Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD)

Progress Reports

January 2006

Presented to CIFASD Steering Committee Meeting
Rockville, MD
February 6-7, 2006

Table of Contents

	Page Number
Administrative Core U24 AA014811	**3**
Informatics Core U24 AA014818	**5**
Pilot Project Core U24 AA014828	**10**
Pilot Project: Buffalo	**11**
Pilot Project: University of Washington	**12**
Pilot Project: University of New Mexico	**14**
Pilot Project: UCSD	**17**
Dysmorphology Core U24 AA014815	**19**
Brain Imaging Core U24 AA014808	**21**
Facial Imaging Core U24 AA014809	**25**
Neurobehavioral Core U24 AA014830	**28**
FASD in San Diego and Moscow U01 AA014834	**31**
Identification of FASD in South African Children U01 AA014790	**35**
International Neuropsychological Study of FASD U01 AA014786	**39**
Multi-Method Intervention Study of FAS in South Africa U01 AA014786S	**41**
Risk Factors for FASD in the Moscow Region U01 AA014835	**44**
Testing FASD Therapeutic Agents: Neonatal Rodent Models U01 AA014829	**46**
Testing FASD Therapeutic Agents in Mouse Models U01 AA014829S	**49**
Photo-labeling of Alcohol Binding Sites on L1 U01 AA014812	**53**
Appendix	**56**

Progress Report for Administrative Core

I. Principal Investigator: Edward P. Riley, Ph.D.

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:

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

The Primary Goals of the Administrative Core are:

1. 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.
2. 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.
3. Convene meetings of the Steering Committee.
4. To coordinate with the Informatics Core to ensure that communication within the projects is effective.
5. 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.
6. Maintain Consortium web site.

IV. Methods:

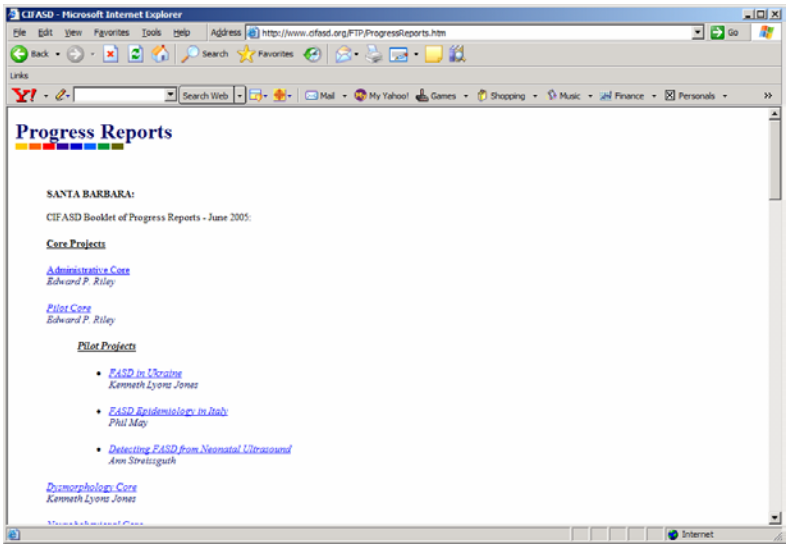
1. Utilizes web based information, regular emails, monthly conference calls and twice annual meetings.
2. Maintains contact with NIAAA through regular phone calls.

V. Accomplishments and Results:

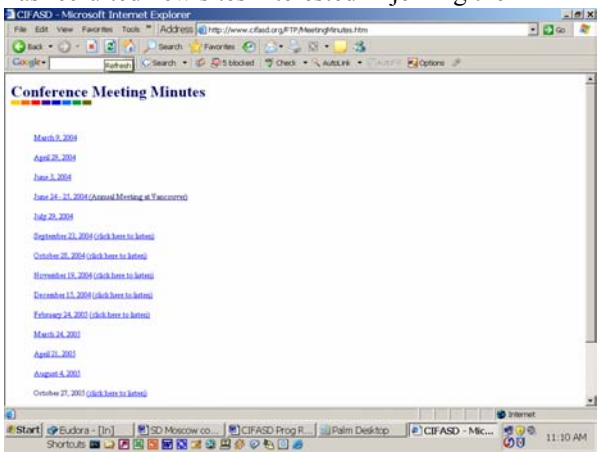
1. The Consortium website is currently functioning. Within the next six weeks, the website will be given a new look that is easier to use, with links to current news relevant to FASD research. We are also adding a page to inform the public of current findings.
2. The website contains forms, manuals, database entry forms, tests, minutes, progress reports, etc.
3. Regular monthly phone conference calls have been convened. These conference calls are available verbatim on the Consortium website.

V. Accomplishments and Results: (continued)

4. A new teleconferencing tool has been established, which will facilitate data sharing and discussion across multiple sites.
5. Twice annual meetings of the Consortium PIs have been convened. Minutes from these meetings are available on the website.
6. Progress reports are created every six months as are links to each component and are available online (see Figure).
7. The Administrative Core has financed parts of projects when funds were not available in the budget of the project. For example, funds were provided to the basic science research to extend projects for an additional year.



8. 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.
9. 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.
10. Five pilot projects were received and reviewed. Supplemental funds for pilot projects have recently been obtained and the projects that receive funding will be determined by the beginning of 2006.
11. Publications are being posted online
12. 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**.** The Administrative Core is also involved in the expansion of the Consortium by funding pilot projects.

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 primary focus in the coming year will be to prepare a contemplated renewal application to NIAAA.

IX. Publications:

Not applicable.

X. Posters and Presentations:

Not applicable.

Progress Report from the Informatics Core

I. Principal Investigator and Key Personnel: Craig A. Stewart, PI, Andrew D. Arenson, Project Manager

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 HIPAA-compliant storage of data to be shared within the CIFASD and tools that facilitate the accomplishment of the scientific mission of CIFASD. The Informatics Core is also providing expert statistical analysis.

IV. Methods:

CIFASD is collecting several different types of data: dysmorphology, 2-D facial images, alcohol and control, neurobehavior, 3-D facial images, and ultrasound. For each of these categories of data, the Informatics Core is working with the rest of the Consortium to accomplish the following:

1. Work with members of the Consortium to define a data dictionary that precisely defines what data is to be stored.
2. Create one or more input tools that allow projects to record their data.
3. 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.
4. Expand the methods for retrieving data to include the ability to retrieve each type of data in turn.

The result is a combination of software tools that allows project members to locally store each of the types of data being collected for the Consortium, upload/submit those 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 completely 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 follows. An asterisk (*) represents status that has changed since the last report six months ago.

Data Area	Task	Status
Dysmorphology	Data Dictionary	**Finished**
	MS Access Input Tool	**Finished**
	Central Repository Schema	**Finished**
	Upload Tool	**Finished**
	Report Tool	**Finished**
Alcohol & Control	Core Data Dictionary	**Finished**
	Core MS Access Input Tool	**Finished**
	Expanded Data Dictionary	**Finished**
	Expanded MS Access Input Tool	**Finished**

Data Area	Task	Status
	Central Repository Schema	**Finished** *
	Upload Tool	**Finished** *
	Report Tool	**Finished** *
Neurobehavior	Data Dictionary	**Finished** *
	MS Access Input Tool	**Finished** *
	Central Repository Schema	**Finished** *
	Upload Tool	**Finished** *
	Report Tool	**Finished** *
3D Facial Images	Data Dictionary	**Finished** *
	Central Repository Schema	**Finished** *
	Upload Tool	**Finished** *
	Report Tool	**Finished** *
2D Facial Images	Data Dictionary	
	Central Repository Schema	
	Upload Tool	
	Report Tool	
Ultrasound	Data Dictionary	**Finished** *
	MS Access Input Tool	*In Progress*
	Central Repository Schema	
	Upload Tool	
	Report Tool	
UCSD Screener	Data Dictionary	**Finished** *
	MS Access Input Tool	*In Progress* *
UCSD Extended Expanded Alcohol & Control	Data Dictionary	**Finished** *
	MS Access Input Tool	*In Progress* *
UCSD Follow-up/Outcome Interview	Data Dictionary	**Finished** *
	MS Access Input Tool	*In Progress* *

Consortium researchers may download MS Access input tools from our web page at http://discover.uits.indiana.edu:8250/downloads/index.html. Example screen images of data entry and retrieval tools we have created are shown in Figures 1 and 2.

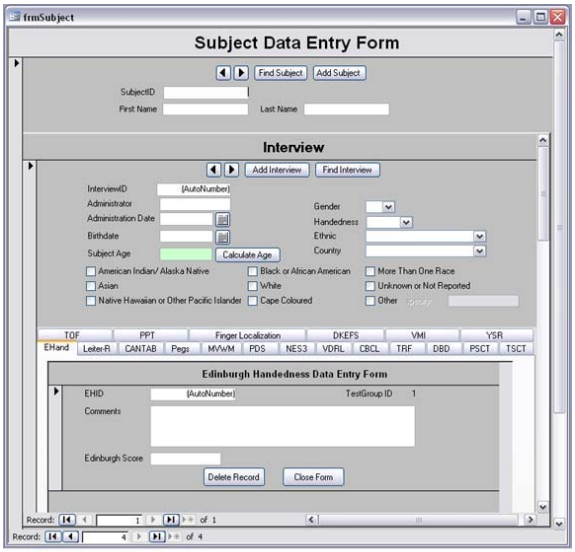


Figure 1. Neurobehavior Data Entry Form. This data entry form includes a data validation tool to decrease data entry errors as well as range checking that makes it impossible to enter a value outside the logically permissible range identified in the data dictionary.

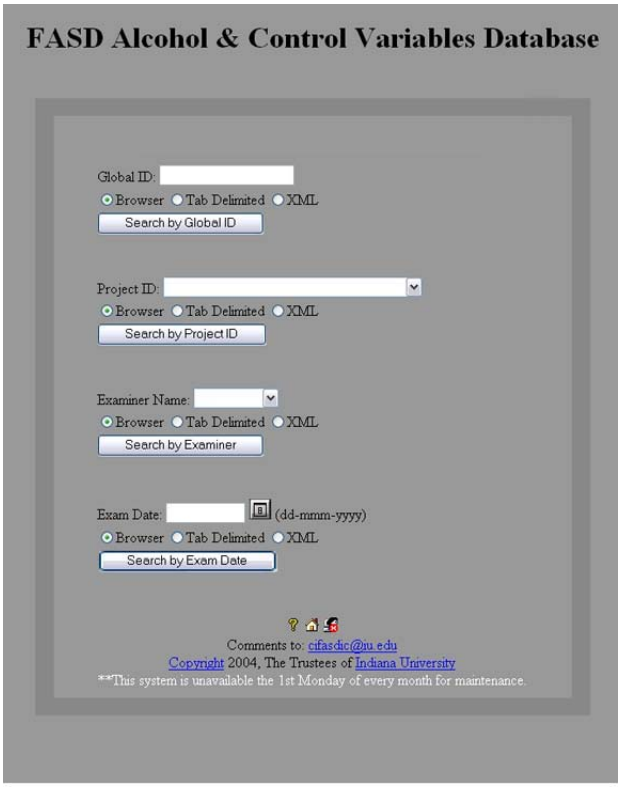


Figure 2. Web-based Alcohol & Control data access and download tool.

V. Accomplishments and Results: (continued)

Data for the Consortium are stored in duplicate – one copy in a robotic tape storage system in Indianapolis, Indiana and a second copy in a robotic tape storage system in Bloomington, Indiana. 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.

VI. Discussion:

The clear goal given to the Informatics Core at the June 2005 meeting was to enable researchers to submit and query the data most relevant to finding immediate results. These data were identified as the 3-D Facial Imaging and Neurobehavior data. The Informatics Core has met these goals, successfully enabling submissions and queries of 3-D Facial Imaging data, including receiving, storing and distributing 3-D model files, and successfully finalizing the Neurobehavior data dictionary, input tool, central schema, submission web page, and query web page.

Finishing the Neurobehavior input tool required writing a custom code to import data from six different computerized tests as well as extensive aesthetic and functional modifications on the over eight hundred variables that span eighteen different subtests. Creating the Neurobehavior input tool was functionally equivalent to developing the Dysmorphology tool eighteen times, plus creating the functionality to tie those eighteen tests together and list which tests were given and/or considered valid, and creating the functionality to either force double-entry for verification purposes or, for a third of those tests, creating custom methods to read data from a variety of data formats generated by different computerized tests. The Neurobehavior input tool has multiple orders of magnitude larger and more complex than the first tools created by the Informatics Core and reflect the growth of specialized expertise in developing tools for managing these types of data gained by the Informatics Core over the course of this project.

Along with meeting these primary goals, the Informatics Core also worked on a number of other software tools. The Alcohol and Control core and expanded variables were added to the central repository with accompanying web upload and query interfaces. As part of a subcontract with UCSD, work was begun on a number of data areas that are either UCSD specific or shared across Consortium projects but of a lower priority than the primary goals. Most notable amongst these is the Ultrasound data dictionary and input tool, which is expected to be used by multiple projects.

VII. How this project interrelates with the aims of the Consortium and with other projects within the Consortium:

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 this may be a model 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 Six Months:

Although the central repository and data input software tools required to manage the most crucial types of data have recently been completed, there is still significant software development to be undertaken. High-priorities for future development are expected to include Ultrasound data, providing SAS-formatted output to web queries, and updating the existing input tools that do not use double-entry verification to have that functionality. Other possible targets include creating a Neurobehavior input tool geared towards younger subjects, updating the Neurobehavior tool to automate the process of determining scaled scores, handling 2-D facial images, and providing for web queries that return results across different data types without manual intervention.

The rush over the last year and the last six months in particular to complete tools has meant that a number of tradeoffs were made in terms of long-term maintainability of the software to allow for the software to be available for use sooner. These tradeoffs, although the right choice under the time-constraints of the collaboration, have

VIII. Plans for the Next Six Months: (continued)

resulted in a relatively brittle set of codes that requires some extensive manual intervention and will require significant rework of code over time to become a truly robust codebase. Much of the code written in the last six months has also not yet been widely used in the field and will likely require urgent modifications and fixes in the coming weeks and months.

IX. Publications: (In print, in press, accepted, submitted that cite the Consortium funding)

Stewart, C.A. 2004. "Bioinformatics: Transforming Biomedical Research and Medical Care." pp. 31-33. Communications of the ACM. November 2004 Volume 47, Number 11.

X. Posters and Presentations: (Please include one copy of any published abstracts)

Flury, L., Rogers J.; Foroud T.; Robinson L.; Moore, E. 2005. "Fetal Alcohol Syndrome and 3-D Facial Imaging: A Preliminary Classification Study". Poster at Research Society on Alcoholism. 26 June 2005. Santa Barbara, CA.

Wheeler, B.C. 2004. "Research and Academic Computing." Presentation at Indiana University Statewide IT Conference. 14 October 2004. Indianapolis, IN.

Presentations were presented at the Indiana University Advanced Visualization Laboratory, including the Indiana State Legislature, Techpoint (an organization representing the IT community in Indiana) along with various groups of researchers and educators, and the NSF.

Progress Report for Pilot Project Core

I. Principal Investigator: Edward P. Riley, Ph.D.

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. There is an emphasis on adding new sites with unique resources or attributes about the population under study that will contribute to the overall functioning of the Consortium. However, providing additional funding for ongoing pilot and regular projects that want to expand based upon current findings from the Consortium is also considered.

IV. Methods:

Initially, four independent projects were funded and funding of these projects has now ended. New projects were solicited from within the Consortium members, as it was decided by the Steering Committee to limit the number of submissions. Proposals were submitted online via the CIFASD website and reviewed by an independent committee, Chaired by Dr. James West. However, it was decided by the CIFASD Steering Committee that funds for these pilot projects should be shifted to the basic science projects for their 04 year. Supplemental monies have recently been secured and it is anticipated that two of the submitted pilot projects will be funded this year.

V. Accomplishments and Results:

Of the original pilot projects funded, three publications, one meeting presentation and one RO1 submission have been achieved.

