can be identified. With the realization that the neurobehavioral profile of FASD might change as a function of age, the tests are designed with specific ages in mind. This Core is the result of many of the psychologists working within the Consortium and listed as Working Group Members.

The Specific Aims of the Neurobehavioral Core are:

- To provide a battery of scientific and psychometrically appropriate neurodevelopmental tests that can be used cross-culturally in the assessment of outcomes specifically affected by prenatal alcohol exposure.
- To establish a testing resource that will manage the test equipment and facilitate training, as appropriate, and monitor reliability of administration of tests, as necessary.
- To establish a collaborative database of testing outcomes to allow the eventual development of a valid neurodevelopmental profile or profiles of FAS and other FASD. Such a profile will allow the more sensitive diagnosis of affected individuals from birth through adulthood and will provide the basis for the development of effective strategies for the treatment of primary disabilities and the prevention of the secondary disabilities that are commonly associated with FASD.

IV. Methods

The Neurobehavioral Core utilized several meetings and conference calls to determine a test batter to be utilized at all sites. It then purchased these tests and made them available to all of the test sites. It has paid for translations, placed them on the website, and is providing training in test administration.

V. Accomplishments and Results

- Convened meetings to develop the neurobehavioral test battery.
- Purchased all tests and forms and distributed them to the test sites.
- Developed a test manual and placed it on the web.
- Placed all of the tests on the web.
- Provided translation services for the test battery
- Developed database to be implemented by informatics core

VI. Discussion

The Neurobehavioral core has helped to facilitate the goals of the consortium and the individual projects collecting relevant behavioral data**.**

VII. Interrelation with Aims of the Consortium and Other Projects

By its very nature, the Neurobehavioral core interrelates with each of the projects where behavioral data are being collected. It has provided materials for investigators in Finland, Moscow, South Africa, New Mexico, and San Diego.

VIII. Plans for the Next Year

The Neurobehavioral Core will continue to provide test materials to each of the sites.

IX. Publications

Not applicable

X. Posters, presentations

Not applicable

Progress Report from San Diego and Moscow

I. Principal Investigator: Sarah N. Mattson, PhD

II. Title of Project: FASD in San Diego and Moscow U01 AA014834

III. Objectives:

The aims of this project are to conduct neuropsychological examinations of children with FASD in three countries (US, Russia, Finland) and neuroimaging studies in two (US and Finland).

IV. Methods

The neuropsychological assessment tools specified by the neurobehavioral core are being used at all three sites. The neuroimaging protocol is specified by the neuroimaging core. Controls for the three sites are derived from the appropriate population at each site. Children in Moscow are primarily ascertained from boarding schools and orphanages that house children with subnormal intellectual functioning. We have previously determined the rates of FAS in this population are very high. Children in San Diego are ascertained from ongoing studies of FASD at the SDSU Center for Behavioral Teratology. Thus, we have the unique opportunity to examine the relationship between FASD, IQ, and living environment in large groups of children. The methodology for the Finnish site is being submitted separately.

V. Accomplishments and Results

San Diego: We have successfully trained personnel to administer all the neuropsychological tests and received IRB approval for the project. We have initiated data collection and have tested 23 children thus far. The details are as follows:

	N	Age	Sex	Ethnicity	Race
FASD	12	12.9 +/- 2.40	6 F/6 M	3 Hispanic	7 White
CON	11	12.7 +/- 2.58	5 F/6 M	3 Hispanic	6 White

These children have been tested using the battery suggested by the neurobehavioral core as well as our site-specific measures of interhemispheric transfer. We have not analyzed any of the neuropsychological data, but results will be forthcoming.

We have also received IRB approval for the MRI portion of the project and have recently initiated data collection using MRI. Thus far, 6 children (2 CON & 4 FASD) have been scanned using the protocol developed in conjunction with Dr. Sowell of the imaging core. In October, Dr. Sowell brought the phantom to be scanned and we were able to obtain phantom images at the San Diego scanner. Data from the scans has not been analyzed yet.

Moscow: We have received IRB approval for this component of the project. We had some difficulty negotiating the contract with our Russian collaborators. However, the contract is now in place. In addition, the Russian pediatricians have seen over 1000 children and Drs. Jones and Robinson have seen nearly 400 of these children. In fact, they just returned from Moscow, where they saw approximately 60 children. Of this population, we have complete neuropsychological testing on 336 children. FAS has been diagnosed in 154 children and 67 are controls. The remaining children either need to be seen by Drs. Jones and Robinson or are pending background information. We have partial neuropsychological data on an additional 86 children. We have completed training administrators on the new neuropsychological test battery and will begin data collection with this battery shortly. In one study that we are preparing for publication, we examined the effect of sex and prenatal alcohol exposure on general intellectual functioning in the Russian sample. Children (8-16 years) with FAS (n = 52) were compared to age and sex-matched control children (CON, n = 48). By design, all of these children had below average IQ scores. We tested differences between boys and girls on FSIQ, VIQ and PIQ scores using analysis of variance (ANOVA) with sex and group (FAS vs. CON) as between-subjects variables. As expected, results revealed no main effect of group on any IQ scales. The main effect of sex (p's $< .01$) revealed lower levels of performance across all IQ scales for girls compared to boys. For FSIQ and PIQ, the sex main effects were qualified by significant sex X group interactions (p's $< .05$), indicating boys with FAS may be less susceptible to adverse effects of prenatal alcohol exposure than

girls with FAS, at least in these Russian institutionalized children. Of course, alternate hypotheses are possible, including possible differences in identification of boys and girls with FAS; perhaps boys with FAS are more likely than girls to be institutionalized due to behavior difficulties, whereas girls may be more likely to be institutionalized due to low IQ scores. Importantly, although both groups in this study were characterized by lower than average IQ scores, only the FAS group showed a specific pattern of functioning with respect to sex.

Finland: The accomplishments for the Finnish subcontract are submitted separately in the next section.

VI. Discussion

Although it is premature to draw any conclusions based on the data collected thus far, we are making good progress at all sites. There is great potential for obtaining a large amount of data at the Moscow site next year. The San Diego site has been collecting data, and we hope to continue at our current pace over the next year.

VII. Interrelation with Aims of the Consortium and Other Projects

This project relates to the overall aim of the consortium project, and more specifically the neurobehavioral core, in that our primary goal is to assess children with FASD and controls and to determine whether a profile of neurobehavioral dysfunction exists in this population. Our project has much strength toward this end. In the Moscow project, we are including a control group that is matched on general level of functioning as well as socioeconomic status and living environment. In the San Diego component, we are also collecting data that are pertinent to the aims of the imaging core.

VIII. Plans for the Next Year

During the next year, we plan to continue our data collection at all sites.

IX. Publications

Pinter, M.N., Lee, K.T., Marintcheva, G., Riley, E.P., and Mattson, S.N. Sex differences in intellectual functioning in Russian children with fetal alcohol syndrome. Paper in preparation, submission expected 1/2005.

X. Posters, presentations

Mattson, S.N., Marintcheva, G., Coles, C.D., and Riley, E.P (2004). Comparison of FAS in Moscow Russia and San Diego, California. Presented at the Twelfth Congress of the International Society for Biomedical Research on Alcoholism, Heidelberg, Germany, September 2004. Alcoholism: Clinical and Experimental Research, 68.

McGee, C.L., Fryer, S.L., Riley, E.P., Coles, C.D., Kalberg, W., Kodituwakku, P.W., May, P.A., and Mattson, S.N. (2004). Adaptive functioning as a function of ascertainment method in children prenatal exposed to alcohol. Presented at the Twelfth Congress of the International Society for Biomedical Research on Alcoholism, Heidelberg, Germany, September 2004. Alcoholism: Clinical and Experimental Research, 68.

Progress Report on Finland

I. Principal Investigator: Ilona Autti-Rämö, Ph.D.

II. Title of Project: Neurobehavioral outcome in adolescents with FASD U01 AA014834S

III. Objectives:

This Finnish research project forms part of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD). Participants are adolescents and young adults that have earlier been assigned diagnoses of Fetal Alcohol Spectrum Disorders (FASD). The diagnoses have been affirmed by an experienced dysmorphologist, Eugene Hoyme, assigned by the CIFASD.

The general aim is to study the status in adolescence of these persons on many levels: educational and societal, cognitive and behavioral, as well as neurobiological. The first specific aim is to evaluate long-term outcome for FASD in Finland. Using questionnaires and in-depth interviews, education and professional activities as well as social functioning and mental well-being of the study group is reviewed. Of particular interest is the extent to which secondary disabilities are influenced by social background and environmental factors. The second specific aim is to compare the neurocognitive profile of the FASD subjects with that of an IQ-matched contrast group. Assessment methods include tests of attention and executive functioning, visual perception, motor functioning as well as memory and learning. The third specific aim is to obtain further information concerning the neurobiological pathology and structural abnormalities underlying impairments specific to FASD. Magnetic resonance spectroscopy (MRS) is carried out on a subgroup in order to study whether possible brain anatomic and metabolic deviance may underlie characteristic impairments of FASD. Results are compared with neuropsychological findings. Furthermore, Magnetic Resonance Imaging (MRI) as well as Magnetic Encephalography (MEG) will be carried out for a smaller subgroup and a control group in order to obtain an understanding of the regional vulnerability of brain development during pregnancy and of the cognitive information processing characteristics of the FASD group.