The major accomplishment in the administration of the Pilot Project Core has been the solicitation and review of new proposals. Although funding was not initially available to support new projects, recent funds will allow us to support two new pilot projects this coming year.

VI. Discussion:

Significant amounts of data have been collected in the pilot projects and are discussed below.

VII. Interrelation with Aims of the Consortium and Other Projects:

See individual project descriptions. New proposals were evaluated based on their potential contribution to the goals of the Consortium.

VIII. Plans for Next Year:

New projects will be funded.

IX. Publications:

None.

X. Posters and Presentations:

None.

Progress Report on Pilot Project: Buffalo

I. Principal Investigator: Luther K. Robinson, M.D.

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

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 this group 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:

We compared the following: 1.) clinical measures of affected subjects 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.

We are using the diagnostic paradigm as set forth by the Dysmorphology Core – No change
Subjects are offered an honorarium of $25.00 – No change

V. Accomplishments and Results:

Our project is completed. We have begun a secondary analysis of the data on the 93.

VI. Discussion:

We have imaged no new patients since September. We had intended to continue and add another disease control group such as Down Syndrome, but we could not engage the local Down Syndrome support group. We also submitted the laser imager to other sites since then. We are continuing with a secondary analysis of our data.

VII. Interrelates with Aims of the Consortium Other Project:

This project contributed data concerning the feasibility of computerized or laser imaging to the clinical diagnosis of FAS. Abstracts from the Imaging Core are addressing correlations between clinical and imaging data. Future clinical-laboratory and laboratory-clinical correlations will address the applicability of the technology to the clinical setting. Certainly, if imaging distinguishes between FAS and Williams phenotypes with sensitivity, specificity, and predictability, then one would expect that further technological developments such as smaller size of hardware or of the software could result in translation of the protocol to the clinical setting.

VIII. Plans for the Next Year:

IX. Publications:

To be submitted.

X. Posters and Presentations:

Abstracts to be submitted for this year's meeting of RSA.

Progress Report on Pilot Project: University of Washington

I. Principal Investigators: Ann P. Streissguth, Ph.D. and Fred Bookstein, Ph.D.

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

III. Objectives:

The overall objective of this project is the development and dissemination of a methodology for very early detection of brain damage from prenatal alcohol exposure within samples of babies known (from perinatal maternal self-report) to have been highly exposed. Detection would occur using ultrasound imaging of the structural brain damage component of FASD, not other channels such as dysmorphology, growth retardation, or developmental delay. Our strategy arose as an extension into this new age range of a strong morphometric signal from brain MR already known to have adequate sensitivity and specificity in adolescents and adults. The challenges included the design and accrual of a suitable high-risk sample, together with comparison subjects, and a semiautomated method of image analysis for the neonatal midsagittal ultrasound frames that substitute for MR imaging in this age range. In addition, as time and funding permit, we attempted to evaluate the neurobehavioral function of these babies and to determine whether babies with atypical CCs would also be rated FAS or deferred based on a clinical dysmorphology exam.

IV. Methods:

We proposed to locate, enroll, and scan 50 mother-baby pairs: 25 recently postpartum mothers reporting prenatal drinking at levels corresponding to the risk threshold of our published paper on the BARC (binge alcohol rating criterion) scale; and 25 non-drinking or only lightly drinking mothers. Babies were scanned between four and seventeen weeks of age. An HDL ultrasound scanner with C8-5 pediatric cephalic transducer grabbed up to 50 freeze frames per baby selected to include the full length of the callosum in or near the midsagittal plane. Raw images were sent to our unit for selection of a subset in a consistent midsagittal plane. The resulting subset was processed by our Edgewarp Software to yield unwarped averaged images for each subject. Some strikingly variable features of these images could be measured by the standard contemporary morphometric tools.

V. Results and Accomplishments:

When the exposure code was broken for the part of the sample that was available as of December 2004, we found that we had valid average images for 7 exposed babies and for 11 unexposed. The variation previously noted as striking proved closely associated with the exposure status of the infant. In particular, one simple angle measurement for four of the exposed infants fell outside its range in the full subsample of unexposed. It is natural to speculate that these might be the four out of the seven who would eventually be diagnosed within the FASD spectrum. A paper explaining all of this in appropriate detail was prepared, submitted to *Alcohol* for publication, revised twice, and accepted. This paper which acknowledges CIFASD support is expected to appear in the December, 2005 issue of *Alcohol* (Volume 36, Issue 3).

Since completing the preliminary study, we have continued to accrue subjects. As of this writing (late December, 2005), we have enrolled a total of 48 subjects; however, only 44 babies have satisfactory image sequences. We will continue to enroll subjects through January 15, 2006. After producing unwarped averaged images for all new subjects (i.e., all those enrolled after December, 2004), we will break the exposure code for this new subsample and attempt to replicate the now-published findings pertaining to the first eighteen.

A subset (N=14) of this extended infant sample (N=44) was examined by Ken L. Jones, M.D., using the resources of the CIFASD Dysmorphology Core and its data forms. Unfortunately, owing to lack of funds, we have not been able to complete assessment of the remaining subjects. Another subset (N=16) received a developmental assessment using the Bayley Scales of Infant Development. Six of these 16 were a year old at the time of developmental assessment. Therefore, they were also evaluated using the A-not-B task, a measure of infant executive function thought to be sensitive to prenatal alcohol exposure and most effectively administered for this purpose when babies are 12 months of age. A large subset (N=32) has received a developmental screening and was evaluated using the

V. Results and Accomplishments: (continued)

Vineland Scales of Adaptive Behavior-II. Their mothers completed a maternal interview to obtain information about the child's health, developmental status, and intervention services.

VI. Discussion:

We have made several attempts to leverage CIFASD's support into additional resources in light of the published (and indeed somewhat surprising) finding sketched above. Here in the United States, attempts to gain further funding for additional samples, and also attempts to gain further funding for confirmation of the suggested diagnosis among the babies of this sample, have all been rejected, including a second pilot submission to the CIFASD Consortium itself. However, we do acknowledge supplemental funding from the University of Washington Alcohol and Drug Abuse Institute (ADAI) and the UW Fetal Alcohol and Drug Unit Royalty Fund and by Grant P200.093/1-VI/2004 from the Austrian Council for Science and Technology to the Institute for Anthropology, University of Vienna, Austria. At the central neonatal ward of the largest hospital in Vienna our intake questionnaire (translated into German) is being used to accrue a completely separate sample of these images for at-risk newborns, and also a much larger sample of unexposed is being archived for a representation of normal variation of this important structure in the newborn brain.

VII. Relation to Other Components of CIFASD:

We have drawn on the CIFASD Dysmorphology Core resources for assessment of our infant subjects by Ken Jones, M.D. as noted above. While other CIFASD sites collect ultrasound images, the Informatics Core does not seem to have a resource for the preservation of those images as an actual archived data source. Currently, no other CIFASD site is using our expertise in morphometric analysis or the informatics of FASD detection. However, such collaborations might well emerge in the course of formulating the new aims for the possible CIFASD renewal in fiscal year 2008.

VIII. Plans for the Next Year:

Our CIFASD pilot study is coming to the end of its promised funding period. Plans for next year are limited to the continued accrual of subjects in the originally proposed study through mid-January 2006, followed by analysis of the complete or nearly complete sample as originally envisioned. We are committed to presenting these findings at the CIFASD semiannual meeting in Winter 2006. If the findings justify an additional peer-reviewed publication, we will pursue one using resources outside CIFASD, as our account will be exhausted by then.

Several sites associated with CIFASD outside the United States have expressed interest in our method of FASD detection by neonatal ultrasound. In view of the gaps of funding through the CIFASD mechanism, these collaborators are being encouraged to work with our co-investigator Fred Bookstein, Ph.D., via his faculty appointment at the University of Vienna, Austria.

IX. Publication:

Bookstein, F. L., Connor, P. D., Covell, K. D. Barr, H. M., Gleason, C. A., Sze, R. W., McBroom, J. A. and Streissguth, A. P. Preliminary evidence that prenatal alcohol damage may be visible in averaged unwarped ultrasound images of the neonatal human corpus callosum. *Alcohol,* in press, 2005.

Progress Report on Pilot Project: University of New Mexico

I. Principal Investigator: Philip A. May, Ph.D.; Mauro Cecanti, M.D. (co-P.I.) Daniela Fiorentino, M.A., La Sapienza, The University of Rome

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:

Wave I Screening. 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 (95.2% overall; 93.3% of preliminary FAS, 91.1% of deferred, and 95.9% of controls) of the mothers from families who consented for their child to be in the study. 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, focus 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 (95.2%) received psychological testing. The test

V. Accomplishments: (continued)

instruments used for that group were the Ravens Colored Matrices and the Rustioni's Evaluation of Language Comprehension.

The Italian team met with the dysmorphology team and epidemiology research team in Albuquerque on the third week of January, 2005. Data on each child (cases and controls) were reviewed, in a formal case conference. A final diagnosis of each child seen was assigned at that time. In the case conference, the results for 145 children were reviewed completely. Using the revised IOM criteria for the diagnosis of FASD published by our group in *Pediatrics* (Hoyme, et al., 2005): 22 of the children had some type of FASD (4 with FAS, 17 Partial FAS, and one ARND); 123 had no FASD; of the 15 children receiving a preliminary diagnosis of FAS from the dysmorphologists, 12 had a final diagnosis of FASD (3 FAS, and 8 Partial); of the 81 originally deferred by the dysmorphologists, 11 were found to have FASD (1 FAS, 9 Partial, and one ARND) which showed how very liberal the FASD form is in deferring children for further testing.

Getting an honest, accurate accounting of drinking from maternal interviews in Italy is proving to be more difficult than in South Africa and about the same as in the United States. Forty-five percent of the FASD diagnoses were made without direct confirmation of drinking from the birth mother.

When these data are presented in the form of an overall prevalence, it is not as simple as dividing the number screened by the number of cases diagnosed. The final estimates of prevalence will have to take a number of factors into account. First, we will use estimation of prevalence to the total sample by calculating a range of rates. Second, the selectivity of a population-based screening system where only 50% of the children had permission to participate presents a challenge. Third, we screened first (Tier I) on the basis of height, weight, head circumference, but also for those children who were believed to have related behavioral problems. Fourth, there are no comparable, in-school studies of FASD in the western world. Finally, several of the randomly-selected controls were found to have FASD. Each of these items presents a factor that needs to be considered in the final estimation of the total prevalence. Our epidemiology manuscript from Wave I raises and attempts to account for these considerations.

Wave II Screening. In March of 2005, the team returned to Italy to complete the second wave of screening with referred children. Of the 910 children randomly selected for the study, 435 consents were obtained. One hundred and eighty five were ultimately identified through the first tier of screening (and the random selection of controls) to receive dysmorphology examinations, and 182 children (including controls) were ultimately located and provided dysmorphology examinations. After the dysmorphology screening, 35 children were suspected of having and FASD and 48 of the selected controls were available. Of these 83 selected for follow-up, new consents were obtained for most of the children. Psychological testing was then completed on all of these children using the WISC and the Pelham Disruptive Behavior Disorder Rating Scale. This testing has been completed and feedback given to families and school personnel.

Maternal interviews on all of the 435 mothers of all of the school children were attempted as in wave one. A total of 408 mothers (93.7%) were interviewed which approaches the efficiency of the first wave.

VI. How this Project Interrelates with the CIFASD:

The data and results from this study will be compared with the other sites involved in 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 participants (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 Agatino Battaglia from Italy in Wave I and Luigi Teranni, an Italian geneticist/dysmorphologist in both Waves I and II.

In the second year of screening, March, 2005, Drs. Hoyme and Robinson were also joined by Melanie Manning, M.D. (Stanford) and Richard Findlay, M.D. (Drew Medical School). As Drs. Manning and Findlay are not members of the Dysmorphology Core, care was taken to team Drs. Hoyme and Robinson with either Dr. Manning or Dr. Findlay each day the teams traveled into the communities to evaluate children. Dr. Hoyme and Dr. Robinson made the final determination regarding diagnosis as representatives of the expert Dysmorphology Core.

VI. How this Project Interrelates with the CIFASD: (continued)

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 the sub-sample of the randomly-selected Italian controls, some of whom were exposed to alcohol in the prenatal period (non growth deficient/non-dysmorphic children) and some of whom were not (alcohol free controls). Thus, the study will provide cases for all children who might be considered candidates for FASD whether they have dysmorphia or not.

VII. Plans for the Next Year:

The results of the Wave II screening will be finalized at the annual case conference to be held in Albuquerque on January 17 -20, 2006. There all cases and controls will be reviewed and a final diagnosis provided to each child.

VIII. Publications:

Two manuscripts have been submitted to *Alcoholism: Clinical and Experimental Research*.

Kodituwakku, P., Coriale, G., Fiorentino, D., Kalberg, W.O., Buckley, D., Gossage, J.P., Deiana, L., Aragon, A.S., Ceccanti, M., May, P. Neurobehavioral Characteristics of Children with Fetal Alcohol Spectrum Disorders in Communities from Italy: Preliminary Results.Alcoholism: Clinical and Experimental Research. Submitted.

May P.A., Fiorentino D., Gossage J.P., Kalberg W.O., Hoyme H.E,. Robinson L.K., Coriale G., Jones K.L., del Campo, Tarani L., Romeo Mkodituwakku P.W., Deiana L., Buckley D. The Epidemiology of FASD in a Province in Italy: Prevalence and Characteristics of Children in a Random Sample, In-School Study. Alcoholism: Clinical and Experimental Research. Submitted

Both manuscripts are under review at the time of this report. These publications report the epidemiological and psychological/developmental testing results of the first wave of the Italian study in the major areas of focus: maternal risk factors, epidemiology, and psychological characteristics in Italy.

IX. Presentations:

A formal presentation of the preliminary research progress findings were made at the RSA meetings in Vancouver in June, 2004 and at RSA in June of 2005.

Since FAS and FASD have not been widely accepted diagnoses and 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 and publication of Wave I data (approximately April to June of 2006).

Progress Report on Pilot Project: UCSD

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

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

III. Objectives:

To identify early markers of prenatal alcohol exposure using standard 2-D prenatal ultrasounds.

IV. Methods:

At four specific time periods during pregnancy, standard 2-D prenatal ultrasound studies will be performed on 80 moderate/heavy drinking pregnant women and a similar number of minimal drinking/abstinent controls. The ultrasound studies will all be transferred electronically to Dr Andy Hull, the prenatal ultrasonographer at the University of California, San Diego (UCSD) who will review each for accuracy. Following delivery, all 160 infants will be examined by specially trained neonatologists and /or geneticists at the delivery hospitals in order to ascertain the status of the infant ie. FAS, Deferred or No FAS. Photographs of each newborn infant will be taken and those felt to have FASD will be evaluated by one of the "expert" dysmorphologists.

V. Accomplishments and Results:

The IRB in Kiev has approved the study as of March 2005 and things are moving ahead. Pilot studies of alcohol consumption by pregnant women have been completed in both Rivne and Kherson. Dr. Lyubov Yertushok, the P.I. of the study in Rivne has met over the last month with all obstetricians in the region to encourage their participation in the study and to recruit moderate/heavy drinking pregnant women. In early April, Dr. Chambers and Dr. Jones met in Kiev with Dr. Ihor Baryliak, the P.I. of the overall study in Ukraine to discuss financial issues, as well as coordination of the project in the two different sites. Dr. Jones met in both Rivne and Kherson with ultrasonographers, neonatologists and geneticists to discuss any problems they had encountered and with Dr. Yevtushok in Rivne and Dr. Onischenko in Kherson to designate a specific individual at each site to enter data, and anticipate and discuss difficulties. While in Rivne, a press conference was held and was attended by numerous members of the press (both newspapers and TV) to publicize the study. Again, in September Dr. Jones and Dr. Chambers met in Kiev with investigators from both Rivne and Kherson to discuss logistic issues relating to recruitment of pregnant women, prenatal ultrasonography, and examination of the prenatally exposed children as well as the controls.

As of November 30, 2005, the following results are available from the two sites in Ukraine: From Medical Genetics Departments: 2,666 women screened, 92 (3.5%) moderate/heavy drinkers, 35 women agreed to take part in study From Prenatal Clinics in Rivne and Kherson: 1140 women screened, 53 (4.5%) moderate/heavy drinkers, 13 women agreed to take part in study From Prenatal Clinics in Rayons: 1781 women screened, 24 (1.3%) moderate/heavy drinkers, 7 women agreed to take part in study **TOTAL**: **5587 women screened, 169 moderate/heavy drinkers, 55 agreed to take part in study as subjects, 55 agreed to take part in study as controls.**

For women in the risk group and the control group, prenatal ultrasounds were performed and videos were taken. These will be sent to Dr. Andy Hull in San Diego over the next few months for his evaluation. As of October 2005, five children of mothers from the moderate/heavy drinkers group have delivered and the children have been examined by both local neonatologists and geneticists, and photos were taken. One of these five children have features of fetal alcohol syndrome.

VI. How this Project Interrelates with the CIFASD:

This project interrelates to the aims of the Consortium by attempting to provide early identification that a baby has been affected prenatally by alcohol in order to get that child into remedial programs at a much younger age. It also interrelates with the project in Moscow (Risk Factors for FASD in the Moscow Region) in that prenatal ultrasonography is also being done in that project.

VII. Plans for the Next Year:

This project will be completed by August 2006. Dr. Jones and Dr. Chambers will make a final trip to Ukraine in May 2006 to validate examinations performed by the neonatologists and geneticists, to meet with project coordinators in Rivne and Kherson, and to begin analysis of the data.

VIII. Publications:

A manuscript documenting the screening interviews will be completed by May 2006. The lead for writing this will be taken by the Ukranian investigators.

IX: **Posters and 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:

The primary objectives of the Dysmorphology Core are as follows:

1. To insure consistency as well as accuracy in diagnosis of FASD at all project sites.Implementation of a standard protocol at each project site.
2. To explore the extent to which various degrees of deficient anthropometric measurements should be used to enhance specificity of diagnosis without loss of sensitivity.
3. To explore possibilities for diagnosis of FASD in the newborn period.
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 clinical diagnosis of FASD with that determined by 3-D photography.

IV. Methods: To perform careful physical examinations, blinded to prenatal alcohol exposure, of each child ascertained at all consortium sites.

V. and VI. Accomplishments, Results and Discussion:

Objective 1: To insure consistency as well as accuracy of FASD at all project sites.

Total data obtained from 8 sites (N=1130 as of December 2005)

- **Clinical Site**	**N**
Luther Robinson, Buffalo	93
Phil May, Rome	545
Sara Mattson, San Diego	74
Sarah Mattson, Moscow	108
Sarah Mattson, Finland	150
Sandra Jacobson, South Africa	151
Tina Chambers, Moscow Region	5
Ann Streissguth, Seattle	14
TOTAL	**1130**

Diagnosis by site

Buffalo: FAS: 23 No FAS: Deferred: 12 Williams syndrome: 12 Controls: 46	Moscow Region: FAS: 0 No FAS: 5	Seattle: FAS: 1 No FAS: 8 deferred: 5
Finland: FAS: 77 Controls: 70	Rome: FAS: 22 No FAS: 456 Controls: 67	South Africa: Diagnosis not yet verified
Moscow: Diagnosis not yet verified	San Diego: FAS: 39 Controls: 35	

Objective 2: To explore the extent to which various degrees of deficient anthropometric measurements should be used to enhance specificity of diagnosis without loss of sensitivity.

This is in the process of being analyzed using new data.

V. and VI. Accomplishments, Results and Discussion: (continued)

Objective 3: To explore possibilities for diagnosis of FASD in the newborn period.

By virtue of the fact that this objective requires a substantial lead time to allow ascertainment of pregnant women, followed by a requisite gestational period, only over the last few months have the first babies born in the two prospective studies (the Moscow Region and the Ukraine studies) been born. The focus of the Dysmorphology Core's activities over the next year is to focus on this objective.

Objective 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.

This is presently being analyzed in the Finish data.

Objective 5: To correlate clinical diagnosis of FASD with that determined by 3-D photography.

This is presently being analyzed.

VII. Interrelation with Aims of the Consortium and Other Projects:

This project interrelates to the aims and other projects within the Consortium by beginning to establish the full spectrum of defects that make up the FASD. This will more effectively be accomplished when data start becoming available from the prospective studies that are part of the Consortium.

VIII. Plans for the Next Year:

Over the next year, we will continue to see all children identified at all Consortium sites. Our focus however will be on the prospective study in the Moscow Region,

IX. Publications:

Hoyme H.E., May P.A., Gossage J.P., Findlay R., Autti-Ramo I., Fagerlund A., Ceccanti M., Fiorentino D., Viljoen D., Khaole N., del Campo M., Jones K.L., Robinson L.K. Concordance/discordance of twins prenatally exposed to maternal alcohol abuse: A classic example of the role of gene-environment interactions in the etiology of birth defects. David W. Smith Workshop on Malformations and Morphogenesis. Iowa City, Iowa. August 2-6, 2005.

Robinson L.K., Wrotniak B., Smistek B., Moore E.S., Riley E.P.. Fetal Alcohol syndrome: Comparison of clinical findings in phenotypically similar disorders. David W. Smith Workshop on Malformations and Morphogenesis. Iowa City, Iowa. August 2-6, 2005.

AuttiRamo I., Fagerlund A., Ervalahti N., Loimu L., Korkman M., Hoyme H.E.. Fetal Alcohol Spectrum Disorders in Finland: Clinical delineation of 77 older children and adolescents. Amer J Med Genet 140A: 137-143, 2006.

May P.A., Fiorentino D., Gossage J.P., Kalberg W.O., Hoyme H.E,. Robinson L.K., Coriale G., Jones K.L., del Campo, Tarani L., Romeo Mkodituwakku P.W., Deiana L., Buckley D. The Epidemiology of FASD in a Province in Italy: Prevalence and Characteristics of Children in a Random Sample, In-School Study. Alcoholism: Clinical and Experimental Research. submitted

Bakhireva L., Jones K.L., Robinson L.K,. del Campo M., Riley E.P., Mattson S.N., Marintcheva G., Chambers C.D. Effectiveness of training pediatricians to diagnose the fetal alcohol syndrome. Pediatrics. submitted.

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:

As of this time, we have made progress in numerous aspects of the proposed function of the Imaging Core for the CIFASD since our last progress report. We have completed our development of functional MRI activation tasks to target brain regions of particular interest in the FASD population. We have begun to study FASD subjects recruited into our prospective imaging studies at UCLA, and we have begun to discuss collaboration on image analysis from data collected by Sarah Mattson, Ph.D., and the San Diego group. Dr. David Shattuck has continued to develop image analysis tools for use on the FASD Consortium imaging data, and we have conducted validation studies to assess the feasibility of using these new tools using data collected by us on FASD and control subjects. Below we describe 3 preliminary studies of several conducted thus far that illustrate our progress. The first study shows the validity of tools developed by Dr. David Shattuck relative to more cumbersome image analysis tools used previously by the CIFASD Imaging Core. The second shows correlations between brain activation and post-test recognition performance in control children during a verbal learning task. This study is described to demonstrate the methods that will be used to evaluate relationships between brain activation and cognitive test performance in the FASD subjects. The third study shows brain activation abnormalities in FASD subjects during the same verbal learning task.

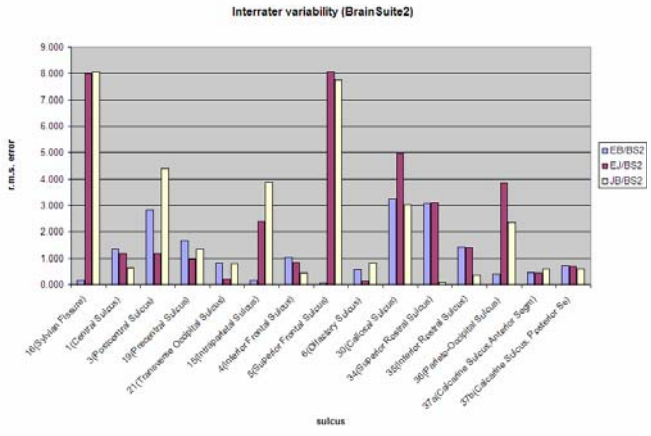


Figure 1: Interrater variability for BrainSuite2.

CIFASD Image Analysis Tool Validity:
This study was conducted to assess both the validity and interrater reliability of the cortical sulcal line delineation used to match cortical patterns across individuals. Three raters (E, B, and J) defined cortical anatomy on one subject using 2 different image analysis software packages. One of the packages, BrainSuite2, was developed by Dr. Shattuck, and the other, Display, was developed by engineers at the

V. Accomplishments and Results: (continued)

Montreal Neurological Institute. As illustrated below, the reliability of the BrainSuite2 Software is quite good, and considerably improved over that of the older Display Software used by the Imaging Core. The increased reliability provides confidence in the use of the tools which also provide a vast improvement in image analysis time for each subject (BrainSuite2 cuts image analysis time approximately in half).

In Figure 1, the graph shows the distance between sulcal points for each rater compared to each of the other raters for each of 15 sulci traced and the numbers were computed as follows:

1.) We had P x Q x R curves, where P = # of programs (2), Q = # of curves(15), R = # of raters(3), represented here as C(p,q,r). 2.) For each pair of raters r1 and r2, and for each landmark, qn, we computed the variance of the minimum distance (MD) between each point on the curve C(p,qn,r1) and the set of points on the corresponding curve C(p,qn,r2) traced by the other rater. Since this metric is not symmetric for r1,r2, we took the average of it, as this is then the variance of the minimum distances between both C(p,qn,r1) and C(p,qn,r2). 3.) We plotted the square roots of these numbers (the RMS MD) for p = BrainSuite2 (graph shown in Figure 1) and p = Display (not shown). EJ/EB/JB reflect comparisons between raters E and J, E and B, and J and B. Generally speaking, these results show that BrainSuite2 produces lower variance for most cases.

Verbal Learning and Memory Task in Normal Children:

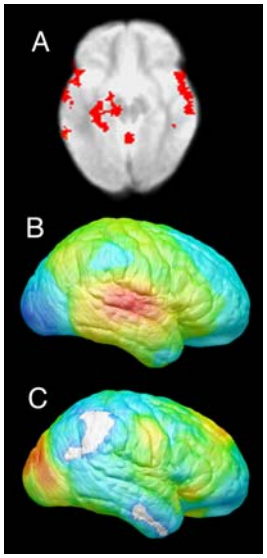


Figure 2: **A.)** FSL Activation map showing average activation for the Learning vs. Rest contrast showing left hippocampal activation. **B.)** Average group activation for the Learning vs. Rest contrast created with LONI Cortical Pattern Matching. **C.)** Correlation map showing the relationship between activation and post-test performance on the learning task (significant in white, right parietal region).

Nine normally developing children between ages of 7 and 15 (mean 11, SD 3, 5 male) were studied as controls for the FASD subjects engaged in a verbal paired associate learning paradigm. Learning-rest and recall-rest contrasts with cluster threshold of Z>2.3 were rendered using FSL. Functional activation volumes for the 2 contrasts were co-registered to high resolution structural images obtained from a Siemens 1.5T magnet. Cortical anatomy was defined in all subjects using tools developed by Dr. David Shattuck, a co-investigator on this project, and cortical pattern matching methods developed by the Laboratory of Neuro Imaging (LONI) were used to match homologous anatomy in both the functional and structural data sets within and across subjects. Statistical and group average maps were created to assess relationships between activation and performance on a post scan recognition test. During cued recall, activation was seen in bilateral medial premotor, inferior frontal, superior temporal cortices, cuneus and left hippocampus (Fig. 2A). In contrast to activation patterns expected based on the adult literature, there was a lack of activation in dorsolateral frontal and parietal cortices (Fig. 2B). The lateralized left hippocampal activation was consistent with a priori hypotheses. A post hoc analysis to explore the relationship between activation during recall and actual recall performance post scan revealed significant negative correlations in the right parietal lobe (uncorrected; see Fig. 2C) where children who recalled fewer word pairs had greater activation. Activation of the left hippocampus confirmed the lateralization of function of this structure in verbal learning during childhood. The lack of activation in frontal and parietal areas may reflect children's use of immature encoding or retrieval strategies. This speculation is consistent with immature executive function in children, and may be related to maturational structural changes that frontal and parietal lobes undergo during the first two decades of life. Poorer recall was associated with activation of the right frontal areas in adults while it is associated with right parietal activation in the present study. These are the same image analysis methods, and the same functional activation task that will be used with increased sample sizes in FASD subjects.

V. Accomplishments and Results: (continued)

Hippocampal Activation Abnormalities During Verbal Learning in FASD Individuals

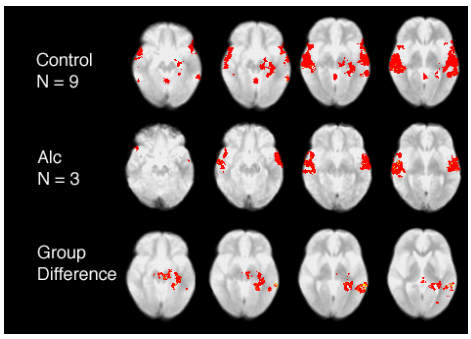


Figure 2: Functional activation maps for control and alcohol exposed children during a the learning vs. rest contrast. Note the complete lack of hippocampal activation in the Alc children.

Three alcohol exposed and 9 age and gender-matched control children had been studied at the time of this analysis. The control children are a subset of those described above, and the task and image analyses for within group averages are also the same as described above. Note the prominent left sided hippocampal activation during verbal learning in the control children, and the complete lack of hippocampal activation in the 3 alcohol exposed children. Both groups show the expected bilateral temporal lobe activation for the auditory verbal task. The group difference shows the statistical significance of decreased hippocampal activation in the alcohol exposed children. More subjects are currently being studied, and if the results maintain their current pattern, these will be very intriguing results in the previously observed neurocognitive pattern of poor verbal learning and memory in FASD subjects.

VI. Integration with Other Cores:

The neurobehavioral cluster, comprising the neurobehavioral and neuroimaging cores will be active in assessing the relationship between brain and behavior variables in our population of children with FASD across sites (San Diego and UCLA, currently). Once more data are collected, we will assess for relationships between the data collected and analyzed within the two cores, using existing statistical tools. 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 thickness, brain activation) at every brain surface point and any measure collected by the various other projects and cores (i.e., neurobehavioral data). The results from the 2nd study described above illustrate the existence of appropriate image analysis tools and our ability to implement them for imaging and neurobehavioral data being collected.

VII. Plans for the Next Year:

During the next year we plan to continue data collection at the UCLA and San Diego locations. Further work will continue on software development in the BrainSuite2 package. We will facilitate installation of image analysis software for use by the San Diego group and support its use. Data analysis will continue during the next year as is consistent with the original specific aims of the Imaging Core.

VIII. Publications

Manuscripts Published:

O'Hare E.D., Kan E., Yoshii J., Mattson S.N., Riley E.P,. Thompson P.M., Toga A.W., Sowell E.R. Mapping cerebellar vermal morphology and cognitive correlates in prenatal alcohol exposure. Neuroreport, 2005 16:1285-1290.

Riley E.P., McGee C.L., and Sowell E.R. Teratogenic effects of alcohol: a decade of brain imaging. American Journal of Medical Genetics Part C, Semin. Med. Genet., 2004, 127C:35–41.

Manuscripts In Preparation:

Shattuck D.W., Sowell E.R., Jolley J., Kan E., Dutton R., and Toga A.W.. Assessing the Validity and Reliability of BrainSuite2. (in preparation)

VIII. Publications: (continued)

Abstracts:

Lu, L.H., O'Hare, E.D., Bookheimer, S.Y., Kan, E., McCourt, S.T. and Toga, A.W. Mapping activation during verbal learning and recall in normally developing children. In preparation for presentation at the 12th Annual Organization of Human Brain Mapping Meeting, Florence, Italy.