IV. Methods

Subjects

Adolescents and young adults diagnosed as FASD (n=60-80) between 8 and 20 years of age are included in this study. The adolescents are recruited from a clinical patient pool in the Hospital for Children and Adolescents, University of Helsinki. Most of the participants have been diagnosed during infancy or before school age. The prenatal alcohol exposure has been verified retrospectively by the mother. To ensure that diagnostic procedures are similar across different projects involved in CIFASD, dysmorphologist Eugene Hoyme from the Dysmorphology Core has examined all children. Medical case records, recent photos as well as photos of the subjects at a younger age (when available) are examined.

In addition, a *normal control group* (n=30) and a *contrast group* (n=30) consisting of same-aged subjects are assessed. The contrast group is to have a comparable cognitive level as the FASD group, according to psychometric test findings performed at a younger age. The contrast group is further specified after scrutinizing the medical records of the FASD group. If the FASD subjects in the sample have a relatively good cognitive level, the contrast group will consist of subjects with ADHD/ADD. If their cognitive level is generally poor, the contrast persons of choice will be those with general learning disorder (LD). The control group and the contrast group are matched with the FASD groups with respect to age, gender and, if possible, SES. In addition, the contrast group will also be matched on IQ.

The control group is recruited by stratified random sampling from a defined population stratum. The distribution of the FASD group on geographical region, gender and age and, if possible, social background (SES) is determined. The appropriate number of control subjects will be sought from the appropriate geographical region. The contrast group is recruited from the files of patients at the Helsinki University Hospital, following the study of medical records of the persons with FASD. First, gender, age and social background (SES), as well as previous IQ data of each FASD subject is determined. Thereafter, a corresponding contrast group is recruited from the selected diagnostic group as obtained from the case files of the Helsinki University Hospital case files.

The subjects for the MRS, MRI and MEG studies are recruited from the older patients to analyze characteristic features of FASD (n=10). Ten age-matched normally performing young adults are recruited for controls.

Assessments

Assessments include in-depth interviews and questionnaires administered to the subjects, their caregivers, teacher, or other adult who knows the subject well. Methods used are the Life History Interview, Vineland Adaptive Behavior Scales-Revised, Questionnaire for evaluating performance and behavior related to attention and executive functions, as well as the Youth Self Report checklist. The overall aim is to obtain insight into the behavioral, psychosocial and educational outcome of FASD children and adolescents. Neuropsychological assessments are undertaken by administering the neuropsychological core battery decided upon by the CIFASD. This includes tests of attention and executive functioning, visual perception, motor functioning as well as memory and learning. Included are also questionnaires administered to parents and teachers.

The MRS study is performed on 10 participants of the FASD group using a 1.5 Tesla Siemens Sonata MR-Imager with a 3D-spectroscopy program at the University Central Hospital, Helsinki. The 3D program allows acquisition of spectral information from 800ml of brain during a 17 min acquisition time. According to preliminary data, the most frequent abnormal structural finding observed visually in MRI is hypoplasia of the cerebellar vermis. Thus, another VOI will be cerebellum.

MEG data from 9 patients with prenatal alcohol exposure and 9 matched (age, gender) controls will be gathered during performance on a task of motor responses (finger movements) to visual cues. The same patient population that was recorded for the MRS pilot data will be used when possible. The analysis will include extraction of the dynamics of activity in the lateral cerebellum and anterior cingulate, areas observed to be abnormal in the MRS pilot study. We will also conduct a coherence analysis to determine if the patients also develop cortico-cerebellar coherence networks during performance on this task. This analysis probes for effective communication and coordination of these two brain areas.

The MRI examinations are performed on 9+9 subjects, the same as in the MEG study. They are purchased from a private neuroradiologic clinic - Teslamed. Anatomical MR images are obtained using a Siemens Vision Symphony 1.5 T scanner. The structural scan consists of a 3-dimensional magnetization prepared rapid gradient echo (3-D MPRAGE) T1-weighted MRI scan (TR, 11.08 ms; TE, 4.3 ms; flip angle 88). The slice thickness being 1.2 to 1.5 mm. Imaging parameters can be altered as a result of image inspection by the Imaging Core investigators.

V. Accomplishments and Results

During the initial phase of this project we have gathered a group of 77 FASD patients who are willing to participate. A brief telephone interview was first conducted with all families. During the summer of 2004, dysmorphologist Eugene Hoyme spent three months in Finland examining all FASD children and adolescents in the group to ascertain diagnoses. After application of the Revised IOM Diagnostic Criteria, 56% of the subjects were diagnosed as having FAS, 27% PFAS, 10% ARND and 7% other diagnoses. Of note, although a family history of mental retardation or birth defects was rare, 47% of the children had one or more sibling who also carried a diagnosis of FAS. 81% of the mothers smoked cigarettes during gestation; other teratogenic exposures were rare (experienced only by 2.5% of the sample). Almost none had undergone genetics evaluation in the past. Almost all (94%) of the subjects had resided in multiple foster placements since early childhood and had been followed regularly by pediatric specialists. Although only 6% were born prematurely, 70% demonstrated prenatal growth deficiency and 45% were microcephalic. Other than growth deficits and the cardinal facial features, the most common major and minor anomalies noted were: camptodactyly (55%), “hockey stick” or other altered palmar creases (51%), refractive errors (40%), strabismus (38%), dental crowding (43%), nail hypoplasia (38%), GU anomalies (22%) and congenital heart defects (18%). “Railroad track” ears were not observed in this population.

A thorough interview was conducted with an adult who knew the participant well. These results are still to be analysed. Methods included the Life History Interview, Vineland Adaptive Behavior Scales-Revised, Questionnaire for evaluating performance and behavior related to attention and executive functions as well as the Youth Self Report checklist.

In addition, all subjects underwent an abbreviated IQ assessment, using six subtests from the age appropriate Wechsler scales, the results of which will be used to match the contrast group and the FASD group with respect to IQ. The same tests will be administered to the participants of the contrast group.

MRS examination has been undertaken on 10+10 subjects. The results of the first 4+4 have been analyzed and the analysis of a further 6+6 participants is being performed. We found evidence of a significant between groups effect ($p = .015$) in the NAA/Cho ratio in the frontal cortex with lower ratios for the FASD group compared to controls. In

the cerebellar vermis the NAA/Cr ratio differed significantly (p=.044) from controls with higher values for the FASD group. No significant differences were found for any other VOIs, apart from a marginally significant (p=.083) caudate nucleus Cho/Cr ratio between groups. To conclude, the preliminary findings from this study show metabolic differences both in the frontal cortex and the cerebellar vermis for a group of FASD young adults compared to controls. Frontal cortex NAA/Cho loss indicates neuronal dysfunction and/or altered glial cell metabolism in the FASD group. Further, the vermis NAA/Cr increase may represent a compensational effort in mitochondrial metabolism.

VI. Discussion

VII. Interrelation with Aims of the Consortium and Other Projects

Prof Eugene Hoyme, a consortium dysmorphologist, examined all Finnish FASD children participating in the study to ascertain diagnoses and to ensure that diagnostic procedures are the same across sites. During 2005 he will examine control and contrast group participants.

The Finnish project is using the Neuropsychological battery common to other sites in the consortium. Our data will be analyzed together with data from other sites.

VIII. Plans for the Next Year

During 2005, we will examine 60 FASD children and 60 control/contrast group children with the consortium neuropsychological battery.

We will finish and publish the articles under preparation (see below). An article on the dysmorphic features in Finnish FASD children and adolescents is being prepared together with Eugene Hoyme. Another article on the correlation between dysmorphic features and IQ is also to be written. Further, we will analyze interview data and write an article regarding social functioning, mental well-being and secondary deficits in the FASD group.

The MEG and MRI studies will be performed on 9+9 subjects.

Eugene Hoyme (possibly in collaboration with another dysmorphologist) will examine all participants in the control and contrast groups.

IX. Publications

Brain Metabolic Deviance in Young Adults with Fetal Alcohol Spectrum Disorders (in preparation)

Fetal Alcohol Spectrum Disorders In Finland: Clinical Delineation of 77 Older Children and Adolescents (in preparation)

Dysmorphic features in Finnish children and adolescents with FASD (in preparation)

The correlation between IQ and dysmorphic features in a Finnish population (in preparation)

X. Posters, presentations

Magnetic Resonance Spectroscopic Imaging on FASD Young Adults – A Pilot Study. Fagerlund, Å., Heikkinen, S., Lundbom, N.,Timonen, M., Autti-Rämö, I., Korkman, M. & Riley, E.P. Oral presentation on the FASSG meeting at RSA in Vancouver, June 2004. Poster presentation at the ISBRA meeting in Mannheim, October 2004.

Brain imaging in FASD. Autti-Rämö, I. Oral presentation on workshop on fetal alcohol syndrome at the ISBRA meeting in Mannheim, October 2004.