O'Hare, E.D., Kan, E., Yoshii, J., Mattson, S.N., Riley, E.P., Thompson, P.M., Toga, A.W. and Sowell, E.R. Cognitive Correlates of Anterior Cerebellar Vermal Dysmorphology in Heavy Prenatal Alcohol Exposure. Program No. 359.14. 2004 Abstract Viewer. Society for Neuroscience, 2004. Online.

O'Hare, E.D., Kan, E., Yoshii, J., Mattson, S.N., Riley, E.P., Thompson, P.M., Toga, AW and Sowell, ER. Mapping Cerebellar Vermal Abnormalities in Severe Prenatal Alcohol Exposure: Localized Dysmorphology of the Anterior Vermis and Primary Fissure. 10th Annual Meeting of the Organization for Human Brain Mapping, Budapest, Hungary. NeuroImage Abstracts, 2004.

Progress Report Facial Imaging Core

I. Principal Investigator and Key Personnel: Tatiana Foroud, Ph.D. – Principal Investigator, Elizabeth Moore, Ph.D. – Co-Investigator, Richard Ward, Ph.D. – Co-Investigator, Shiaofen Fang, Ph.D. – Co-Investigator, Jeffrey Huang, Ph.D. – Co-Investigator

II. Title of Project: Facial Imaging Core

III. Objectives:

The goal of this core is to analyze 3-D images created from laser scans of individuals of variable ethnicity, age and history of alcohol exposure provided by each of the participating study sites. Analyses of 3-D facial images will be developed and utilized for more effective clinical diagnosis of FAS, as well as the more broadly defined FASD. In addition, these studies will generate important insight regarding the changes that occur in the face both prenatally and postnatally that produce the clinical features associated with FAS and thereby provide improved understanding of the pathophysiological effects of ethanol on human development.

IV. Methods:

Data for the Facial Imaging Core is collected at the CIFASD sites. Facial imaging data has been collected from subjects in Buffalo, Finland, San Diego and South Africa. Dr. Elizabeth Moore is responsible for meshing the separate subject images and generating a 3-D image. Dr. Moore then takes a series of indirect anthropometric measurements on each 3D image. Once completed, a shaded rendering of the 3-D images and an xml file with the measurements are stored on a secure server accessible to the other investigators. These images are then used for a number of different analyses performed by various core investigators.

V. Accomplishments and Results:

Specific Aim 1: Train and supervise personnel at each recruitment site to ensure collection of standardized data.

Training has been completed for all site personnel in Buffalo, Finland, San Diego and South Africa. Protocols are in place to ensure that training of future collection sites will occur prior to shipping the camera. All data collected from each new site is reviewed with very regular frequency during the initial stages of collection to ensure that any difficulties with data collection are resolved quickly and efficiently without compromising the project.

Specific Aim 2: Analyze the 3-D facial imaging data to identify the measurements that most efficiently differentiate alcohol exposed from control subjects.

The focus is currently on pursuing two different hypothesis. The first is developing an FAS facial profile which will be compared across the races. Using data from Finland and the United States, differences among Caucasians are being delineated. These results are then being contrasted with the facial profile developed from FAS subjects from South Africa who are mixed race. The second hypothesis being tested is the comparison, again across ethnicity, of the facial features that distinguish FAS and controls.

Initial methods and results were presented in the poster session for the 2005 meeting of the Research Society on Alcoholism: Fetal Alcohol Syndrome and 3-D Facial Imaging: A Preliminary Classification Study, Flury L.; Roger J.; Foroud, T.; Robinson L., Moore, E.

Another abstract was presented at the poster session for the 2005 International Genetic Epidemiology Society meeting: Fetal Alcohol syndrome and 3-D facial imaging: A preliminary classification study. Flury L.; Roger J.; Foroud, T.; Robinson L., Moore, E.

Abstracts are currently being prepared for submission this week to the 2006 meeting of the Research Society on Alcoholism. These include: 1.) Facial features associated with FAS using 3-D facial imaging; and 2.) Facial features distinguishing FAS from controls using measures from 3-D facial imaging.

V. Accomplishments and Results: (continued)

Specific Aim 3: Utilize algorithms and methods derived from the emerging field of Automated Facial Recognition (AFR) to extract and identify the most discriminating higher order surface features from 3-D facial images, with the goal of developing an automated method of identifying facial features diagnostic of prenatal alcohol exposure.

Analyses performed to address this specific aim have already been initiated. New advanced geometry analysis techniques have been developed and specifically applied to the classification of subjects with FAS.

These methods and results have been submitted as a manuscript: Fang S., Fang J., Huang J., Tuceryan M., Moore E., Robinson L., Mattson S.N., Ramo I.A., Foroud T., Riley E.P., Li, T.K. Digital Geometry Image Analysis for Medical Diagnosis. IEEE Transaction on Visualization and Computer Graphics.

Further analyses are being proposed to address automatic FAS diagnosis technique using 3-D facial image analysis. This is basically the automatic method documented in the IEEE paper. We take all the points on a face and then use an automatic filtering method to select a subset that exhibit the greatest difference between FAS and non-FAS faces. This subset will then be treated as a feature vector that is analyzed using a Support Vector Machine technique and a Radial Basis Function Network technique. The success rate here is between 89% to 95% (under different validation tests and analysis methods).

A second set of analyses are being prepared using visualization to assist facial feature analysis for FAS diagnosis. This is a new technique we are working on right now, with some success. The idea is to first visualize a "difference map" on the face to show significance of "differences" between a group of FAS faces and a group of non-FAS faces. Based on this visualization, the user can then select the regions on the faces that have more significant "differences". Various geometric features will then be computed on these regions for feature analysis, using similar feature analysis methods. The success rate so far is between 87.5% to 90%. The advantage here is that the results are more intuitive as it explicitly regions on the face that FAS-sensitive.

Abstracts are currently being prepared for submission this week to the 2006 meeting of the Research Society on Alcoholism. These include: 1.) An automatic FAS diagnosis technique using 3-D facial image analysis; and 2.) Using visualization to assist facial feature analysis for FAS diagnosis.

Specific Aim 4: Combine the results from the direct and higher order measurements derived from the 3D facial imaging with variables collected from other study domains to improve the power to accurately discriminate alcohol exposed from control subjects and to better understand the pathophysiological effects of ethanol on human development.

We have not yet begun analyses of Specific Aim 4. We need to first complete analyses of the existing facial imaging data and then proceed to more sophisticated, higher order analyses.

VI. Discussion:

The core has made excellent progress working with the CIFASD sites to increase the rate and quality of data collection. Data collection is complete in Finland and Buffalo. Data collection continues in South Africa and San Diego. Analyses are actively being pursued. Issues remain regarding obtaining critical data from the data repositories. However, these issues are being resolved.

VII. How this project interrelates with the aims of the Consortium and with other projects within the Consortium:

The Facial Imaging Core has interacted with each of the projects collecting data to determine which sites were interested and able to collect facial imaging data. More focused interaction has occurred with those sites collecting facial images. This core has also relied heavily on the Informatics Core which stores the meshed, shaded 3-D images and the anthropometric measurements.

VIII. Plans for the Next Year:

Data collection will continue in South African and San Diego. It is still to be determined where the camera will be placed for a long period of time. The focus of the coming year will be data analysis.

IX. Publications: (In print, in press, accepted, submitted that cite the Consortium funding.)

Amit J., Huang J., Fang, S. and Riley, E.P. Using Facial Images to Diagnose FetalAlcohol Syndrome (FAS), In Proc. of IEEE International Conference on Information Technology: Coding and Computing, ITCC, 2005.

Fang S., Fang J., Huang J., Tuceryan M., Moore E., Robinson L., Mattson S.N., Ramo I.A., Foroud T., Riley E.P., Li, T.K. Digital Geometry Image Analysis for Medical Diagnosis. IEEE Transaction on Visualization and Computer Graphics, submitted.

X. Posters and Presentations: (Please include one copy of any published abstracts.)

Flury, L., Rogers; J., Foroud, T., Robinson, L., Moore, E.. Fetal Alcohol Syndrome and 3-D Facial Imaging: A Preliminary Classification Study. Poster at Research Society on Alcoholism. Santa Barbara, CA, June 2005.

Progress Report Neurobehavioral Core

I. Principal Investigator: Edward P. Riley, Ph.D.

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.

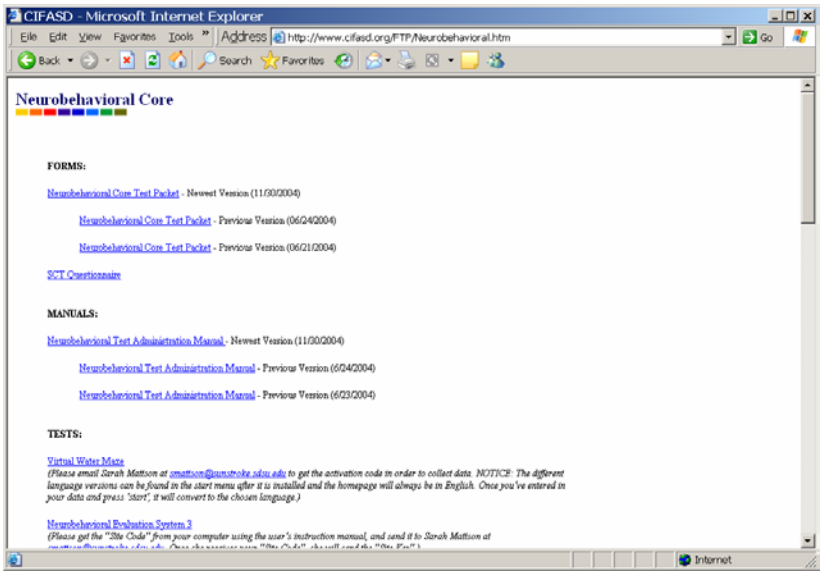
The goals of this Neurobehavioral Core are to assist individual sites in the appropriate neurobehavioral assessment of FASD and to provide for common behavioral assessment instruments to be used across sites so that converging data can be identified. With the realization that the neurobehavioral profile 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:

1. 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.
2. 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.
3. 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 battery 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.

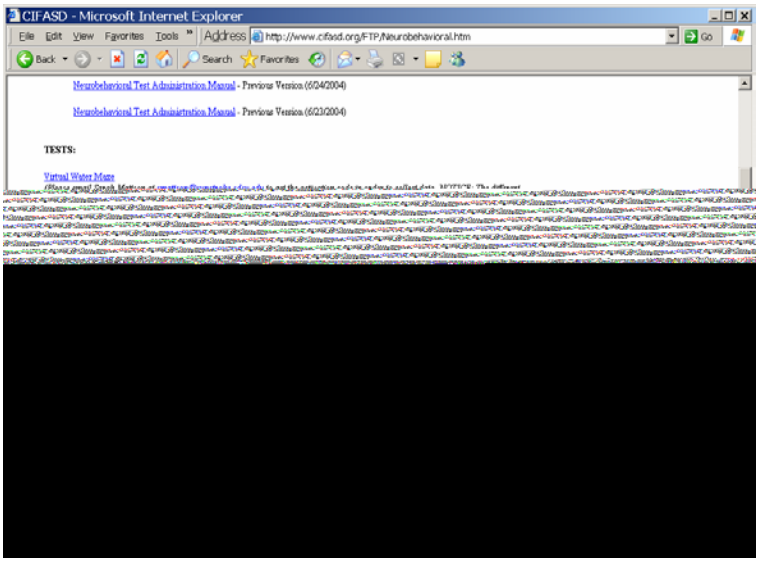


V. Accomplishments and Results:

1. Developed a test manual and placed it on the web.
2. Placed all of the tests on the web.
3. Provided translation services for the test battery.
4. Developed database to be implemented by informatics core. A guide to using the neurobehavioral database is being created and is being distributed.
5. Video clips of each test are available on the website.
6. Distributed updated Administration Manual and Test Packet.

V. Accomplishments and Results: (continued)

7. A Scoring Manual has been created and is being distributed.
8. The creation of Helpful Hints sheets: For each test is a one-page sheet filled with helpful hints and answers to questions many sites have asked.
9. The creation of a FAQ sheet for each test.
10. Conducted one training for two testers from the University of New Mexico.



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:

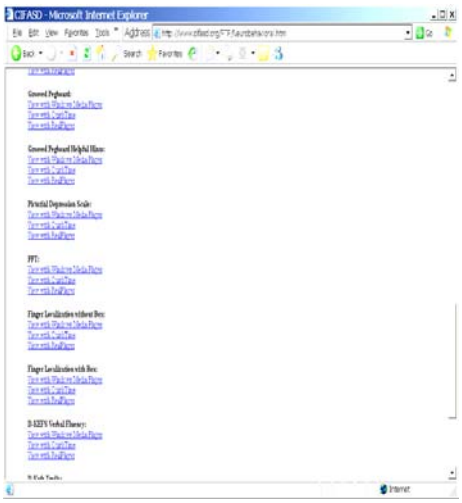
The Neurobehavioral Core will continue to provide test materials and technical support to each of the sites. We are also planning to monitor data reliability and validity by reviewing videotapes and test packets from 5% of all protocols administered. The methodology for the procedure is outlined in the appendix.

IX. Publications:

Not applicable.

X. Posters and Presentations:

Not applicable.



Progress Report from San Diego and Moscow

I. Principal Investigator: Sarah N. Mattson, Ph.D.

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 (USA, Russia, Finland) and neuroimaging studies in two (USA and Finland).

IV. Methods:

There are three sites for this project: San Diego, CA, USA, Moscow, Russia, and Helsinki, Finland. At all sites, children with FASD and controls are being recruited and assessed using four components of the Consortium: dysmorphology, 3-D photography, neurobehavior, and neuroimaging. The neuropsychological assessment tools specified by the neurobehavioral core are being used in all three sites. The neuroimaging protocol is specified by the Neuroimaging Core.

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. Children in Helsinki were ascertained from a clinical patient pool in the Hospital for Children and Adolescents, University of Helsinki. Controls for the three sites are derived from the appropriate population at each site. In Moscow, they are selected from the same orphanages and boarding schools as the children with FASD. In San Diego, they are selected from the pool of participants followed by the CBT. In Helsinki, there is a control group (CON) and a contrast group (CONT). These groups differ in that the CONT group will have a comparable cognitive level as the FASD group. Given our control and contrast groups, we have the unique opportunity to examine the relationship between FASD, IQ, and living environment in large groups of children. The complete progress report for the Finnish site is included as an appendix to this progress report, however, where possible, a summary of all three sites is presented.

V. Accomplishments and Results:

San Diego Recruitment: We have been collecting data for nearly 18 months. In that time, we have recruited 95 children for participation. The details of this sample are as follows:

San Diego					
	N	Age	%Female	%Hispanic	%White
FASD	58	13.02 +/- 3.05	44.83	18.97	58.62
CON	37	12.09 +/- 2.78	45.95	21.62	72.97

Of these children, 76 have been tested (39 FASD, 37 CON), 74 (47 FASD, 27 CON) have been examined for the Dysmorphology Core, 67 (32 FASD, 35 CON) have had 3-D and 2-D photos taken, and 29 (13 FASD, 16 CON) have had an MRI.

Helsinki Recruitment: Our Finnish collaborators have been collecting data for several months. In that time, 150 children have been identified or recruited for participation. The details of the tested sample have not changed and are as follows:

Finland					
	N	Age	Sex	Ethnicity	Race
FASD	76	13.3 +/- 3.6	45 F/31 M	Finnish	White
CON	40	13.5 +/- 3.6	22 F/18 M	Finnish	White
CONT	30	13.3 +/- 2.9	16 F/14 M	Finnish	White

Of these, most have had 3D photos taken, 140 (70 FASD, 40 CON, 30 CONT) have been tested, 150 (80 FASD, 40 CON, 30 CONT) have been examined for the Dysmorphology Core, and 20 (10 FASD, 10 CON) have had MRS examinations. Four children in the FASD group remain to be tested. Otherwise data collection is complete.