Progress Report – South African Follow-up

I. Principal Investigator—Sandra W. Jacobson, PhD

II. Title of Project—Identification of FASD in South African Children U01 AA014790

III. Objectives

The principal goals of this CIFASD component are to improve diagnosis of FASD by advancing our understanding of core deficits and their neural substrates and of moderator variables that influence fetal vulnerability. In this project, we are evaluating our prospectively-recruited, longitudinal cohort in Cape Town, South Africa at 5 years of age. Recent studies have documented an extremely high rate of FAS in the Cape Coloured (mixed ancestry) community in Cape Town, which we confirmed during the infant phase of this study. Data from that phase and data from our 14-year longitudinal study of moderate-to-heavy prenatal alcohol exposure in Detroit suggest that arithmetic and executive function are two developmental endpoints that are particularly vulnerable to fetal alcohol exposure. The specific aims of this project are:

1. To administer new tests of arithmetic and executive function based on the contemporary models derived from event-related potential (ERP) and neuroimaging studies, in order to determine which elements of these neurobehavioral domains appear to be core deficits of FASD.
2. To administer an eyeblink conditioning paradigm, currently being used in a CIFASD project with laboratory animals, to examine neural processes that may mediate the effects of prenatal alcohol exposure on cerebellar and hippocampal function. Eyeblink conditioning is a culturally neutral, nonverbal learning procedure in which a conditioned stimulus, typically a pure tone, is temporally paired with an unconditioned stimulus, a brief air puff to the eye that elicits a reflexive blink. Because the neural circuits involved in this learning paradigm have been documented in considerable detail, it provides an excellent opportunity to advance our understanding of fetal alcohol effects on brain development through collaborative studies conducted in parallel in children and laboratory animals.
3. To test the hypothesis that two moderator variables that have been shown to increase fetal vulnerability to alcohol exposure—maternal age and the absence of the *ADH1B*2* allele —can improve the identification of FASD in prenatally-exposed children.
4. To evaluate the usefulness of two tasks administered during the infant phase of this research—infant numerosity and A-not-B—for early diagnosis of FASD by assessing their predictive validity in relation to the specific elements of arithmetic and executive function found to be associated with prenatal alcohol exposure during early childhood.
5. To determine the degree to which the dysmorphology examination, following the protocol developed by the Dysmorphology Core, and photographs taken using a new 3-dimensional camera, following the protocol developed by the Facial Imaging Core, may make it possible to detect differences among FAS, ARND, and controls and improve the validity of FASD diagnosis by detecting subtle craniofacial anomalies in children with ARND.

IV. Methods

Approximately 150 children, whose mothers were recruited during pregnancy, are being evaluated at 5 years of age. Half of the children were heavily exposed to alcohol *in utero* (at least 14 standard drinks per week or 5 drinks/occasion at least twice per month); half were born to mothers who abstained or drank at very low levels. A very large proportion (57.3%) of the heavy drinking mothers in this cohort met DSM-IV criteria for alcohol dependence or abuse, and almost one-quarter of their infants (24.4%) met criteria for full FAS. The child and his/her mother are transported to our laboratory at the University of Cape Town for 4-hour assessments on 2 consecutive days. These assessments include innovative tests that focus on development of number processing and executive function during the preschool period developed by Elizabeth Spelke at Harvard University and Philip Zelazo at University of Toronto, respectively; eyeblink conditioning, which is being studied in collaboration with Mark Stanton, University of Delaware; and several neurobehavioral tests being administered by other CIFASD projects, including the Leiter International Performance Scale-Revised, the Beery Test of Visual-Motor Integration, the Grooved Pegboard Test, and the Edinburgh Handedness Inventory. Alcohol-related dysmorphology is assessed according to the CIFASD protocol by Nathaniel Khole, a pediatrician trained by Kenneth Lyons Jones, and we are

planning to take 3-dimensional digitized photos following the protocol developed by the CIFASD Facial Imaging Core. A blood sample obtained from each child is analyzed for the presence of the *ADH1B*2* allele, lead concentration, and several indicators of iron deficiency anemia. A broad range of control variables are examined, including maternal education and IQ, smoking and illicit drug use during pregnancy, quality of intellectual stimulation provided by the parents, maternal depression and psychopathology, and current maternal drinking, smoking, and drug use, which will permit statistical control for potential confounding influences in all evaluations of prenatal alcohol effects.

V. Accomplishments and Results

Implementation of the project began in January, 2004 (the 4th month of the first grant year), as planned. During the first 3 months, our Cape Town-based research staff succeeded in locating all but one of the families from the first half of the cohort, and all those who were contacted agreed to participate in the follow-up assessment. During this first year of the project, we have worked intensively with Mark Stanton of the University of Delaware to implement the eyeblink conditioning paradigm and analyze the data it has generated. We also consulted with Elizabeth Spelke at Harvard and Philip Zelazo at University of Toronto to implement their innovative preschool assessment tasks and worked intensively with Andrea Hay, our clinical psychologist in Cape Town, to develop procedures for administering this challenging test battery to our cohort of culturally very disadvantaged children. The child and maternal assessments began in May and to date (December, 2004), 26 children have been evaluated.

We have just completed a preliminary analysis of the eyeblink conditioning data for the first 24 children in the cohort, and they are impressively consistent with the findings in the animal literature. One important challenge was helping the children feel sufficiently at ease to participate in the task, which involves wearing a special helmet with a nozzle that intermittently delivers a puff of air to the child's eye causing him/her to blink. In this phase of the study, we are administering the "short delay" conditioning procedure, which entails a 650-ms delay between the onset of the tone and the air puff. The relation between fetal alcohol exposure and short delay conditioning was highly significant, $\chi^2 = 7.83$, $\underline{p} < 0.01$. All six of the children born to women who abstained during pregnancy or drank at very low levels learned to associate the tone with the air puff; whereas none of the children with FAS were conditioned in this paradigm. This finding is consistent with evidence from the animal studies indicating a conditioning deficit only at very high levels of prenatal alcohol exposure.

VI. Discussion

The eyeblink conditioning data being generated by this project provide a unique opportunity to work collaboratively with Charles Goodlett of Indiana University, a CIFASD colleague who is using this paradigm with laboratory rats. This collaboration to study the adverse effects of prenatal alcohol exposure on classical conditioning and cerebellar function simultaneously in an animal model and a human cohort has considerable potential to improve understanding of the neural substrates of this important aspect of FASD. As detailed below, we have recently received seed money funding to implement functional magnetic resonance imaging (fMRI) at the University of Cape Town for the first time, which will make it possible for us to perform assessments when the children in this cohort reach 8 years of age that will be comparable to those being collected at other CIFASD sites. In a second prospective Cape Town cohort, which we will begin to recruit this coming February 2005, we will have the opportunity to administer the CIFASD prenatal ultrasound protocol developed by Andrew Hull of the University of California, San Diego, that is currently being implemented in the Ukraine. If we can find the needed supplemental funding, this assessment will provide an opportunity for cross-cultural corroboration of any abnormalities that are detected in the Ukraine.

VII. Interrelation with Aims of the Consortium and Other Projects

Because our project is the only one assessing preschool-age children, only a subset of the battery developed by the Neurobehavioral Core for school-age children can be administered. The Leiter Scale will provide a measure of overall intellectual function, analogous to an IQ score, which will permit comparison of the severity of the effects of prenatal alcohol exposure in this and other CIFASD populations. The innovative neuropsychological assessments being implemented in this project are designed to provide new insights into the effects on two of the domains of cognitive function—number processing and executive function—that are most commonly affected by prenatal alcohol exposure. We anticipate that data to be generated by these assessments will provide an important contribution to the development of the diagnostic schema for FASD envisioned in the CIFASD mission statement, both by providing new information about the specific aspects of arithmetic and executive function that are most vulnerable to fetal alcohol exposure and by providing tests that can be used for diagnostic assessment with

preschool-age children. In addition, this cohort will provide the sole opportunity to examine the degree to which algorithms that may be developed to identify FASD-related facial dysmorphology in older children may apply during the preschool-age period as well. One unique feature of this cohort is that it provides data on quantity and pattern of drinking during pregnancy obtained prospectively during pregnancy that can be related directly to long-term developmental outcomes.

VIII. Plans for the Next Year

Data collection, scoring, and data entry will be the principal focus of this study during the next year. We expect to assess approximately 75 additional children and mothers during that period. We also anticipate the arrival of the 3-dimensional camera in Cape Town, which will permit us to assess the children in our cohort using this new technology. Last month, we learned that Sandra Jacobson will be awarded a Fogarty International Research Collaboration Award from NIH to implement fMRI at the University of Cape Town, and we have also received a seed money grant from Wayne State University to supplement this award. The latter funds were used to bring a physicist from University of Cape Town to the U.S. for training this fall, and we will be initiating a pilot study with a cross-sectional sample of 10-12 year olds to be recruited this coming spring. The pilot data from this study will provide the basis to apply for NIH funding to assess our longitudinal CIFASD cohort at 8 years of age following protocols that will provide data that can be compared and potentially pooled with neuroimaging data from other CIFASD sites.

IX. Publications

Because data collection in this prospective, longitudinal study has been ongoing for only 6 months, no publications have been generated to date.

X. Posters, presentations

The first poster reporting data generated by this project will be submitted next month for presentation at the Research Society on Alcoholism this coming summer.

Progress on International Neuropsychological Study of FASD

I. Principal Investigator: Philip A. May, Ph.D.

Co-Principal Investigators: C. Adnams, M.D., P. Kodituwakku, Ph.D., W. Kalberg

II. Title of Project. International Neuropsychological Study of FASD U01 AA014786

III. Objectives

The long term goal of this research is to delineate cognitive and emotional profiles of children exposed to substantial amounts of alcohol prenatally and with valid and substantial symptoms of FASD. The specific aims are:

1. To administer a neurobehavioral core test battery to children with confirmed prenatal alcohol exposure from a community in South Africa and on a number of American Indian reservations in the US. The data gathered through this test battery will eventually be combined with those collected at other international research sites.
2. To test a specific statistical model of neurocognitive functioning (e.g. radex model) in children with prenatal alcohol exposure. Radex or hierarchical models of cognitive abilities posit that complex tasks that are at the top of the hierarchy load on what is known as general or 'g' factor. We hypothesize that those complex tests at the top of the hierarchy highly discriminate between children with substantial prenatal alcohol exposure and normal controls.