V. Accomplishments and Results: (continued)

Moscow Recruitment. Data collection was slow to start in our Moscow site because the contracts were not signed until April of 2005 and no testing can be conducted during the summer. However, the psychologists have been trained on the testing battery and recruitment has begun. Previously identified children in targeted orphanages comprise the potential recruitment pool for this study. All children will be examined by the dysmorphology pool and examined using the neurobehavioral core battery and hopefully have 3-D pictures taken. This sub-project took longer than expected to get started, and data collection has been slow. As of 31 December 2005, 35 children have been tested using the neurobehavioral core battery and 108 children have been examined by the Dysmorphology Core (Jones/Robinson/Del Campo). We are in the process of entering and verifying these data into the dysmorphology database.

Neurobehavioral Testing. Preliminary examination of the neurobehavioral data collected from 73 children (37 with FASD and 36 Controls; 3 were excluded from analysis) in San Diego and children from the Finnish and Moscow sites. The data from Helsinki (27 with FASD and 25 Controls) and Moscow (10 with FASD and 5 Controls) are the same that were available at the last progress report. All data were collected using the battery suggested by the neurobehavioral core and some preliminary results are included in Appendix 2.

MRI Evaluations: In San Diego, we have evaluated 29 children using MRI and data analysis is underway.

3-D Facial Imaging: In San Diego, we received the 3-D camera at the beginning of April 2005. Since that time, we have evaluated 67 children and transferred these data to Elizabeth Moore.

The 3-D camera was in Finland since last summer and was recently been returned to the imaging core. In that time, nearly 150 children were been examined.

We were hoping to get a 3-D camera to Moscow in the Fall, once data collection is stable and consistently occurring. However, since this is not yet the case, no plans are in place, although we are still hopeful it will be possible.

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. In Helsinki, data collection is nearly complete. In San Diego, we plan to continue collecting data over the next year. The Moscow site remains slow but has great potential for the next year to obtain a large amount of data. With these three sites, we feel we have demonstrated our ability to collect data in varied locations.

VI. How this project interrelates to the aims of the Consortium and with other projects within the Consortium

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 function exists in this population. We have many strengths 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. The Helsinki project also included a contrast group matched for intellectual functioning. In the San Diego component, we are also collecting data that are pertinent to the aims of the Imaging Core.

VII. Plans for the Next Year:

During the next year, we plan to continue our data collection at 2 of our 3 sites. We will continue collecting 3-D photo, dysmorphology, and neurobehavioral data at the San Diego site, and dysmorphology and neurobehavioral data at the Moscow site. Data collection is complete at the Helsinki site, although much remains to be done in terms of scoring and verifying neurobehavioral data. We also hope to get a 3-D camera to Moscow. The neurobehavioral database is near completion and we plan to get all data entered from all three sites during 2006. The San Diego data are for the most part scored and verified and are waiting to be entered. The Helsinki data are partially scored; the remaining files need to be scored and all need to be verified and entered. The Moscow data are partially scored.

VIII. Publications: (In print, in press, accepted, submitted that cite the Consortium funding. Please include one copy of each).

Fetal alcohol spectrum disorders in Finland: Clinical delineation of 77 older children and adolescents. Autti-Rämö, I. , Fagerlund, Å., Ervalahti, N., Loimu, L., Korkman, M., Hoyme, H.E. *American Journal of Medical Genetics* Part A (to be published January 2006, html version can be read on line).

VIII. Publications: (continued)

Brain metabolic alterations in adolescents and young adults with fetal alcohol spectrum disorders. Fagerlund, A., Heikkinen, S., Autti-Rämö, I., Korkman, M., Timonen, M., Kuusi, T., Riley, E.P., Lundbom, N. (Under peer review in Alcoholism Clin Exp Res).

Relationship between dysmorphic features and general cognitive functioning in children with fetal alcohol spectrum disorders. Ervalahti, N., Hoyme, H.E., Fagerlund, A., Autti-Rämö, I., Loimu, L., Korkman, M. (to be sent for peer review in January-February).

IX. Posters and Presentations: (included in previous report):

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

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

Hoyme, H.E., Fagerlund, Å, Ervalahti, N, Loimu, L, and Autti-Rämö, I. Fetal alcohol spectrum disorders in Finland: Clinical delineation of 77 older children and adolescents and comparison with affected North American children. Paper presented at the Western Society for Pediatric Research February 2005.

We are planning on submitting an abstract to the 2006 RSA meeting, but it is not yet complete. It is an analysis of data from San Diego and Moscow.

Appendix 1

Finish Project:

Data acquisition:

MRS studies on ten FASD and ten control adolescents have been performed. Dysmorphological studies have been conducted on 80 FASD (some excluded from the Consortium project) 40 control and 30 contrast children and adolescents aged 8 to 20. This study included also the dysmorphology score of Aase. Neuropsychological studies have been performed on 70 FASD , 40 control and 30 contrast children and adolescents aged 8 to 20. For most of the children 3D pictures have been taken. Four FASD need yet to be tested neuropsychologically, these will be performed January-February 2006. Finish focus has been to evaluate the prevalence of secondary disabilities as well risk and protective factors in the lives of individuals with FASD using qualitative interviews, Life History Interview, developed for individuals with FASD, Child Behavior Checklist (CBCL) and the Vineland Adaptive Behavior Scale (VABS).

Consortium data entry:

All dysmorphological data has been forwarded to the Consortium project. Most of the 3-D pictures have been sent to the Dysmorphology Core. The rest will be sent early January.

The neuropsychological test results need to be double checked and corrected according to the new instructions. The checking procedure is expected to take up to two months, starting from early January. Expected date to start entering neuropsychological data into the Consortium files early March.

Own data analysis

The results of the MRS studies have been analyzed and a scientific paper has been written which has been sent for publication. The clinical usefulness of the Aase score and the consequences of using the new revised IOM criteria in the finish population have been analyzed and written in the form of a scientific paper already accepted for publication. The correlation between Aase score and IQ in the finish FASD population has been analyzed and an article is underway to be sent for peer review. The evaluation of how environmental factors affect emotional well-being and social competence on a long-term perspective in children and adolescents with FASD is also under way.

Current own publications:

Fetal alcohol spectrum disorders in Finland: Clinical delineation of 77 older children and adolescents. Autti-Rämö, I., Fagerlund Å, Ervalahti, N., Loimu, L., Korkman, M., Hoyme, H.E. American Journal of Medical Genetics Part A (to be published January 2006, html version can be read on line)

Brain metabolic alterations in adolescents and young adults with fetal alcohol spectrum disorders. Fagerlund, A., Heikkinen, S., Autti-Rämö, I., Korkman, M., Timonen, M., Kuusi, T., Riley, E.P., Lundbom, N. (Under peer review in Alcoholism Clin Exp Res).

Relationship between dysmorphic features and general cognitive functioning in children with fetal alcohol spectrum disorders. Nina Ervalahti, H. Eugene Hoyme, Åse Fagerlund, Ilona Autti-Rämö, Leena Loimu, Marit Korkman (to be sent for peer review in January-February).

Planned own publications:

Neuropsychologist, Åse Fagerlund will base her thesis on the results from the Finnish project. Her main interest is to compare the test results of the FASD children and the IQ matched contrast group. We have had several questionnaires in addition to the core neuropsychological tests. The tests results of the questionnaires will be correlated with some core neuropsychological tests for each study group. We shall ask the Consortium for permission to use some of the core tests for this part of our study. Neuropsychologist, Åse Fagerlund has just gone on maternity leave and the expected date for continuing the data analysis is Fall 2006.

Progress Report – South African Follow-up

I. Principal Investigator: Sandra W. Jacobson, Ph.D.

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. Data from that phase and from our 14-year longitudinal study of moderate-to-heavy prenatal alcohol exposure in Detroit point to arithmetic and executive function as two developmental endpoints that appear to be 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 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 170 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 during the first trimester of pregnancy); 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 based on a tentative diagnosis performed during infancy. 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 and Philip Zelazo at University of Toronto, respectively; eyeblink conditioning, which is being studied in collaboration with Mark Stanton at University of Delaware; and several 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 Dr. Nathaniel Khaole, a pediatrician trained by Dr. Kenneth Lyons Jones, and 3-dimensional digitized photos following the protocol developed by the CIFASD Facial Imaging Core were obtained this past fall. A blood sample obtained from each

IV. Methods: (continued)

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:

To date, we have completed the 5-year assessments of 101 of the children in this longitudinal cohort. Attrition to date has been remarkably low—5.6%, due primarily to 6 deaths and a few cases in which the family has moved away from Cape Town.

This Fall Eugene Hoyme, M.D., and Luther Robinson, M.D., two of the dysmorphologists from the CIFASD Dysmorphology Core, and Elizabeth Moore, Ph.D., a co-principal investigator on the CIFASD Facial Imaging Core, spent 2 weeks in Cape Town. We organized a 6-day clinic at a local church in Hanover Park, where many of the participating children live, in which 151 of the children from the cohort (76.6%) were assessed for dysmorphology and 3-dimensional facial imaging. Unlike previous clinics that have generally been held in the children's schools or at a hospital, this clinic involved our staff's transporting mothers and their children from all over the metropolitan Cape Town area to a central clinic site within a very short time period. Each child was evaluated using the standard CIFASD dysmporphology protocol by either Dr. Hoyme or Dr. Robinson, and two-thirds of the children were evaluated by both. Drs. Hoyme and Robinson met with the three PIs of this project: Drs. Sandra Jacobson, Christopher Molteno, and Joseph Jacobson, in a case conference at the end of every other clinic day to review the data obtained for each of the children and arrive at a tentative diagnosis. There was substantial agreement between Drs. Hoyme and Robinson on their assessments of all dysmorphic features, particularly palpebral fissure length and the philtrum and vermillion ratings. There was also substantial agreement between them and Dr. Khaole, who had already evaluated 71 of the children during their comprehensive 5-year evaluations, which are scheduled to coincide with the child's 5th birthday. Each child was also photographed by Dr. Moore using the 3-dimensional camera following the CIFASD Facial Imaging Core protocol and received a t-shirt with the study logo in appreciation for having participated in the clinic. Upon completion of the clinic, Dr. Sandra Jacobson reviewed the dysmorphology and growth measurements in relation to the maternal reports of pregnancy drinking, most of which had been obtained contemporaneously during pregnancy.

VI. Discussion:

The "gold standard" dysmorphological evaluation of the children in this cohort by two of the internationally most respected dysmorphologists was a critically important element of this project. Due to the difficulty of obtaining reliable FAS diagnoses, our reports of a very high incidence of FAS based on the tentative diagnoses made during infancy needed to be confirmed. The findings that we will be reporting based on these "gold standard" clinic data confirm that the rate of FAS is virtually as high in the relatively well-nourished urban Cape Coloured Cape Town population as among the rural residents of Wellington, South Africa. Dr. Sandra Jacobson is currently using the results from the clinic data to identify the neurobehavioral deficits that are most frequently seen in children meeting these gold standard facial and growth criteria for FAS and PFAS. Her preliminary analyses point to several key features that appear to be specifically associated with the FAS/PFAS diagnoses and are consistent with findings they have previously reported for alcohol-exposed children in their Detroit cohort. These neurobehavioral features have the potential to advance our ability to diagnose both FAS and ARND in infancy; that is, determine which of the children exposed to alcohol during pregnancy are affected in terms of their CNS function. Once the 5-year evaluations have been completed on the bulk of this cohort during this coming spring, a similar analysis will be performed to identify the neurobehavioral markers of FASD during the preschool period. The ability to better diagnose these disorders during infancy and early childhood will facilitate referral for appropriate early intervention for the affected children.

VII. Interrelation with Aims of the Consortium and Other Projects:

Our Cape Town cohort was the last of the CIFASD sites to participate in a diagnostic and 3-D photographic clinic (September-October 2005). Complete data from the dysmorphology clinic were submitted to the CIFASD central repository on December 22, 2005. Dr. Moore brought the facial imaging data with her directly from Cape Town to Indianapolis and is currently performing the required pre-processing procedures. Dr. Moore has informed us that our data set is the largest she has received from the various CIFASD projects, and we believe that the dysmorphology data set we have provided is probably one of the largest for the Dysmorphology Core, as well. Both data sets will be merged with those provided by other CIFASD projects for papers to be written by the dysmorphology and Facial Image Core investigators, respectively, in collaboration with the CIFASD project PIs. Because our cohort is the only one performing assessments on preschool-age children, it 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.

Because the children in this cohort have not yet begun formal schooling, 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.

VIII. Plans for the Next Year:

Data collection on 138 children will be completed by April 30, 2006. An additional 32 children will reach their 5th birthday gradually during the course of the following 12 months. We are trying to save funds from this year's budget to carry forward through April 30, 2007, to permit completion of the data collection. Once the data collection on the bulk of the cohort has been completed at the end of April 2006, 5-year data will be available on a sufficiently large number of children in each of the diagnostic groups to evaluate the neurobehavioral characteristics of FASD during the preschool period. Based on these findings, we are planning to submit an R01 application on June 1, 2006, to follow up these children at 8 years of age. We have already been funded by NIH/Fogarty to conduct a pilot study in preparation for this grant proposal.

IX. Publications:

As noted above, given the importance of assessing sufficient numbers of heavily exposed children to achieve adequate power, most of the analyses of the data are scheduled to be performed after the first 138 children have been evaluated. Last month, we published a report from the infant phase of this study linking pregnancy drinking to visual acuity at 6 months of age. This paper appeared in *The Journal of Pediatrics* together with an editorial commenting on its significance and substantial international press coverage including an op ed article in a prominent South African newspaper. A paper based on the meconium data from this cohort was already published in *The Journal of Pediatrics*, and we are currently working on a paper relating the 5-year diagnoses to the infant findings and another paper relating these diagnoses to the 5-year eyeblink conditioning data.

Carter, R.C., Jacobson, S.W., Molteno, C.D., Viljoen, D., Chiodo, L.M., & Jacobson, J.L. (2005). Effects of prenatal alcohol exposure on infant visual acuity. The Journal of Pediatrics, 147, 470-476.

X. Posters and Presentations:

Data from this project were reported in posters at the Research Society on Alcoholism meeting in Santa Barbara, CA, in June 2005 and in talks to the Nutrition Society of South Africa, Western Cape Branch in May 2005 and the American Society for Addition Medicine in Washington, DC, and the Fetal Alcohol Spectrum Disorder Conference in Cape Town, both of which were held in October 2005.

X. Posters and Presentations: (continued)

Croxford, J., Jacobson, J.L., Jacobson, S.W., Marais, A.S., Molteno, C.D., and Viljoen, D.L. (2005). Estimated peak blood alcohol concentration as an indicator of fetal alcohol risk. Alcoholism: Clinical and Experimental Research, 29, 228.

Jacobson, S.W., Stanton, M.E., Hay, A.M., Burden, M.J., Fuller, D.S., Molteno, C.D., Viljoen, D.L., and Jacobson, J.L. (2005). Impaired short delay eyeblink conditioning in children with FAS: Preliminary findings. Alcoholism: Clinical and Experimental Research, 29, 233.

Jacobson, S.W., Jacobson, J.L., Molteno, C.D., and Viljoen, D.L. (May, 2005). Drinking in pregnancy and fetal alcohol syndrome in Cape Town, South Africa. Nutrition Society of South Africa, Western Cape Branch, Medical Research Council, Cape Town, South Africa

Jacobson, J.L., Jacobson, S.W. (2005, October). Core neural deficits and effect modifiers of fetal alcohol spectrum disorder: Detroit and Cape Town research findings. American Society for Addition Medicine, Washington, DC.