IV. Methods

We propose to attain these specific research goals using a case-control design, in which the performance of alcohol-exposed children will be compared with that of controls on a carefully selected test battery. The ability to draw valid conclusions within this research design hinges on the success of accounting for a multitude of variables that directly and interactively influence social-cognitive development in children with prenatal alcohol exposure. Therefore, in the current research project we plan to compare alcohol-exposed children to a group of non-exposed children matched for a range of factors including socio-economic status, ethnicity, sex, age, and maternal drinking level and pattern (e.g., quantity, frequency and timing, binge vs. chronic, and peak BAC estimates). Information on family history of alcoholism, especially on paternal drinking history, will also be ascertained to determine the comparability of the groups with respect to genetic influences.

The work scope of the New Mexico award, with a subcontract to The University of Cape Town, under the NIAAA-supported Consortium of International Collaborative Research (CIFASD), includes the neuropsychological testing of 300 children using: 1) the core test battery that is being designed by a collaborative group of neuro-behaviorists under the guidance of the CIFASD Behavioral Core and 2) instruments that are designed to test a specific statistical model of neurocognitive functioning (radex model) with the aim to further define the cognitive dysfunction in alcohol-affected children.

Because the budget period was reduced from five years to three, and due to the fact that educational interventions were added to this grant, the number of human subjects has changed. In the neuropsychological study the numbers are: 100 children with FAS and 100 controls for the South African study and 50 American Indian children with FAS and 50 American Indian controls in the Northern Plains.

V. Accomplishments and Results

Because of the collaborative nature of this project, parallel activities have ensued over the past year in preparation for the core testing to begin. First, the Behavioral Core group of the CIFASD has held a series of meetings to determine the battery of tests that will be administered to the children targeted by this work scope. As the battery and test administration manual was in development most of the first year of funding, project staff at UNM and at UCT have worked throughout the year to initiate and finalize the following activities in preparation for beginning the test administration and data collection:

1) IRB approval was acquired from both the IRB of the Health Sciences Center of UNM and the Ethics Committee of UCT.
2) Subcontracts were finalized and administered across both universities.

3) Philip May, Ph.D. has requested an audience with tribal councils in American Indian sites to present the project to those communities and gain approval for the project to begin. These meetings have been completed in two of the project sites and only tentative approval has been granted.

4) Piyadasa Kodituwakku, Ph.D., has had continued contact with, and has attended formal meetings of the Behavior Core of the CIFASD to develop and finalize the battery to be used in this project.

5) Philip May, Ph.D., Piyadasa Kodituwakku, Ph.D., and Wendy Kalberg, M.A., CED, made a first site visit to South Africa in March, 2004 to participate in strategic planning, plan for the implementation of the study, and to define job descriptions and the training activities of local staff.

6) Colleen Adnams, M.D., in collaboration with UNM staff, has hired the following well-qualified project staff:

    a. Bernice Castle, MA. in Clinical Psychology and Registered Psychometrist - Ms. Castle has been hired to coordinate the activities of both the Neuropsychological study as well as the Multi-Method Intervention study.

    b. Sean September, M.A., Registered Psychometrist - Mr. September has been hired to conduct the bulk of the testing with the children enrolled in the study.

7) The UNM team completed a second site visit in September, 2004 accomplishing the primary goal of training the staff in the neuropsychological battery and assuring that the battery specific to the testing of the radex model is consistent across both sites, US and South Africa.

8) Test battery video-tapes will be completed soon to ensure reliability and formal testing will begin soon.

VI. Discussion

Progress is right on track for the South African portion of the study, as the staff, protocols and training are all in place. Furthermore, the children with FAS and Partial FAS are those who were diagnosed in the NIAAA-funded South African FAS epidemiology study, so the gold standard cases for testing are all identified and access is in place.

Progress is a little slow at the Plains reservation sites, as each tribal council has to be approached individually for permission to perform the neuropsychological tests on the children previously diagnosed though the NIAAA-funded study of prevention and epidemiology among these same communities. Thus far, meetings with two of the tribal councils have indicated an ambivalence about participation. But we believe that formal approvals will be forthcoming once some demonstrations of the actual tests can be presented to the council members and some images from the 3-D camera can be shown as well.

VII. Interrelation with Aims of the Consortium and Other Projects

This project is collecting the core neurobehavioral data outlined by the CIFASD behavioral core personnel. All testing will be done according to the stated manual administration procedures. These data will then be combined with the data of the other sites to compare the abilities of alcohol-exposed children across multiple sites and cultures.

VIII. Plans for Next Year

The South Africa team and the New Mexico team plan to submit a videotape of the administration of the test battery to San Diego State University for their review. These videotapes will be submitted soon, although the administration of the tests in Afrikaans presents a challenge to those English speakers who will review them. Once the approval of the videotape is given, both sites will begin to administer the protocol to the affected and control children.

IX. Publications

None

X. Posters, presentations

None

Progress Report for Intervention Study in South Africa

I. Principal Investigator: Philip A. May, Ph.D.

Co-Principal Investigators: C. Adnams, M.D., P. Kodituwakku, Ph.D., W. Kalberg,

II. Title: Intervention Study in South Africa U01 AA014786S

III. Objectives

The Multi-Method Intervention Study is aimed at determining the degree to which three specific intervention methods are successful in remediating the effects of prenatal alcohol exposure in affected children. The following three intervention methods are being tested: 1) cognitive control therapy 2) family intervention, and 3) specific linguistic and literacy training. Another aim of the study is to determine the degree to which combinations of above methods improve academic skills and behavior in alcohol-exposed children. Finally, the study aims to assess the effects of three mediating variables (self-efficacy, attention, meta-cognitive skills) and three moderating variables (child's IQ, life stress, maternal education) on therapeutic outcomes.

IV. Methods

This work scope targets 80 Grade 2/3 children at 10 schools from Wellington III Epidemiology study. Of these 80 children, 65 have a confirmed diagnosis of FAS or Partial FAS and 15 were 'deferred' on initial diagnosis and all had confirmed prenatal exposure to alcohol. The children have been randomly assigned to one of 4 groups (three treatment groups, one control group). The analysis will focus on clinical, statistically significant improvements.

The University of Cape Town is collaborating with The University of Stellenbosch and professionals from the target community to implement the aforementioned interventions. The South African intervention team is comprised of the following individuals:

Colleen Adnams, M.D., Co-Investigator (UCT)
Pharyn Sorour, SLP (UCT)
Mariechen Perold, M.Ed. Psych. (Univ. of Stellenbosch)
Rubin Adams, B.Ed., (Paar School District, Western Cape Schools)
Petra Engelbrecht, Ph.D. (University of Stellenbosch)

V. Accomplishments and Results

In March 2004, the Co-investigators (Dr. May, Dr. Kodituwakku, and Ms. Kalberg) traveled to South Africa to participate in strategic planning for the commencement of the Intervention study. In the start-up year, the South African team has met regularly to design the pretest protocol, design and finalize intervention protocols, and hire staff to drive the daily activities of the project. Bernice Castle, M.A., and Sean September, M.A. were hired to provide expertise to both the Intervention Study as well as the Neuropsychological Study.

The current progress on the intervention protocol has included recruitment of 80 children for the study. One hundred percent (100%) consent has been obtained for the 80 children identified to participate in the study. Baseline testing has been completed for all children who will be participating in the study. The baseline testing battery for the intervention children includes: 1) two reading tests, 2) a spelling test, 3) two mathematics tests, 4) two language tests, 5) Cognitive Control Battery, and 6) visual acuity and auditory screening. The baseline testing also includes behavior measures through questionnaires completed by parents and teachers. Finally, process measures of the participant's behavior are being collected through qualitative classroom observations.

Results of the preliminary (baseline) testing data are tabled below:

Treatment components have commenced for the three intervention methods. Linguistic interventions are being conducted two times a week for 45 minutes each session. Cognitive Control Therapy is being delivered one hour per week, and the Parent Groups have begun to meet and will meet a total of 20 times over the course of the intervention time period.

South African Intervention Cohort Pretest Results

Test	Mean age (mths) M	F	Mean age - mths (SD) Males	Mean age – mths (SD) Females
Reading	115	113	90.1 (16.1)	87.7 (14.0)
Spelling	115	113	87.0 (19.4)	88.5 (20.1)
Grammar	115	113	72 (19.1)	63 (24.1)
Maths Add	115	113	77.0 (12.0)	76.8 (11.1)
Maths Sub	115	113	77.4 (13.6)	72.6 (9.8)
Maths all	114		75.9 (11.5)	75.9 (12.1)

Reading passages comprehension	% correct Males - Mean (SD)	% correct Females - Mean (SD)
Level 1	34.5 (35.4)	39.0 (32.0)
Level 2	17.1 (18.7)	19.5 (16.2)
Level 3	5.4 (2.6)	19.5 (16.2)
Level 4	0.0	0.0

VII. Discussion

VIII. Interrelation with Aims of the Consortium and Other Projects

This is the only study in the consortium that provides intervention with affected children in a school setting. As such, it is unique in the consortium and also in the extant literature. However, other investigators in the consortium are examining, through neuropsychological tests and neuroimaging, the developmental strengths and weaknesses of children with various levels of FASD. Their studies will be enhanced, and (vice versa) ours will also be enhanced, by sharing information on development and learning from multiple sources of inquiry and multiple environments. As new insights are forthcoming from the consortium, these insights can be adapted to classroom interventions, and insights from our interventions will help guide basic research on the development of FASD children. Finally, classroom interventions can be implemented at other sites if this efficacy trial is successful.