Jacobson, S.W., Jacobson, J.L., and Molteno, C.D. (2005, October). Why do we need more research? Drinking in pregnancy and fetal alcohol syndrome in Cape Town, South Africa. Fetal Alcohol Spectrum Disorder Conference, Cape Town, South Africa.

Progress on International Neuropsychological Study of FASD

I. Principal Investigator: Philip A. May, Ph.D., Co-Investigator: Colleen Adnams, M.D., Piyadasa Kodituwakku, Wendy Kalberg, M.A.

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

III. Objectives:

The long term goal of this research is to delineate cognitive, behavioral 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 United States. 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 proposed 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 250 children using the core test battery that is being designed by a collaborative group of neuro-behaviorists under the guidance of the CIFASD Behavioral Core and analysis with 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 FASD and 100 controls for the South African study and 50 children with FASD and 50 controls in the Northern Plains. For the educational intervention, 80 children with FASD and 24 non-alcohol exposed control children are enrolled.

V. Accomplishments and Results:

Due to the collaborative nature of this project, parallel activities have been pursued over the past year in preparation for the core testing to begin. The Behavioral Core group of the CIFASD has completed development of the battery of tests that will be administered to the children targeted by this work scope. The test administration and scoring manuals have now also been developed. Simultaneously, project staff at UNM and at UCT have worked throughout the two years to initiate and finalize the following activities in preparation to begin the test administration and data collection:

1. IRB approval has been acquired and updated.
2. Subcontracts are ongoing between the University of New Mexico and University of Cape Town.

V. Accomplishments and Results: (continued)

3. Philip May, Ph.D. and Wendy Kalberg, M.A., CED made a site visit to South Africa in late September and early October 2005, to observe testing and refine training of local personnel and engage in progress meetings with site personnel working on the neuropsychological study.
4. Bernice Castle, M.A., is a Clinical Psychologist and Registered Psychometrist. She is overseeing the day-to-day activities of both the International neuropsychological study and the Multi-Method Intervention study in South Africa.
5. Sean September, M.A., Registered Psychometrist, has completed the neuropsychological testing of a total of 56 children with the CIFASD neuropsychological battery in South Africa. Of those tested, 33 are affected children (13 females and 20 males) and 23 are non-exposed controls (12 females and 11 males). The cohort of children participating in the South African study will be derived from participants in three waves of epidemiological studies in the same high-risk geographical community.
6. The New Mexico staff has begun to test children in the plains communities. To date, 21 children have been tested; 6 affected children and 15 control children have been assessed on the full battery.
7. Test battery validity video-tapes have been prepared by South Africa and the New Mexico staff. These tapes have been submitted to San Diego for review. In addition, Alfredo Aragon, Ph.D., and Jason Blankenship, Ph.D. will attend training in San Diego with Sarah Mattson, Ph.D., and Jill Van der Velde the week of January 9th, 2006.
8. Three clinics, dedicated solely to testing children on the neuropsychological battery in the plains, are being planned by the New Mexico staff. Those clinics will be held in February, March, and April, 2005. Bernice Castle from South Africa and Jill Vander Velde from San Diego have been asked to help with the testing for two of those clinic weeks. Ms. Castle will work with the project for one week in March. Negotiations are being completed for Ms. Vander Velde to work with the project for the week of April 17th.
9. David Buckley, M.A., has been designated as the contact person in New Mexico for data management for the neuropsychological study. He is interfacing with San Diego and Indiana regarding the data base readiness.

VI. Discussion:

VII. How this Project Interrelates to the Aims of the Consortium:

This project is collecting the core neurobehavioral data outlined by the CIFASD Behavioral Core personnel. All testing is being done according to the stated manual of administration procedures. A quality assurance and training conference is being held in January, 2006, in San Diego. Alfredo Aragon, Ph.D. and Jason Blankenship, Ph.D. will travel to San Diego for the two-day conference.

VIII. Plans for Next Year:

The South Africa team and the New Mexico team plan to complete all testing for the neuropsychological study by June, 2006. When the web-site is finalized, the New Mexico staff will begin to upload the neuropsychological data that is required.

IX. Publications:

No manuscripts have been prepared for submission to journals to date.

Multi-Method Intervention Study of FASD in South Africa

I. Principal Investigator: Philip A. May, Ph.D.**,** Co-Investigator: Colleen Adnams, M.D., Piyadasa Kodituwakku, Wendy Kalberg, M.A.

II. Title of Project. Intervention Study of FASD 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 variable (child's IQ, life stress, maternal education) on therapeutic outcomes.

IV. Methods:

This work scope targeted 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). In addition, a 5th group of 24 unexposed controls is included in the study. The analysis will focus on clinically, statistically significant improvements.

The University of Cape Town is collaborating with Stellenbosch University 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., (Western Cape Schools)
Petra Engelbrecht, Ph.D. (University of Stellenbosch)

The interventions are being administered by Anna Kotze, Speech and Language Therapist: Language and Literacy; Wilma Rossouw, M. Ed. Psych: Cognitive Control Therapy, and Ellick Williams M.Ed., and Rubin Adams: Parent Intervention.

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 both years of the project, the South African team has met regularly to design the pretest protocol, design and finalize intervention protocols, hire staff to drive the daily activities of the project, and oversee the first 10 months of the intervention activities. Bernice Castle, M.A., and Sean September, M.A., both provide expertise to both the Intervention Study as well as the Neuropsychological Study.

One hundred and three (103) children were recruited for the study. Ninety eight (98%) consent has been obtained for them. Baseline testing has been completed for all children who will be participating in the study. 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 and self-efficacy measures through questionnaires completed by parents and teachers. Parents in the family-intervention group also completed additional questionnaires and interviews. Finally, process measures of the participant's behavior are being collected through qualitative classroom observations.

V. Accomplishments and Results: (continued)

Some results of the preliminary (baseline) testing data for children with FASD are tabled below:

South African Intervention Cohort Pretest Results

Test	Mean age (mths) M F	Mean Mental Age (mths) (SD) Males	Mean Mental 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)
Math Add	115: 113	77.0 (12.0)	76.8 (11.1)
Math Sub	115: 113	77.4 (13.6)	72.6 (9.8)
Math 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

Comparison between the baseline results for children with FASD and the unexposed control group showed significant differences in all areas tested (language, reading, spelling and math). These data were presented in a poster at the 2005 RSA meeting.

V. Accomplishments and Results: (continued)

Treatment components have commenced for the three intervention methods, and 10 months of activity have been completed for the Cognitive Control therapy and Language and Literacy components. Language and Literacy interventions are being conducted two times a week for 30 minutes each session. Cognitive Control Therapy is being delivered for sessions of one hour once per week. The Parent Groups commenced later than the classroom interventions because many parents in this group are seasonal agricultural laborers who were unable to commit to intervention sessions at that time. There was significant attrition of parent participation in this intervention resulting in regularly low attendance numbers. These parents experience overwhelming personal and environmental constraints which progressively diminished participation in spite of significant practical support being provided to enable attendance at the sessions, and thus the intervention was terminated in June 2005 after 12 - 15 sessions. Final interviews, completion of questionnaires and group discussions will be undertaken with the Parent Group. The information and data will be analyzed to determine the psycho-social, economic and environmental factors contributing to the difficulty in sustaining this form of intervention in such a group. It is hoped that these data can inform future research endeavors and services in high risk communities overwhelmed by adverse social circumstances and poverty, and in which alcohol plays a negative role. With their consent, the Parent Group's children remain in the study, to undergo scheduled assessments at midpoint and at the end of the study.

The mid-intervention (9 - 10 months) re-assessment for the Intervention Study was completed on 16 December. The core Intervention battery has been administered to all children participating in the study, and scoring and data analysis is underway. The mid-intervention results will be presented at the 2006 CIFASD and RSA meetings in Baltimore.

VI. How this Project Interrelates with the CIFASD:

This is the only project within CIFASD which directly addresses interventions in children with FASD.

VII. Plans for the Next Year:

Intervention methods will continue through next year. The interventions will be administered for a period of 18 months after which a final assessment will be undertaken. All proposed methods will continue to be implemented.

VIII. Publications:

The baseline and preliminary data were presented, and a publication resulted from a symposium at the ISBRA Conference, Germany September 2004:

Stromland K, Mattson S.N., Adnams C.M., Autti-Ramo I., Warren K.R. Fetal Alcohol Spectrum Disorders: An International Perspective: Alcohol: Clinical Experimental Research 2005; 29: 1121-1126**.**

No other publications have been drafted to date. However, by the end of February of 2006, a manuscript will be produced which will summarize the results at midpoint (after 9 months) of the project interventions. Positive and statistically significant changes may already have occurred in the mean scores of the children in the Cognitive control group and also the Language and Literacy intervention.

IX. Presentations:

Colleen Adnams, M.D., presented the rationale, methodology and preliminary (baseline) data at the ISBRA conference in Germany in late September, 2004, in addition to a similar presentation to the CIFASD group at the June, 2005 RSA meetings.

Poster: Adnams, C, Kodituwakku PW, Kalberg W, Adams R, September S, Perold MD, Sorour, P, Kotze A, Castle B, Engelbrecht P, May P. Cognition and behaviour of children with FASD in South Africa: preliminary data from a pilot intervention study. Scientific Meeting of the Research Society on Alcoholism, 25-30 June 2005, Santa Barbara, USA. Alcohol Clin Exp Res 2005 :29, 5. The mid-intervention assessment findings will be presented at the 2006 RSA meeting.

Progress Report from Moscow Neonatal Project

I. Principal Investigator: Tina Chambers, Ph.D.

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

III. Objectives:

1. 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.
2. 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 on 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:

Since January, 2005, Report

1. Bayley training continues with on-site consultant; two all-day sessions held with psychologists May 26, and June 7, 2005.
2. Completion and translation of data abstraction form and data dictionary for ultrasound measures to be used in Moscow and Ukraine; completion and translation of second interview and outcome abstraction forms; additional screens added to expanded alcohol and control database for nutritional measures and biomarkers of exposure.
3. Data collection and data entry continues for screening; database contains information on approximately 500 pregnancies.

V. Accomplishments and Results: (continued)

4. Analysis of data provide the following: 95% of pregnant women report being ever drinkers; approximately 22% qualify as exposed subjects; 33% as controls.
5. Recruitment of subjects for first maternal interview initiated.
6. Site visit to two Moscow nutrition laboratory facilities by Carl Keen – March, 2005.
7. Meeting with CRDF staff in Moscow to organize consulting and arrangements for collaborations with other institutions in Moscow.

VI. Discussion:

Since January, we have set up subcontracts with two nutrition laboratories and with collaborators at the Friendship University, and site visited these laboratories to determine if capacity to store samples and perform required procedures.

Screening results in the Moscow study sites continue to indicate that even if subjects are under-reporting alcohol use, that the proportion of pregnancies eligible for recruitment support a more rapid pace of enrollment than originally projected, although we anticipate requesting an unfunded fourth year extension for this project.

VII. Interrelation to the Aims of the Consortium and to Other Projects in the Consortium:

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 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 continue 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 well-advanced in competence to perform the one-year assessments.

IX. Publications:

We are in the process of preparing a manuscript describing the alcohol consumption patterns and other demographics of the screened sample data collected to date.

X. Posters and Presentations:

None.

Progress Report from Basic Science: Therapeutic Agents

I. Principal Investigator: Charles R. Goodlett, Ph.D.

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 neuroprotective agents for their 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). Candidate agents are tested for their ability to block neuronal cell death in the cerebellum following binge alcohol exposure in neonatal rats [postnatal day (PD) 4], and for their ability to protect against subsequent deficits in eyeblink classical conditioning typically induced by neonatal binge alcohol exposure. Additional studies assess candidate protective agents using a neonatal C57BL/6 (B6) mouse model, evaluating both cerebellar and forebrain damage in C57BL/6 mice. New studies have been planned using prenatal exposure in C57BL/6 mice to assess long-term protection of behavior and brain structure by activity-dependent neuroprotective peptides (e.g., .L-NAP).

IV. Methods:

The studies completed to date have tested two candidate agents, vitamin E and L-NAP, for their potential to protect against structural or functional damage the cerebellum induced by binge-like neonatal alcohol exposure in rats. Anatomical studies have assessed 1.) acute activation of caspase-3 in the cerebellum using immunohistochemical localization and western blotting, and 2.) counts of Purkinje cells and neurons of the deep nuclei. Ongoing studies are developing additional markers of the time course of cerebellar Purkinje cell death, i.e., Fluoro-Jade B. Behavioral studies have used eyeblink conditioning procedures in juvenile and adult rats to assess the extent of alcohol-induced deficits (and potential protection) in standard Pavlovian delay conditioning, a form of associative learning known to depend on functional plasticity in specific cerebellar-brain stem circuits. In addition, ongoing studies are evaluating effects in the "trace" eyeblink conditioning task, a task 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 of trace conditioning may permit assessment of potential protection against hippocampal-forebrain circuits, to complement our cerebellar-specific analyses already underway.

Additionally, studies in B6 mice are ongoing which mice are treated with a heavy binge dose of alcohol either on postnatal day (PD) 7 or on PD 7-9, and tested on two variants of eyeblink conditioning (acquisition and reversal of conditional discrimination; trace conditioning) and on spatial learning in the Morris water maze. These studies can potentially identify behavioral consequences of developmental effects of alcohol on the forebrain-hippocampal circuits. Dr. Zhou has confirmed that binge alcohol exposure on PD 7 causes significant apoptotic-like neuronal death in the hippocampal and limbic cortical circuitry, and these studies can provide evidence for correlations between the structural damage and behavioral function.

V. Accomplishments and Results:

Neither vitamin E (Tran et al., 2005) nor L-NAP protected against neonatal alcohol-induced cerebellar cell death. Vitamin E also failed to prevent deficits in acquisition of Pavlovian eyeblink conditioning. Despite these negative findings, the experimental approach can still be usefully applied to other potential therapeutic agents. In that regard, it was recently reported at the 2005 Society for Neuroscience meeting that nicotinamide can dramatically protect against alcohol-induced apoptotic cell in the mouse forebrain and prevent subsequent deficits in water maze navigation (Ierac & Herrera, 2005). Based on their findings, in the coming year we will assess whether nicotinamide can protect against alcohol-induced structural and functional damage in the neonatal rat cerebellum.

As part of this project, alcohol-induced deficits have now been confirmed in Pavlovian "trace" eyeblink conditioning, and that the deficits in trace conditioning are even more severe than deficits for standard delay conditioning procedures. 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 a sensitive measure of learning deficits in fetal alcohol spectrum disorders.

V. Accomplishments and Result: (continued)

In the studies involving B6 mice (with Drs. Zhou and Stanton), ethanol 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. Yet, in the discrimination reversal task, the ethanol-treated groups did not show the expected deficits in reversal of discrimination training, even though an unexpected enhancement of responding to both conditioned stimuli (the new CS+ and the former CS+) during reversal was observed. New studies will assess whether the typical profile of hippocampal damage can be produced in this neonatal mouse model using 3 days of binge alcohol exposure on PD 7-9.

VI. Discussion:

The lack of protective effects by L-NAP in the neonatal rodent exposure models—both in our studies and in those of Dr. Zhou—compared to the potent protective effects in the gestational rodent exposure models, imply that different mechanisms of damage are involved and that L-NAP may be more effective in preventing alcohol-induced damage occurring during the first half of human pregnancy. 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). The potential of for vitamin E to protect against alcohol-induced damage to hippocampal and/or limbic cortex will be evaluated in the coming year.

If the 3-day alcohol binge exposure on PD 7-9 in B6 mice result in permanent deficits in hippocampal-dependent learning (trace conditioning; spatial learning), then studies of neuroprotection using this mouse model may be particularly useful in developing therapeutic applications relevant to human FASD.

VII. Interrelation with the aims of the Consortium and other Projects:

This project is closely interrelated with that of Dr. Zhou's work. Both labs produced data that activity-dependent neuroprotective peptides (e.g., L-NAP) failed to protect against alcohol-induced brain damage in neonatal rodent models. This suggests that these peptides may not be effective against apoptotic neuronal death induced by binge drinking later in pregnancy. However, the effectiveness of L-NAP in protecting against embryonic neuroteratogenesis demonstrated by Dr. Zhou indicates significant protection of the embryonic-fetal brain. Planned behavioral and neuroanatomical studies in adult mice (following prenatal manipulations) will attempt to confirm and extend this encouraging possibility. The collaborative studies on mouse behavioral endpoints, in collaboration with Dr. Stanton, will continue to be based on mutually informative neuroanatomical, pathological, and behavioral outcomes.

An important link between the basic science component and the human components is through translation research focused on eyeblink classical conditioning. The ongoing eyeblink conditioning work in the South African cohort is directly related to our basic science effort. The behavioral and neurobiological data can move most quickly in the animal studies, including potential interventions or treatments. Should the animal model studies identify a potentially effective treatment against alcohol-induced brain damage, the Consortium may consider potential applications to at-risk human populations at the Consortium sites. In addition, it may be possible to link variation in the extent of functional damage in FASD cases to the relative impairment on trace and delay conditioning. For example, the demands on neural systems underlying these two learning tasks differ, given that 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).

The L-NAP animal model work now focuses exclusively on prenatal rodent models, and the key advances will involve 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 are identified as being crucial to neuroprotective actions of L-NAP, localizing the site of action and mechanisms of L-NAP can be pursued through direct collaboration with Drs. Miller and Charness.

VIII. Plans for the Next Year*:*

1. Assess whether nicotinamide (vitamin B3 complex) can protect the neonatal rat cerebellum against alcohol-induced structural and functional damage, including delay eyeblink conditioning.
2. Assess whether vitamin E or nicotinamide can protect the neonatal rodent forebrain against alcohol-induced structural and functional damage, using trace eyeblink conditioning and/or discrimination reversal training, spatial learning, and hippocampal cell counts.
3. 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.

IX. Publications:

Articles

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, 2005, 29:117-129.

Goodlett, C.R., Horn, K.H., and Zhou, F.C. Alcohol teratogenesis: Mechanisms of damage and strategies for intervention. Experimental Biology and Medicine, 2005: 230:394-406.

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 peptide analogues NAPVSIPQ (NAP) or SALLRSIPA (SAL) of Activity Dependent Neurotrophic Protein 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:

1.) Testing the protective effect of NAP on growth retardation and microencephaly, and NAP / SAL on reduction of serotonin neurons in prenatal liquid diet model.

We have previously shown that prenatal alcohol exposure (PAE) with a liquid diet containing 25% ethanol-derived-calories (EDC) compromised a midline neural tube development and reduced the number of serotonin neurons in the midline rostral raphe, including dorsal and median raphe in C57BL/6 (B6) mice. The SAL peptides were found to protect against fetal body and brain weight reduction area of brain region reduction, and cortical thinning (Zhou et al., 2004). In the current year studies, NAP was tested for microencephaly, and NAP and SAL were tested for midline defect and serotonin neuron reduction. 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 the midline structure and the serotonin neurons were measured.

2.) Use the multiple-day NAP treatment to test the NAP protective effect against binge alcohol exposure induced apoptosis in the limbic brain and striatum.

We have previously tested the effect of single-day L-NAP treatment against apoptosis 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) were performed. We found NAP does not reduce the apoptotic neurons in the alcohol-induced high apoptotic regions, such as the limbic forebrain and cortices of the neonatal brain. To explore this extended treatment in multiple-days in the current year a 3-day treatment was test along with daily alcohol (or saline) treatment from P7-P9. All pups were perfused at P9 for immunocytochemical staining of the active subunit of caspase-3 for analysis of apoptosis.

3.) Explore ways of facial image data acquisition in mouse model

The liquid diet prenatal model of B6 mouse as the treatment in the Methods section above (1) were used for preliminary analysis of facial dysmorphology. The embryos at E15 were taken and photographed with a stereomicroscope and the facial features such as head size, eye distance, and nose size were measured in 2-D.

V. Accomplishments and Results:

1a. Effect of NAP on growth retardation and microencephaly

On Body and Brain Weight and brain volume. In agreement with our previous report (Zhou et al, 2003 2004), prenatal alcohol exposure in the current paradigm reduced body and brain weight as well as the head size, of the developing embryos. NAP treatment increased the fetal body weight ($p<0.01$) from ALC group, and restored brain weight ($p<0.01$) from the level of ALC group to the levels comparable to PF and Chow groups. NAP pretreatment also increased the prenatal alcohol exposure reduced forebrain volume from the level of ALC group ($p<0.01$) to the level comparable to those of PF and Chow group. NAP increases

V. Accomplishments and Results: (continued)

prenatal alcohol exposure reduced size of brain regions in ganglionic eminence, diencephalon, septal nucleus, hippocampus, and amygdala to levels similar to those of PF and Chow group. Alcohol also reduced the thickness of the medial frontal and cingulate cortices. In agreement with previous observation that prenatal alcohol exposure causes thinning of cortices in the differentiating molecular layer and intermediate zone but not in the germinal layer of the ventricular and subventricular zone. NAP-treatment increases thickness in the differentiating zone of cortex, but does not the germinal zone. No difference was found between PF and Chow in the above parameters. We further demonstrated that the serotonin receptor 5-HT1A is actively expressed in the differentiating zone but not the germinal zone of the above regions. This active expression is subsided when differentiation is completed (Patel and Zhou, 2005). Since 5-HT serves as signal for forebrain differentiation, If NAP / SAL increase serotonin, it may provide protection through mediating serotonin-5-HT1A signaling.

On Neural Tube Opening and Ventricle Enlargement. Fetal alcohol exposure reduced cell mass in neural tube and the timely formation of midline tissue particularly in the roof and floor plate. The thinning of floor and roof plate leads to intermittent opening especially in the regions of future lateral and 3rd ventricles. (Zhou et al 2003). NAP increases the cell mass and thus reduced the size and frequency of the roof and floor opening. Fetal alcohol exposure enlarged ventricular size in the ALC brain as compared with that of PF throughout the lateral and third ventricles. NAP attenuated the enlargement of both the lateral and III ventricles.

1b. Effect of NAP on Serotonin Neurons

The E15 fetal 5-HT-im neurons were counted through the rhombencephalon which contains 5-HT neurons in entire rostral raphe. In this range of tissue, the ALC had an average of 20-30% fewer estimated 5-HT-im neurons, as compared to that of PF and Chow. We have seen a similar reduction in our previous studies (Zhou et al, 2001; 2002). No statistical difference was found between PF and Chow. Between the ALC+NPA group and the ALC+SAL group, the SAL has greater effect in increasing the number of 5-HT-im neurons from the level of ALC group. The NAP+Chow group has a similar level to that of Chow group. In analyzing the subnuclei of the dorsal raphe, the effect of peptide treatment is predominantly in the dorsal raphe nucleus.

2. Multiple-day treatment on binge neonatal model

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

3. ***Mouse facial measurement***

Prenatal alcohol exposure in the current paradigm reduced head size, eye distance, and nose width in ALC group as compared those with the Chow and PF groups. Both SAL and NAP protected against the alcohol-reduction of head size and nose width but does not protect against eye distance in ALC+SAL and ALC+NAP groups, as compared those with ALC group. No difference was found between PF and Chow in the above parameters. There are significant increases of gross malformation or missing of eyes in ALC group as compared with PF and Chow. Both SAL and NAP protected against the effect of alcohol-increased gross eye malformation. ALC group significantly increased deformation of limbs, and both ALC group and liquid diet (PF) delayed segregation of digits (webbed digits) as compared with Chow groups. Both NAP (ALC+NAP) and SAL (ALC+SAL) protected against the alcohol or dietary effect.

VI. Discussion:

The ability of SAL or NAP to antagonize the prenatal alcohol-induced retardation of forebrain growth and midline serotonin neurons at midgestation, without itself inducing noticeable abnormalities, suggests its

VI. Discussion: (continued)

potential for use as a protecting agent against alcohol neuroteratogenesis and fetal alcohol spectrum disorders. Data collected to date indicates that NAP or SAL seems effective at the prenatal stages on fetal 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 short (one day) or extended (3 days) treatment. The NAP treatment of the current dose and pattern in the postnatal stage suggests alternative treatments. On the other hand, the SAL or NAP is found as a potential protectant in antagonizing the prenatal alcohol exposure caused facial or limb dysmorphogenesis at midgestation.

Since the NAP / SAL protects against the prenatal alcohol exposure induced serotonin reduction, and 5-HT1A expression temporal and spatially coincides with the NAP/SAL protection in differentiating zone of the forebrain, together they support the hypothesis that a mechanism of NAP or SAL's protection against forebrain growth retardation is mediated through the serotonin and 5-HT1A signaling pathway.

VII. Interrelation with the aims of the Consortium and other Project:

1. The current in vivo study on NAP/SAL is in agreement with the in vitro study of NAP/SAL with respect to the protective effect on cultured cells and embryos by Dr. Charness. Our study will gain insight from Dr. Miller's mechanistic study of the protective effect of NAP and SAL on culture cells.
2. 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 apoptosis in the cerebellum of the neonatal rat model. We are collaborating with Drs. Goodlett and Mark Stanton for the study on the behavioral paradigm of the neonatal binge alcohol exposure model. In light of effective protection of NAP/SAL in prenatal model, we will pursue the behavioral paradigm with Drs. Goodlett and Mark Stanton for behavioral deficit screening.
3. Our pilot data indicates that (a) the prenatal alcohol exposure induced facial dysmorphology and (b) the potential effect of NAP/SAL on protecting against prenatal alcohol exposure induced facial dysmorphology. The potential application of the mouse model will be an interesting interaction with the human facial FAS/ FAE projects pertaining to the determination of the early phase of facial dysmorphology. Furthermore, it will serve as a model for a potential NAP/SAL treatment on this parameter.

VIII. Plans for the Next Year:

1. Testing pilot study for mechanism of NAP/SAL on protection against prenatal alcohol exposure
2. Testing alternative protecting agents for pre- and postnatal alcohol exposure; this includes one or multiple of the following compounds: Glutathione, serotonin1A agonist buspirone, Vitamin B3, or brain derived neurotrophic factor (BDNF).
3. Pilot study of Facial imaging in 3-D analysis for comparison with human facial dysmorphology. The facial dysmorphology study is beyond the current funding. Seeking a support will be necessary to pursue this study.

IX. Publications:

Patel T., Zhou F.C., (2005) Ontogeny of 5-HT_{1A} Receptor Expression in the Developing Hippocampus, Brain Res, Develop. Brain Res., 157(1): 42-57.

Zhou F.C., Sari Y., Powrozek T. Activity Dependent neurotrophic protein agonist, NAP, protects against fetal alcohol exposure induced brain growth retardation, in preparation.

Zhou F.C., Powrozek T., and Tonade, R. Activity Dependent neurotrophic peptides protects against fetal alcohol exposure compromised serotonin deficit, in preparation.

X. Posters and Presentations:

Zhou, F.C. Sari, Y. Powrozek, TA. Agonist of activity dependent neurotrophic factor, L-NAP, antagonizes the alcohol compromised midgestation fetal growth, microencephaly and ventricle enlargement. Abstract. Soc. Neurosci. (2004) 34, 944.11

Powrozek T.A. and Zhou F.C. Activity Dependent Neurotrophic Antagonizes the facial and limb dysmorphology in liquid diet model of C57BL mice fetal alcohol syndrome Res. Soc. on Alcoholism 29(2005) P130A.

Zhou F.C., Alcohol as a liability to brain development- the Fetal alcohol syndrome in US-China joint Workshop "Healthy People 2020 - Alcohol, Obesity and Diabetes" 10, 2005.

Progress Report for Basic Science: Therapeutic Agents

I. Principle Investigator: Keith W. Miller**,** DPhil, Subcontract to Michael Wilkemeyer, PhD.

II. Title of Project: Photo-labeling 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 □M), incubated for 10 minutes in the dark and then irradiated for 30–40 minutes at 365 nm. We previously reported photolabeling mL1–1–6—Fc with [^{3}H]3-azioctanol and identifying by HPLC two cyanogen bromide (CNBr) fragments that incorporated tritium. These were identified by their mass but could not be sequenced. We have now switched to a new protocol. Photolabeled L1 was reduced with dithiothreitol and the free cysteines alkylated with iodoacetamide to prevent cross linking. This digested and "linearized" L1 was applied to a 7% polyacrylamide gel, which separated two bands that were digested with trypsin, eluted and subject to mass spectrometric analysis. We have also changed to a more powerful FT/MS/MS spectrometer to sequence these fragments. 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). Finally, identified peaks can be sequenced by collision–activated MS/MS.

V. Accomplishments and Results:

Aim 1: A synthesis we had worked out last year for [^{3}H]3-azibutanol has now been accomplished by Amersham/GE. Unfortunately, they initially purified the isotopically labeled compound in methanol/water and were unable to provide it in a suitable solvent. We then devised an HPLC protocol which should enable them to produce it in aqueous solution. The second synthesis may have been successful, but they were unable to characterize it. We are currently carrying out HPLC analysis and photolabeling trials. If these are successful we will purchase their product and commence studies on Aim 1.

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. The new protocols we introduced (see above) this year have now led to the identification of several sites of photoincorporation of 3-azioctanol. We have identified and sequenced 7 peptides in the L1-Fc protein that were photolabeled. The location of these sites is as follows: 1.) Residue E33 within the N-terminus just before the Ig1 domain; 2.) residue E81 in the Ig1 domain; 3.) residue Y417 at the C-terminal end of the Ig4 domain; 4.) residue E463 in the Ig5 domain; 5.) residue E561 in the Ig6 domain; 6.)

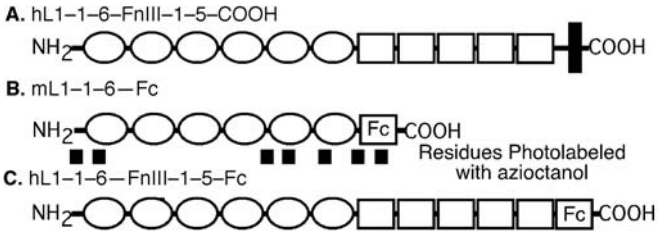


Fig. 1 Fusion proteins referred to. **A.** Full length human L1, consisting of 6 Ig (ovals) & 5 Fn (oblongs) domains plus a transmembrane C–terminus (Ig= immunoglobulin; Fn= fibronectin-like type III). **B.** Mouse model L1 contains 6 Ig domains with an added Fc domain to aid purification (Fc=Fc region of human Ig1). Black squares indicate approximate position of residues photolabeled by 3-azioctanol. **C.** Human L1 with an Fc added and the transmembrane region deleted.

residue E618 in the junction between L1 and the Fc region, and 7.) residue E672 in the Fc domain of the construct.

Other data suggest that under our standard photolabeling conditions Ng-CAM does not photoincorporate [^{3}H]azioctanol. Because Ng-CAM is part of the same superfamily as L1, this suggests that photoincorporation into the latter exhibits some selectivity. Consistent with our observations, the homology between these two proteins is weak in the regions photolabeled.

Protein production: We are currently moving our studies from mouse to human polypeptide, such as the complete extracellular region of human L1 (immunoglobulin domains 1-6 and fibronectin-like type III domains 1-5) fused in frame with the Fc region of human immunoglobulin isotype 1 (hL1–Ig1–6–FnIII-1–5–Fc) shown in Fig. 1C. Cells stably transfected with this construct were obtained from Dr. Cynthia Bearer. We are using the purification strategy developed by Dr. Martin Grumet, (Haspel et al, 2001), who provided the original mouse L1-Fc chimeric protein described above. Yields as high as 1-2 mg of recombinant polypeptide/L of tissue culture medium have been reported. However, after one semi-successful preparation, we were unable to obtain sufficient yield of the recombinant hL1-Fc polypeptide (<100 ug/liter of tissue culture media). To improve the yield, we tried a system employed by Dr. Bearer, who used an artificial capillary cartridge system (Cellmax, Spectrum labs) that concentrates secreted proteins and reduces the volume of culture medium 10–fold. Preliminary experiments using this culture system have produced a yield of ~2 mg/L, but with reduced purity (using the same purification scheme as described above) and on a very small scale (10mL). We have been unable to scale-up production using this system.