VII. Plans for Next Year

Intervention methods will continue through next year. The interventions will be administered for a period of 18 months.

VIII. Publications

None

IX. Presentations

Colleen Adnams, M.D. presented the methodology and progress report at RSA in Vancouver in June. She also presented the rationale, methodology and preliminary (baseline) data at the ISBRA conference in Germany in late September, 2005. A summary of the later presentation is to be published in Alcoholism: Clinical and Experimental Research.

Progress Report from Moscow Neonatal Project

I. Principal Investigator: Tina Chambers, PhD

II. Title of Project: Risk Factors for FASD in the Moscow Region U01 AA014835

III. Objectives:

- To measure the birth prevalence and range of alcohol-related physical features and neurobehavioral impairment among children born to pregnant women in the Moscow Region who report consuming moderate to heavy amounts of alcohol by utilizing methods designed to permit earlier diagnosis of alcohol-related effects.
- To evaluate the contribution of maternal nutritional factors to increased risk for prenatal growth deficiency, neurobehavioral impairment, and alcohol-related physical features in infants prenatally exposed to moderate to heavy amounts of alcohol by conducting a randomized trial of a micronutrient supplementation intervention and measuring micronutrient levels in maternal blood.

IV. Methods

This project involves a cohort study design with women recruited at first prenatal visit in Ladies Consultation Services feeding into one of four delivery hospitals located in lower income areas of the Moscow Region. Women are screened for quantity and frequency of alcohol use at time of conception and currently, and for symptoms of abuse. Women who screen positive are offered enrollment, and the next eligible unexposed woman is offered enrollment as a control. All subjects are interviewed at the time of enrollment and again at 32 weeks gestation regarding alcohol use and potential predictors, modifiers and confounders.

Two of the four hospitals have been selected for the nutritional intervention. At these hospitals, at the time of enrollment, women are provided standard, commercially available prenatal vitamins to be taken daily throughout the remainder of pregnancy. Women in the non-intervention sites are given standard advice regarding prenatal care, but not provided vitamins.

All subjects provide blood samples at the time of enrollment and again at 32 weeks gestation. These samples are used to assess blood levels of nutrients and to perform CDT, MCV and GGT tests as biomarkers of exposure, as well as DNA abstraction and banking for future genotyping. In addition, all women provide a saliva sample at enrollment and at 32 weeks which is used as a marker for current blood alcohol level.

Outcome measures include medical record abstracted information on birth size, birth complications, etc. and a specialized neonatologist's physical exam using the dysmorphology core's standard exam protocol. These examinations are supplemented by 2-D facial photographs.

Subsequent outcome measures include 6 month and 1 year neurobehavioral assessments using the Bayley, and a second standardized physical exam at one year of age.

V. Accomplishments and Results

1. Study protocol finalized with Moscow Region Ministry of Health
2. Contractual negotiations finalized with CRDF DEFINE and first year subcontract monies now being distributed according to budget
3. U.S. OHRP FWA and approval of ethics committee in Moscow
4. Human Subjects approval obtained in U.S. and Moscow
5. Two training sessions held with interviewers, neonatologists, psychologists, and ultrasonographers
6. Two training sessions held with data manager and project coordinator
7. Screener access database developed and available for distribution
8. Expanded alcohol and control database developed with bioinformatics core and available for distribution
9. Laboratory methods finalized and protocol for preparation and transportation of samples
10. Study materials translated, and translated copies of Bayley instruction manual and testing forms obtained
11. Office equipment for coordinating center purchased, four Bayley kits, one DVD video camera, four digital cameras, and one DVD video recorder purchased for the project
12. Screening for study eligibility initiated November, 2004. In the two weeks, at two sites, 80 women screened, and 10% qualified as alcohol exposed

13. Screener database populated with continued screening at all four sites through December, 2004. Results to be analyzed for modifications to selection criteria
14. Project Program Assistant, Ludmila Bakhireva, has selected analysis of predictive value of multiple biomarkers of exposure in this population for her doctoral dissertation and has assisted with expanding this portion of the project
15. Recruitment to begin 1/6/05
16. Second formal training session for Bayley testers scheduled for February, 2005

VI. Discussion

A number of unexpected obstacles have had to be overcome in implementing this project in a manner that meets the scientific goals of the study while working within the cultural and bureaucratic challenges of this particular country. Thus, the need for the Ministry of Health and other collaborators in the Moscow Region to structure this project so that it builds capacity within their infrastructure has required some modifications to the protocol that would otherwise be performed more efficiently by working outside their system. However, we feel strongly that building these kinds of relationships will yield better cooperation and ultimately a better result for this project and any future research in this setting.

A number of other ancillary features of this project have been proposed in the last several months, including ophthalmologic evaluations of infants, additional experimental biomarkers of exposure, and additional neurobehavioral tests. Each of these additional pieces appears to be reasonable and feasible to incorporate into the study protocol. However, we have focused on establishing the core study protocol on firm ground as a top priority, and have elected to wait until this is accomplished before considering additional study components.

The pilot screening experience in the Moscow study sites suggests that our initial estimate that 2.5% of screened subjects would meet the alcohol exposure criteria was quite conservative and, in fact, this estimate has been exceeded by four-fold. If 10% of women qualify on first prenatal visit, as the pilot data suggest, we feel confident that recruitment of the required sample is achievable at the selected study sites.

VII. Interrelation with Aims of the Consortium and Other Projects

This study relates to the consortium goal of informing better intervention and treatment approaches by contributing to the outline of an improved diagnostic schema through prenatal ultrasound, standardized physical examinations that may allow for earlier diagnosis, and standardized neurobehavioral assessments among infants across a range of exposures. This study further relates to the consortium goal of informing better intervention and treatment by conducting a nutritional intervention trial. Finally, this study relates to the consortium goal of identifying the full range of effects of prenatal alcohol exposure by following a large cohort defined by a range of levels of prenatal exposure and assessing infants for a broad spectrum of physical features and neurobehavioral effects. This study relates to other projects in the consortium through the prenatal ultrasound measures that are being implemented in other sites, through the standard physical exam protocol established by the dysmorphology core, through the standard assessment of alcohol and control variables and through the bioinformatics core by using the common definitions of these variables in both prospective and retrospective samples included in the consortium.

VIII. Plans for the Next Year

The plans for the next year are to recruit the first half of the total sample and to conduct preliminary analysis of the prevalence and patterns of alcohol use in this population based on the broadly sampled screening data, to preliminarily assess biomarkers of exposure in the recruited sample as they relate to maternal self report, to preliminarily assess maternal nutritional status in exposed versus unexposed pregnancies, to preliminarily assess prenatal ultrasound markers in exposed versus unexposed pregnancies, and to preliminarily assess the prevalence of structural features in the newborn period. We also plan in the next year to have the neurobehavioral testers fully trained to perform the six-month assessments, and to be competent well in advance to perform the one-year assessments.

IX. Publications

We have not yet published any data from this project.

X. Posters, presentations

We anticipate submission of an abstract to RSA on the initial screening data that has been collected between November and January.

Progress Report from Basic Science: Therapeutic Agents

I. Principal Investigator: Charles R. Goodlett, PhD

II. Title of Project: Testing FASD Therapeutic Agents: Neonatal Rodent Models U01 AA014829

III. Objectives:

The long-term goal of this project is to test therapeutic agents that may have potential to protect against alcohol-induced developmental alterations in brain structure and behavioral function, using rat and mouse models of fetal alcohol spectrum disorders (FASD). One set of studies tests whether L-NAP or other molecules will protect against activation of caspase-3 in the cerebellum following binge alcohol exposure on postnatal day (PD) 4, thereby limiting alcohol-induced apoptotic cell death of Purkinje cells. A second set of studies determines whether candidate therapeutic agents will protect against eyeblink classical conditioning deficits associated with the cerebellar cell loss induced by neonatal binge alcohol exposure. The third set extends the analysis to the neonatal mouse model, to determine whether candidate therapeutic agents will protect against alcohol-induced cerebellar and/or forebrain damage in C57BL/6 mice, sufficient to prevent deficits in an eyeblink conditioning task that is known to include a forebrain circuitry component.

IV. Methods

The studies completed to date have focused on structural and functional indicators of damage to the cerebellum induced by binge-like exposure to alcohol during the early postnatal period (the "3rd trimester equivalent" in rats). Four sets of studies have been initiated and are in different stages of completion. The first set asked whether vitamin E (an antioxidant) could protect against alcohol-induced cerebellar cell death, using western blot and immunohistochemical analyses of activation of the caspase-3 "executioner" protease in the cerebellum on postnatal day 4. In addition, other rats were treated on postnatal days 4-9, with or without vitamin E, and tested as juveniles for deficits in acquisition of conditioned responding using standard ("delay") Pavlovian conditioning procedures, a simple associative learning task known to depend on specific cerebellar-brain stem circuitry. After training was completed, counts of cerebellar Purkinje neurons and neurons of the deep nuclei were performed to assess whether structural protection could be detected.

Studies of L-NAP, a small peptide know to have neuroprotective effects in other models of neuronal death, involved alcohol treatments on postnatal day 4 (with or without L-NAP pretreatment), and assessment either of acute (8-hour) activation of caspase-3 in the cerebellum, or counts of Purkinje neurons (on postnatal day 10) to determine potential protection against alcohol-induced Purkinje cell death.

Additional studies were performed using eyeblink conditioning procedures in juvenile and adult rats to compare the extent of alcohol-induced deficits in the standard delay conditioning task, a variant that is known to require cerebellar plasticity (and not involve forebrain circuits), with deficits in the "trace" conditioning task, a variant that is known to require additional contributions and interactions of forebrain/hippocampal circuits with the cerebellar circuitry essential for all Pavlovian eyeblink conditioning. These studies were developed to permit assessment of potential protection against hippocampal-forebrain circuits, to complement our cerebellar-specific analyses already underway.

Fourth, studies were initiated in which C57BL/6 (B6) mice were treated with a heavy binge dose of alcohol on postnatal day 7, and they will be tested on another variant of eyeblink conditioning (acquisition and reversal of a conditional discrimination), which can potentially identify selective effects of alcohol on the forebrain-hippocampal circuits that interact with the cerebellar circuits. Because Dr. Zhou has shown in this B6 model that alcohol causes significant apoptotic-like effects in neurons in the hippocampal, cingulate and retrosplenial cortex, this behavioral study tests the hypothesis that the alcohol-induced forebrain damage will be associated with deficits in reversal of the conditional discrimination, but that original acquisition of the discrimination (which does not require hippocampal processes) will be unaffected (since cerebellar apopotosis or cell loss is not evident with these postnatal day 7 treatments).

V. Accomplishments and Results

The first set of studies tested whether vitamin E had potential as a neuroprotective treatment, and the research in now in press in *Alcoholism: Clinical and Experimental Research.* Three key alcohol-induced endpoints were evaluated: reductions in alcohol-induced activation of caspase-3 in the cerebellum (on postnatal day 4); deficits in

acquisition of standard delay eyeblink classical conditioning (as juveniles); and loss of neurons in the cerebellum. In all cases, vitamin E pre-treatment failed to produce any protection against the damage or deficits induced by neonatal alcohol (see Tran et al., in press).

A second set of studies evaluated whether pre-treatment with L-NAP afforded protection against alcohol-induced damage to the neonatal rat cerebellum. L-NAP failed to prevent the expression of the active subunit of caspase-3 in the cerebellum on postnatal day 4. Counts of Purkinje cell survival (in postnatal day 10 rats, following treatments on postnatal day 4) are currently underway to determine whether L-NAP might still promote cerebellar cell survival in the face of an alcohol-induced insult.

A third set of studies has been completed that extends the analysis of alcohol-induced deficits in Pavlovian eyeblink conditioning to a hippocampal-dependent form of learning, i.e., trace conditioning. Studies of groups given binge neonatal alcohol exposure on postnatal days 4-9 indicate that regardless of whether training occurs as juveniles or adults, the alcohol-induced deficits on trace conditioning are more severe than deficits for standard delay conditioning procedures, although both forms of conditioning are impaired. This suggests that variants of eyeblink conditioning that require forebrain neural processes (in addition to the essential cerebellar-brain stem circuit that is obligatory) may provide more sensitive measures of learning deficits in fetal alcohol spectrum disorders.

A fourth set of studies has been initiated to provide a long-term behavioral follow-up to the observation by Dr. Zhou that a single-day, binge alcohol exposure in B6 mice (2.5 g/kg, injected twice, two hours apart, on postnatal day 7) produced substantial neuronal apoptosis in hippocampal and limbic forebrain circuits. Mice have now been treated and will be shipped to Dr. Stanton's laboratory in Delaware for testing when they are adults. The mice will be tested on acquisition and reversal of a conditional discrimination, in which we predict (based on the cell death in forebrain regions) that there will be deficits on reversal but not on acquisition of this task.

VI. Discussion

The antioxidant interventions had no significant protective effect against cerebellar damage in the neonatal rat model, an outcome that has since been confirmed by other laboratories. It appears that antioxidant interventions during the brain growth spurt are not likely to ameliorate the damaging effects of heavy binge drinking on the cerebellum (later in pregnancy). However, this does not rule out some potential neuroprotection in other brain regions, most importantly in the hippocampus, and we will pursue that in future studies. Likewise, L-NAP was ineffective in our studies of neonatal alcohol exposure, consistent with the lack of effects found in Dr. Zhou's neonatal mouse studies.

Since the alcohol-induced deficits in eyeblink conditioning were significantly more severe for trace conditioning than delay conditioning, we believe that the trace procedures may be reflecting the combined effects of alcohol-induced damage to the forebrain and the cerebellum-brain stem. This is particularly relevant to potential applications to human studies. It may be possible to link variation in the extent of functional damage in FASD cases to the relative impairment on these two forms of learning. For example, the demands on neural systems underlying these two learning tasks differ, since trace conditioning requires hippocampal-medial temporal circuits (a form of declarative memory) whereas delay conditioning does not (a "non-declarative" task requiring just the cerebellar-brain stem circuitry).

VII. Interrelation with Aims of the Consortium and Other Projects

This project is closely interrelated with that of Dr. Zhou, and daily or weekly discussions and planning occurs to compare the mouse and rat outcomes. Both labs produced data that activity-dependent neuroprotective peptides failed to protect against alcohol-induced brain damage in neonatal rodent models. This suggests that these peptides may not be effective against damage induced by binge drinking later in pregnancy. However, the effectiveness of L-NAP in protecting against embryonic neuroteratogenesis indicates that additional efforts should be pursued (in both rat and mouse models) to confirm and extend this encouraging possibility. The collaborative studies on mouse behavioral endpoints (both neonatal and, to be added, prenatal models) will continue to be based on mutually informative neuroanatomical, pathological, and behavioral outcomes.

Perhaps the most important link between the basic science component and the human components is through translation research focused on eyeblink classical conditioning. Preliminary work is underway in the South African cohort, and exploration and comparison of outcomes and potential uses of the rodent and human data sets are a priority. Since the behavioral and neurobiological data can move most quickly in the animal studies, including

potential interventions or treatments, it is likely that this is one example where the animal model studies can test and inform approaches that can be considered for at-risk human populations at the Consortium sites.

As the L-NAP animal model work moves forward (now to be focused on prenatal rodent models), the key questions are the identification of the target sites of action and mechanisms for the protective effects of these peptides. Once the relevant developmental time periods and brain developmental processes crucial to neuroprotective actions of L-NAP are identified, localizing the site of action and mechanisms of L-NAP can be pursued through interactions with Drs. Miller and Charness.

VIII. Plans for the Next Year

1. Test neuroprotective peptides and antioxidants for their potential to protect against hippocampal damage, even though cerebellar damage is refractory to these compounds.
2. Develop behavioral and neuroanatomical studies in adult mice and rats following prenatal alcohol exposure, to evaluate whether L-NAP can provide long-term protection against alcohol-induced structural and functional brain damage. Included in this will be an effort to develop a collaboration with the Johns Hopkins Center for Neurogenetics for high-throughput behavioral screening.
3. Expand the effort to find effective interventions in the neonatal rodent models, including developing a collaborative effort with Dr. Jennifer Thomas (SDSU) to assess effects of post-treatment dietary choline supplementation on hippocampal and cerebellar-dependent learning processes.

IX. Publications

Tran, T.D., Jackson, H.D., Horn, K.H., and Goodlett, C.R. Vitamin E Does Not Protect Against Neonatal Ethanol-Induced Cerebellar Damage or Deficits in Eyeblink Classical Conditioning in Rats. Alcoholism: Clinical and Experimental Research, in press

X. Posters, presentations

Progress Report for Basic Science: Therapeutic Agents

I. Principal Investigator: Feng C. Zhou, PhD

II. Title of Project: Supplemental Project: Testing FASD Therapeutic Agents in Mouse Models U01 AA014829S

III. Objectives:

The long-term goal of this project is to test therapeutic agents that may protect against fetal alcohol spectrum disorders (FASD). The objectives of these studies are to examine how the Activity Dependent Trophic molecules, NAP or SAL, protect against: (1) the neural tube defect that is cascaded in the midline forebrain; (2) brainstem damage induced in prenatal models of alcohol exposure; and (3) apoptosis in the neonatal model of alcohol binge exposure.

IV. Methods

The NAP or SAL peptides are tested in C57BL/6 (B6) mice with alcohol administered prenatally via liquid-diet alcohol consumption. We have previously shown that brain weight, brain size, and the forebrain area are reduced with this prenatal alcohol exposure, and that the midline neural tube development is compromised in B6 mice treated with a liquid diet containing 25% ethanol-derived-calories (EDC). The SAL or NAP peptides are known to prevent cell death in cell culture and fetal demise induced by heavy binge exposure in mice. In our studies, groups of time-pregnant B6 dams were randomly assigned to either alcohol consumption (ALC, 25% EDC liquid diet) from gestation day 7-14 (E7-E14), pair-fed (PF) liquid diet control, or chow-fed (Chow) control, or alcohol liquid diet consumption also treated with injections of a short form of ADNP, L-SAL (ALC/SAL, 20μg/day, i.p.), or L-NAP (ALC/NAP, 20μg/day, i.p.). The embryos were taken at E15 and brain morphometric measures were taken.