Dr. Grumet has recently provided additional stably transfected NIH/3T3 cell lines that express various subsets of the extracellular domains of human L1, fused in frame to the Fc domain. These cell lines include hL1–Ig1–6–Fc, hL1–Ig1–2–Fc, hL1–Ig1-3–Fc, hL1–Ig1-4–Fc, hL1–Ig1-6+2–Fc (all 6 Ig domains and the first 2 fibronectin type III domains of human L1 fused to the Fc sequence) and HEK293 cells expressing mouse hL1–Ig1-6+5–Fc (i.e. hL1–1–6–FnIII–1–5–Fc; Fig. 1C). We have had much better success with scaling up production of recombinant protein from these cell lines. For example, the hL1–Ig1–6–Fc cell line produces large quantities of recombinant polypeptide with high purity, using the strategy outlined above. From one preparation we obtained ~2 mg/L of tissue culture media with little contamination, as judged by SDS-PAGE and silver staining. Preliminary preparations using the cells expressing the mL1–1–6–FnIII–1–5–Fc, gave a high level of purity and a good yield; with 4 ml of conditioned tissue culture medium we obtained ~5 □g (which extrapolates to ≥ 1.2 mg/L).

VI. Discussion:

Where are these sites on L1? There is no structure of L1 but structures of some related proteins have been reported. Therefore, we conducted a homology modeling study. The sequence of mL1 (Ig1–4) was submitted to the server. The best match was found with the axonin-1 structure, which includes Ig1–4. Our sequence was threaded on this backbone and structural refinement performed to allow for the different residues. More refined modeling of this predicted structure is ongoing, but the preliminary model is very encouraging. It place E33 and Y417, on Ig1 and Ig4 respectively very close together, likely they are two residues in a single pocket formed by the two Ig domains. Furthermore, two residues, L120 & G121, in which mutations in humans are associated with neurological disorders, are adjacent to this pocket. Thus, this site likely has functional significance. Also of interest is that E81 forms the bottom of a groove suitable for binding alcohols.

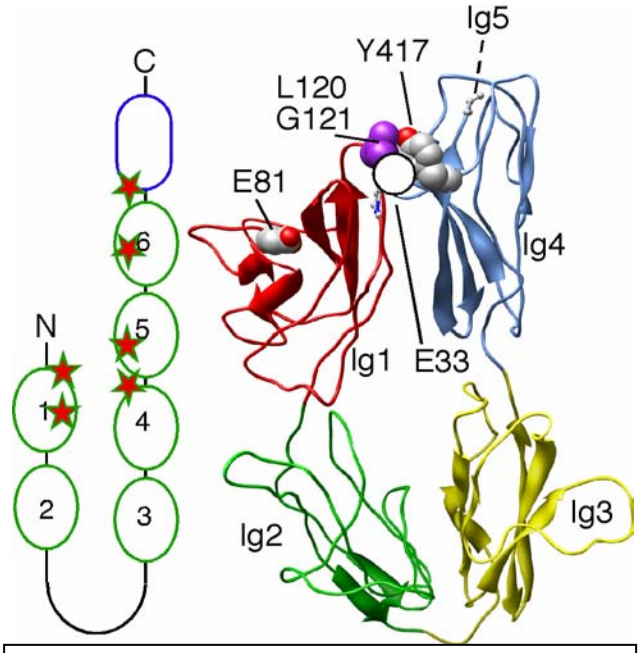


Fig. 2. Photolabeled residues. **Left panel**: the six Ig domains are arranged according to one model and the stars indicate the approximate location of photolabeled residues. **Right panel**: The homology model of Ig1–4. Side chains are only shown if they were photolabeled by 3-azioctanol (grey & red, E81, Y417. E33 is indicated by a white sphere) or are pertinent mutations (purple, L120, G121). Note that E33 & Y417 and L120 & G121 are clustered together.

VII. How this project interrelates to the aims of the Consortium

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 to provide more detailed information about the structure of the alcohol binding sites in order to guide rational drug development.

VIII. Plans for the Next Year

Many of the technical problems encountered last year have been overcome this year. The photolabeled sites identified on mouse L1 look sufficiently promising to encourage us to continue with new studies on human L1

Specific Aim 1: Photolabeling with the agonist [^{3}H]3-azibutanol will commence if characterization of the ligand is successful. Initial work will involve photolabeling human L1, digestion, purification and sequencing 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, and consideration will be given to using the simpler construct hL1–Ig1–6–Fc. Once this is resolved pharmacological characterization of the different sites will be commenced and FT/MS/MS studies will be performed to enable comparison with the mouse protein.

IX. Publications:

None.

X. Posters and Presentations:

None.

Appendix

IX. Publications in Print or in Press:

Amit J., Huang J., Fang, S. and Riley, E.P. Using Facial Images to Diagnose FetalAlcohol Syndrome (FAS), In Proc. of IEEE International Conference on Information Technology: Coding and Computing, ITCC, 2005.

AuttiRamo I., Fagerlund A., Ervalahti N., Loimu L., Korkman M., Hoyme H.E.. Fetal Alcohol Spectrum Disorders in Finland: Clinical delineation of 77 older children and adolescents. Amer J Med Genet 140A: 137-143, 2006.

Bookstein, F. L., Connor, P. D., Covell, K. D. Barr, H. M., Gleason, C. A., Sze, R. W., McBroom, J. A. and Streissguth, A. P. Preliminary evidence that prenatal alcohol damage may be visible in averaged unwarped ultrasound images of the neonatal human corpus callosum. *Alcohol,* in press, 2005.

Carter, R.C., Jacobson, S.W., Molteno, C.D., Viljoen, D., Chiodo, L.M., & Jacobson, J.L. (2005). Effects of prenatal alcohol exposure on infant visual acuity. Journal of Pediatrics, 147, 470-476.

Goodlett, C.R., Horn, K.H., and Zhou, F.C. Alcohol teratogenesis: Mechanisms of damage and strategies for intervention. Experimental Biology and Medicine, 2005: 230:394-406.

Patel T, Zhou F.C., (2005) Ontogeny of 5-HT_{1A} Receptor Expression in the Developing Hippocampus, Brain Res, Develop. Brain Res., 157(1): 42-57.

Riley E.P., McGee C.L., and Sowell E.R.. (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., Repasky, R.,and Arenson, A., 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.

Stromland K, Mattson S.N., Adnams C.M., Autti-Ramo I., Warren K.R. Fetal Alcohol Spectrum Disorders: An International Perspective: Alcohol: Clinical Experimental Research 2005; 29: 1121-1126**.**

O'Hare E.D., Kan E., Yoshii J., Mattson S.N., Riley E.P,. Thompson P.M., Toga A.W., Sowell E.R. (2005) Mapping cerebellar vermal morphology and cognitive correlates in prenatal alcohol exposure. Neuroreport 16:1285-1290.

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, 2005, 29:117-129.

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.

Publications Submitted:

Bakhireva L., Jones K.L., Robinson L.K,. del Campo M., Riley E.P., Mattson S.N., Marintcheva G., Chambers C.D. Effectiveness of training pediatricians to diagnose the fetal alcohol syndrome. Pediatrics. submitted.

Brain metabolic alterations in adolescents and young adults with fetal alcohol spectrum disorders. Fagerlund, A, Heikkinen, S., Autti-Rämö, I., Korkman, M., Timonen, M., Kuusi, T., Riley E.P., Lundbom, N. Alcoholism Clin Exp Res, submitted.

Fang S., Fang J., Huang J., Tuceryan M., Moore E., Robinson L., Mattson S.N., Ramo I.A., Foroud T., Riley E.P., Li, T.K. Digital Geometry Image Analysis for Medical Diagnosis. IEEE Transaction on Visualization and Computer Graphics. submitted.

Publications Submitted: (continued)

Kodituwakku, P., Coriale, G., Fiorentino, D., Kalberg, W.O., Buckley, D., Gossage, J.P., Deiana, L., Aragon, A.S., Ceccanti, M., May, P. Neurobehavioral Characteristics of Children with Fetal Alcohol Spectrum Disorders in Communities from Italy: Preliminary Results.Alcoholism: Clinical and Experimental Research Submitted.

May P.A., Fiorentino D., Gossage J.P., Kalberg W.O., Hoyme H.E,. Robinson L.K., Coriale G., Jones K.L., del Campo, Tarani L., Romeo Mkodituwakku P.W., Deiana L., Buckley D. The Epidemiology of FASD in a Province in Italy: Prevalence and Characteristics of Children in a Random Sample, In-School Study. Alcoholism: Clinical and Experimental Research. Submitted

X. Abstracts, Posters and Presentations:

Flury, L. J. Rogers; T. Foroud; L. Robinson; E. Moore. Fetal Alcohol Syndrome and 3-D Facial Imaging: A Preliminary Classification Study. Poster at Research Society on Alcoholism. Santa Barbara, CA, June 2005.

Robinson L.K., Wrotniak B., Smistek B., Moore E.S., Riley E.P.. Fetal Alcohol syndrome: Comparison of clinical findings in phenotypically similar disorders. David W. Smith Workshop on Malformations and Morphogenesis. Iowa City, Iowa. August 2-6, 2005.

Bookstein,F.L.; Connor, P.D. ,. Covell, K.D., Barr, H.M., Gleason, C.A. Streissguth, A.P., Sze, R.W. , McBroom, J.A.: "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

Hoyme H.E., May P.A., Gossage J.P., Findlay R., Autti-Ramo I., Fagerlund A., Ceccanti M., Fiorentino D., Viljoen D., Khaole N., del Campo M., Jones K.L., Robinson L.K. Concordance/discordance of twins prenatally exposed to maternal alcohol abuse: A classic example of the role of gene-environment interactions in the etiology of birth defects. David W. Smith Workshop on Malformations and Morphogenesis. Iowa City, Iowa. August 2-6, 2005.

Croxford, J., Jacobson, J.L., Jacobson, S.W., Marais, A.S., Molteno, C.D., and Viljoen, D.L. (2005). Estimated peak blood alcohol concentration as an indicator of fetal alcohol risk. Alcoholism: Clinical and Experimental Research, 29, 228.

Jacobson, S.W., Stanton, M.E., Hay, A.M., Burden, M.J., Fuller, D.S., Molteno, C.D., Viljoen, D.L., and Jacobson, J.L. (2005). Impaired short delay eyeblink conditioning in children with FAS: Preliminary findings. Alcoholism: Clinical and Experimental Research, 29, 233.

Jacobson, S.W., Jacobson, J.L., Molteno, C.D., and Viljoen, D.L. (May, 2005). Drinking in pregnancy and fetal alcohol syndrome in Cape Town, South Africa. Nutrition Society of South Africa, Western Cape Branch, Medical Research Council, Cape Town, South Africa

Jacobson, J.L., Jacobson, S.W. (2005, October). Core neural deficits and effect modifiers of fetal alcohol spectrum disorder: Detroit and Cape Town research findings. American Society for Addition Medicine, Washington, DC.

Jacobson, S.W., Jacobson, J.L., and Molteno, C.D. (2005, October). Why do we need more research? Drinking in pregnancy and fetal alcohol syndrome in Cape Town, South Africa. Fetal Alcohol Spectrum Disorder Conference, Cape Town, South Africa.

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.

X. Abstracts, Posters and Presentations: (continued)

O'Hare E.D., Kan E., Yoshii J., Mattson S.N., Riley E.P., Thompson P.M., Toga A.W., Sowell E.R. (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, E.D., Kan, E., Yoshii, J., Mattson, S.N., Riley, E.P., Thompson, P.M., Toga, A.W. and Sowell, E.R.. 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. The baseline and preliminary data were presented, and a publication resulted from a symposium at the ISBRA Conference, Germany September 2004:

Stromland K, Mattson S.N., Adnams C.M., Autti-Ramo I., Warren K.R.. Fetal Alcohol Spectrum Disorders: An International Perspective: Alcohol: Clin Exp Res 2005; 29: 1121-1126**.**

Poster: Adnams, C, Kodituwakku PW, Kalberg W, Adams R, September S, Perold MD, Sorour, P, Kotze A, Castle B, Engelbrecht P, May P. Cognition and behaviour of children with FASD in South Africa: preliminary data from a pilot intervention study. Scientific Meeting of the Research Society on Alcoholism, 25-30 June 2005, Santa Barbara, USA. Alcohol Clin Exp Res 2005 :29, 5. The mid-intervention assessment findings will be presented at the 2006 RSA meeting.

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, Ph.D. Platform presentation at the June 2004 Teratology Society Meetings in Vancouver, Canada: "Ultrasound Imaging 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, Ph.D.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, Ph.D. 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

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

Flury, L. J. Rogers; T. Foroud; L. Robinson; E. Moore. 2005. "Fetal Alcohol Syndrome and 3-D Facial Imaging: A Preliminary Classification Study". Poster at Research Society on Alcoholism. 26 June 2005. Santa Barbara, CA.

Wheeler, B.C. 2004. "Research and Academic Computing." Presentation at Indiana University Statewide IT Conference. 14 October 2004. Indianapolis, IN.

X. Abstracts, Posters and Presentations: (continued)

Presentations in the Indiana University Advanced Visualization Laboratory, including the Indiana State Legislature, Techpoint (an organization representing the IT community in Indiana), various groups of researchers and educators, and the NSF.

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

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

Hoyme, E.H., Fagerlund, Å, Ervalahti, N, Loimu, L, and Autti-Rämö, I. Fetal alcohol spectrum disorders in Finland: Clinical delineation of 77 older children and adolescents and comparison with affected North American children. Paper presented at the Western Society for Pediatric Research Feb 2005.

Powrozek T.A. and Zhou F.C. Activity Dependent Neurotrophic Antagonizes the facial and limb dysmorphology in liquid diet model of C57BL mice fetal alcohol syndrome Res. Soc. on Alcoholism 29(2005) P130A.

Zhou F.C., Alcohol as a liability to brain development- the Fetal alcohol syndrome in US-China joint Workshop "Healthy People 2020 - Alcohol, Obesity and Diabetes" 10, 2005

Zhou, F.C. Sari, Y. Powrozek, TA. Agonist of activity dependent neurotrophic factor, L-NAP, antagonizes the alcohol compromised midgestation fetal growth, microencephaly and ventricle enlargement. Abstract. Soc. Neurosci. (2004) 34, 944.11

Lu, L.H., O'Hare, E.D., Bookheimer, S.Y., Kan, E., McCourt, S.T. and Toga, A.W. Mapping activation during verbal learning and recall in normally developing children. In preparation for presentation at the 12th Annual Organization of Human Brain Mapping Meeting, Florence, Italy.

O'Hare, E.D., Kan, E, Yoshii, J, Mattson, S.N., Riley, E.P., Thompson, P.M., Toga, A.W and Sowell, E.R. Cognitive Correlates of Anterior Cerebellar Vermal Dysmorphology in Heavy Prenatal Alcohol Exposure. Program No 359.14. 2004 Abstract Viewer. Society for Neuroscience, 2004. Online.

O'Hare, E.D., Kan, E., Yoshii, J., Mattson, S.N., Riley, E.P., Thompson, P.M., Toga, AW and Sowell, ER. Mapping Cerebellar Vermal Abnormalities in Severe Prenatal Alcohol Exposure: Localized Dysmorphology of the Anterior Vermis and Primary Fissure. 10th Annual Meeting of the Organization for Human Brain Mapping, Budapest, Hungary. NeuroImage Abstracts, 2004.

Relationship between dysmorphic features and general cognitive functioning in children with fetal alcohol spectrum disorders. Nina Ervalahti, H. Eugene Hoyme, Åse Fagerlund, Ilona Autti-Rämö, Leena Loimu, Marit Korkman (to be sent for peer review in January-February)