L-NAP was also tested for its protective potential in a model of binge alcohol exposure in neonatal B6 mice, in which two injections of alcohol (2.5 g/kg per injection) or saline control injections were given, two hours apart on postnatal day 7 (P7). Just prior to the alcohol (or saline) treatment, half of the pups of each group were injected with L-NAP and the others injected with saline. At designated times later on P7, the pups were perfused with formaldehyde for immunocytochemical staining of the active subunit of caspase-3, a marker for apoptosis.

V. Accomplishments and Results

Treatment with L-SAL was found to have widespread protective effects, reducing alcohol-induced deficits at E15. The fetal body weight of the ALC/SAL group was significantly increased above the level of the ALC group ($P<0.01$), but not to the level of PF and Chow groups. The brain weight was increased in ALC/SAL from the level of ALC ($P<0.05$) to levels comparable to PF and Chow. The brain circumference also increased in ALC/SAL vs. ALC ($p<0.01$), to a level comparable to PF and Chow. L-SAL injections reduced the alcohol-induced enlargement of the opening in the floor plate of the neural tube and the occlusion of ventral canal in brainstem, but did not affect the roof plates. There were general increases in the size of a number of actively developing brain regions including the basal ganglia*, septal nucleus**, diencephalons*, hippocampus**, and amygdala**, as well as increases in the cortical thickness of the medial frontal** and cingulate cortices* in ALC/SAL as compared to ALC (*=$p<0.05$; **=$p<0.01$). Importantly, these increases attributable to SAL pre-treatment restored these measures to PF and Chow levels. No difference was found between PF and Chow subjects in the above parameters.

Recently, new data indicate that L-NAP produces similar effects in increasing fetal body weight (ALC/NAP group from that of the level of the ALC group, $P<0.01$), fetal brain weights ($P<0.01$), and cortical thickness. Treatment with L-NAP concurrently with alcohol treatment significantly increased medial-frontal and cingulate cortical thickness as compared to that of alcohol-only groups ($P<0.01$); the medial-frontal cortex increased to the PF and Chow control levels. These findings suggest that L-NAP is also effective in protecting against the above tested effects of alcohol exposure during prenatal development.

The one-day-binge alcohol exposure with two doses of 2.5g/kg/day (s.c.) in the neonatal mouse model produced substantial apoptosis in the limbic system and striatum, as demonstrated by marker caspase-3 immunocytochemistry. The one-day NAP treatment along with the one-day-binge alcohol exposure, however, did not show a protective effect against the apoptosis.

VI. Discussion

The ability of ADNF-SAL or NAP to antagonize the alcohol-induced retardation of growth of the forebrain and midline neural tube at midgestation, without itself inducing noticeable abnormalities, suggests its potential for use as a therapeutic agent against alcohol-induced neuroteratogenesis and fetal alcohol spectrum disorders. Data collected to date indicate that NAP or SAL seem effective at the prenatal stages of mouse brain growth (comparable to the human 1st and 2nd trimesters of brain development), but not at early postnatal stages (the "3rd trimester equivalent") with one-day-binge alcohol exposure and one day peptide treatment. A multiple-day NAP treatment will be applied in advance of neonatal binge alcohol exposure, to further test the protective potential of NAP against apoptosis in the limbic brain and striatum. The differential effect of NAP on pre- and post-natal alcohol effects will be compared after the confirmation of results from the latter experiment.

VII. Interrelation with Aims of the Consortium and Other Projects

The current in vivo study on NAP/SAL is in agreement with the in vitro studies of NAP/SAL by Dr. Charness, with respect to the protective effect on cultured cells and embryos. Our study will gain insight from Dr. Miller's mechanistic study of the protective effect of NAP and SAL on cultured cells. In addition, we are comparing data in the current mouse model with that of the rodent model by Dr. Goodlett, who also found no protective effects of L-NAP against cerebellar damage in the neonatal rat model. We are also interacting with Dr. Goodlett in developing behavioral paradigms for future tests of NAP/SAL. In conjunction with Dr. Goodlett, we have begun to breed a cohort of postnatal binge-alcohol exposure mice for Dr. Mark Stanton for behavioral deficit screening.

Our pilot data indicates that mouse embryonic head size is altered by prenatal alcohol exposure. We are interested in extending our investigation to explore, with our liquid diet model, if facial parameters are altered at the prenatal stage when neural crest cells are contributing to the facial construction. The potential application of the mouse model to human facial FASD projects examining the early phases of facial dysmorphology is of great interest. Furthermore, it will serve as a model for potential NAP/SAL treatment on this parameter.

VIII. Plans for the Next Year

1. Testing the protective effect of NAP or SAL on reduction of serotonin neurons with the prenatal liquid diet alcohol model.
2. Use the multiple-day NAP treatment to test NAP's protective effect against binge alcohol exposure induced apoptosis in the limbic brain and striatum.
3. Explore ways of acquiring facial image data in the mouse model.

IX. Publications

Zhou FC, Sari, Y, Powrozek T, and Spong CY. A neuroprotective peptide antagonizes fetal alcohol exposure compromised brain growth. J Mole Neurosci, 24 (2004)189-199.

X. Posters, presentations

Progress Report for Basic Science: Therapeutic Agents

I. PI: Keith W. Miller, DPhil., Subcontract to Michael Wilkemeyer, PhD.

II. Title of Project : Photolabeling of alcohol binding sites on L1 U01 AA014812

III. Objectives

Specific Aim 1. We will test the hypothesis that there are alcohol agonist sites on the L1 adhesion molecule using 3–azibutanol, an alcohol that inhibits L1–mediated cell adhesion just like ethanol.

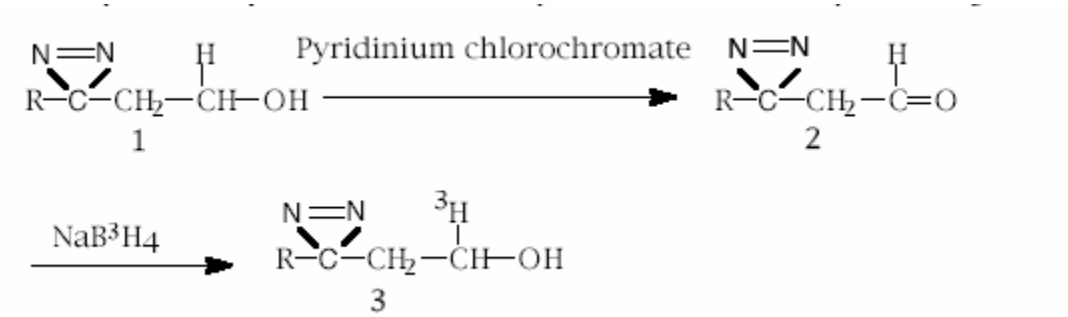
Specific Aim 2. We will test the hypothesis that there are alcohol antagonist sites on the L1 adhesion molecule using 3- and 7-azioctanol, newly developed photoreactive diazirine derivatives of octanol. We have shown that 3-azioctanol, like octanol itself, antagonizes ethanol's inhibition of L1–mediated cell adhesion.

IV. Methods

L1 (0.5 nmoles) is mixed with 3-azioctanol (10–1,000 μM), incubated for 10 minutes in the dark and then irradiated for 30–40 minutes at 365 nm. The photolabeled sample is reduced with DTT, alkylated with iodoacetamide, lyophilized, digested with CNBr for 24 h and again lyophilized. Samples are resuspended in acetonitrile:water (50:50) and applied to a microcapillary POROS 10R2 chromatography column (75 μm x 360 μm; Perceptive Biosystems, Framingham, MA) packed to 15 cm and butt–connected to a fused silica nanospray tip (5 μm) and eluted with a gradient of 5–100% acetonitrile in 120 minutes at a flow rate of ~0.2 μL/min into an ion trap mass spectrometer with electrospray ionization (LCQ, Finnigan MAT, San Jose, CA). Mass spectra were acquired from m/z 300 to 2000 with a maximum ejection time of 400 ms. This generates a huge amount of data. The ion current chromatogram is analyzed extensively to yield groups of mass/charge ratios (charge envelopes) that each represents a single peptide fragment. This can then be deconvoluted to yield the molecular weight of the peptide and that of peptide plus photoincorporated alcohol (if any). In addition, identified peaks can be sequenced by collision–activated MS/MS.

V. Accomplishments and Results

Aim 1: A synthesis for [3H]3-azibutanol has been worked out and tested using non- tritiated sodium borohydride. The synthesis worked well and protocols were then developed for doing the reaction on a microscale suitable for work incorporating radioactivity. Recently, purified precursor (compound # 2 below) went to Amersham for tritiation.



Aim 2: Full length L1 (Fig. 1A) is a membrane protein and therefore difficult to work with. Photolabeling work to date has been carried out on the truncated protein shown in Fig. 1B and kindly provided by Dr. Grumet. We have photolabeled this with 1 μM [3H]3-azioctanol, digested it with cyanogen bromide (CNBr) and purified the fragments by HPLC. Two peptides incorporated tritium, and 10 μM of the NAP peptide reduced azioctanol photolabeling. Identifying these two fragments unequivocally and defining which of the 850 amino acid residues have photoincorporated 3-azioctanol has proved difficult. We have photolabeled with up to 1 mM 3-azioctanol and performed mass spectrometry both on samples separated by off line HPLC and on samples separated by online HPLC. We have preliminary identification of two

peptides that appear to have photoincorporated 3-azioctanol. Currently, a lot of effort is going into refining the algorithms to analyze the data after online HPLC mass spectrometry and to sequence these peptides.

In addition, we are investigating alternative strategies. One is to separate CNBr fragments by size prior to online HPLC/MS/MS. Another, which is yielding some success, is to digest L1 with trypsin instead of CNBr.

Protein production: We plan to continue the photolabeling using the complete extracellular region of human L1 fused in frame with the Fc region of human immunoglobulin isotype 1, added to facilitate purification (Fig. 1C). Therefore, we have been developing methods to produce this protein. Dr. Cynthia Bearer has provided NIH/3T3cells that express and excrete this recombinant protein. We have cultured these cells on plates and collected the conditioned culture medium, containing secreted L1-Fc. Proteins are precipitated from pooled media by adding ammonium sulfate, centrifuging and dialyzing against appropriate buffer. They are loaded on a DE52 ion-exchange column, and the L1–Fc molecule is eluted with high salt (between 0.2 and 0.4 M KCl) and then affinity-purified using Protein Asepharose linked beads. The yield in our hands to date is ~100–200 µg L1/L.

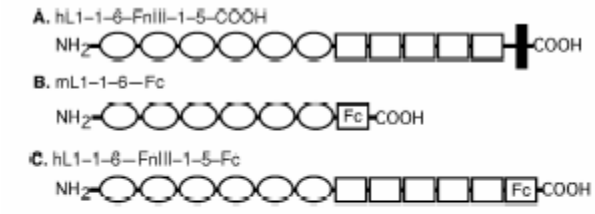


Fig. 1 Chimeric proteins referred to. **A.** Full length human L1, consisting of 6 Ig (ovals) & 5 Fn (oblongs) domains plus a transmembrane C–terminus (Ig= immunoglobulin; Fn= fibronectin-like type III). **B.** Mouse model L1 contains 6 Ig domains with an added Fc domain to aid purification (Fc=Fc region of human Ig1). **C.** Human L1 with an Fc added and the transmembrane region deleted.

VI. Discussion

Although good progress is being made, this project is still at an intensely technical stage of its development. Work to date is encouraging but no definitive data can yet be presented.

VII. Interrelation with Aims of the Consortium and Other Projects

This project is one of the Consortium's basic science projects. Its first long-term goal is to test the hypothesis that there are alcohol binding sites on L1. Should this be so, then the intellectual framework for seeking pharmacological antagonists to ethanol will have been established. The second long-term goal is to establish whether antagonists act competitively or allosterically to attenuate ethanol's action. This will define the quality of antagonism that might be expected. The third long term goal is provide more detailed information about the structure of the alcohol binding sites in order to guide rational drug development.

VIII. Plans for next year

Specific Aim 1: Photolabeling with the agonist [3H]3-azibutanol will commence once Amersham has synthesized the ligand. Initial work will involve photolabeling, digestion and purification of fragments. The techniques employed will be chosen based on ongoing experiments with 3-azioctanol.

Specific Aim 2: We will switch to using the complete extracellular domain of human L1 (Fig. 1C). We anticipate that this larger protein will be corresponding difficult to analyze and that, therefore, initially efforts will go into refining techniques.

Protein production: Dr. Bearer has obtained similar overall yields of the L1-Fc, but from greatly reduced volumes of cell culture medium (20-100 ml), by culturing her cells on an artificial capillary cartridge system (Cellmax, Spectrum labs). We will use this to improve our yield of L1-Fc/liter of medium. So doing will greatly enhance our ability both to successfully carry out the biochemical analyses following photolabeling and to conduct pharmacological studies to characterize the significance of alcohol binding sites identified by photolabeling.

IX. Publications

None

X. Posters, presentations None

Appendix

Publications

Riley EP, McGee CL, and Sowell ER. (2004) Teratogenic effects of alcohol: a decade of brain imaging. American Journal of Medical Genetics Part C (Semin. Med. Genet.) 127C:35–41.

Stewart, C.A., R. Repasky, and A. Arenson. 2004. Open source tools for computational biology. Tutorial handbook. SC2004 conference, November 2004, Pittsburgh, PA. Tutorial notes may be downloaded from rac.uits.indiana.edu

Stewart, C.A. 2004. Bioinformatics: transforming biomedical research and medical care. Communications of the ACM 47(11): 31-33.

Tran, T.D., Jackson, H.D., Horn, K.H., and Goodlett, C.R. Vitamin E Does Not Protect Against Neonatal Ethanol-Induced Cerebellar Damage or Deficits in Eyeblink Classical Conditioning in Rats. Alcoholism: Clinical and Experimental Research, in press

Zhou FC, Sari, Y, Powrozek T, and Spong CY. A neuroprotective peptide antagonizes fetal alcohol exposure compromised brain growth. J Mole Neurosci, 24 (2004)189-199.

Posters, presentations

CIFASD Informatics Core. 2004. Indiana University display at Indiana Health Industry Forum, June 2004. Indianapolis, IN

CIFASD Informatics Core. 2004. Electronic poster included as part of Indiana University display at the SC2004 Conference, November 2004. Pittsburgh, PA.

Bakhireva, L., Jones, K. Robinson, L., Riley E., Mattson, S., Marintcheva, G., Chambers, C. Effective Training of Pediatricians to Diagnose Features of the Fetal Alcohol Syndrome in Russia Sample. Research Society on Alcohol. Vancouver, 2004

O'Hare ED, Kan E, Yoshii J, Mattson SN, Riley EP, Thompson PM, Toga AW, Sowell ER (2004) Localized dysmorphology of the anterior vermal lobule and primary fissure in severe prenatal alcohol exposure (abstract). 11th Annual Meeting of the Organization for Human Brain Mapping, Budapest, 2004 (In Press).

O'Hare, ED, Kan, E, Yoshii, J, Mattson, SN, Riley, EP, Thompson, PM, Toga, AW and Sowell, ER. Cognitive Correlates of Anterior Cerebellar Vermal Dysmorphology in Heavy Prenatal Alcohol Exposure. 34th Annual Meeting, Society for Neuroscience, San Diego, CA.

Mattson, S.N., Marintcheva, G., Coles, C.D., and Riley, E.P (2004). Comparison of FAS in Moscow Russia and San Diego, California. Presented at the Twelfth Congress of the International Society for Biomedical Research on Alcoholism, Heidelberg, Germany, September 2004. Alcoholism: Clinical and Experimental Research, 68.

McGee, C.L., Fryer, S.L., Riley, E.P., Coles, C.D., Kalberg, W., Kodituwakku, P.W., May, P.A., and Mattson, S.N. (2004). Adaptive functioning as a function of ascertainment method in children prenatal exposed to alcohol. Presented at the Twelfth Congress of the International Society for Biomedical Research on Alcoholism, Heidelberg, Germany, September 2004. Alcoholism: Clinical and Experimental Research

Colleen Adnams, M.D. presented the methodology and progress report at RSA in Vancouver in June. She also presented the rationale, methodology and preliminary (baseline) data at the ISBRA conference in

Germany in late September, 2005. A summary of the later presentation is to be published in Alcoholism: Clinical and Experimental Research.

Fred L. Bookstein. Platform presentation at the June 2004 Teratology Society Meetings in Vancouver, Canada: "Ultrasound Imasging of the Neonatal Corpus Callosum is Feasible and Useful" presented by. (Although the earlier printed abstract described only pilot data, the oral presentation included the first analysis and findings from the CIFASD project.)

Fred L. Bookstein. Presentation at the Fetal Alcohol Study Group Meeting of the Research Society on Alcoholism June 2004 Meeting in Vancouver, Canada: "Detecting FASD with Prenatal Ultrasound". Presentor: (other authors: Ann Streissguth).

: Fred L. Bookstein Presentation at the 25th Anniversary Celebration of the University of Washington Department of Statistics. Sept 2004, Seatle, Washington. Presentor. Hour-long talk of which 1/4 was on our CIFASD project

4. Presentation at the Konrad Lorenz Institute for Evolution and Cognition Research, Altenberg, Austria. October 2004. Presentor: Fred L. Bookstein. Hour-long talk of which 1/4 was on our CIFASD project.

Publications submitted or in preparation

Fred L. Bookstein,; Paul D. Connor, Kristi D. Covell, Helen M. Barr, Christine A. Gleason, Ann P. Streissguth, Raymond W. Sze, Jenny A. McBroom: "Prenatal Alcohol Damage Can Be Visible in Averaged Ultrasound Images of the Neonatal Corpus Callosum." by Children's Hospital and Regional Medical Center, Seattle, Washington. , Submitted 12/30/04

O'Hare, ED, Kan, E, Yoshii, J, Mattson, SN, Riley, EP, Thompson, PM, Toga, AW, and Sowell, ER (2005). Mapping Cerebellar Vermal Morphology and Cognitive Correlates in Prenatal Alcohol Exposure. Submitted.

Pinter, M.N., Lee, K.T., Marintcheva, G., Riley, E.P., and Mattson, S.N. Sex differences in intellectual functioning in Russian children with fetal alcohol syndrome. Paper in preparation, submission expected 1/2005.

Brain Metabolic Deviance in Young Adults with Fetal Alcohol Spectrum Disorders (in preparation)

Fetal Alcohol Spectrum Disorders In Finland: Clinical Delineation of 77 Older Children and Adolescents (in preparation)

Dysmorphic features in Finnish children and adolescents with FASD (in preparation)